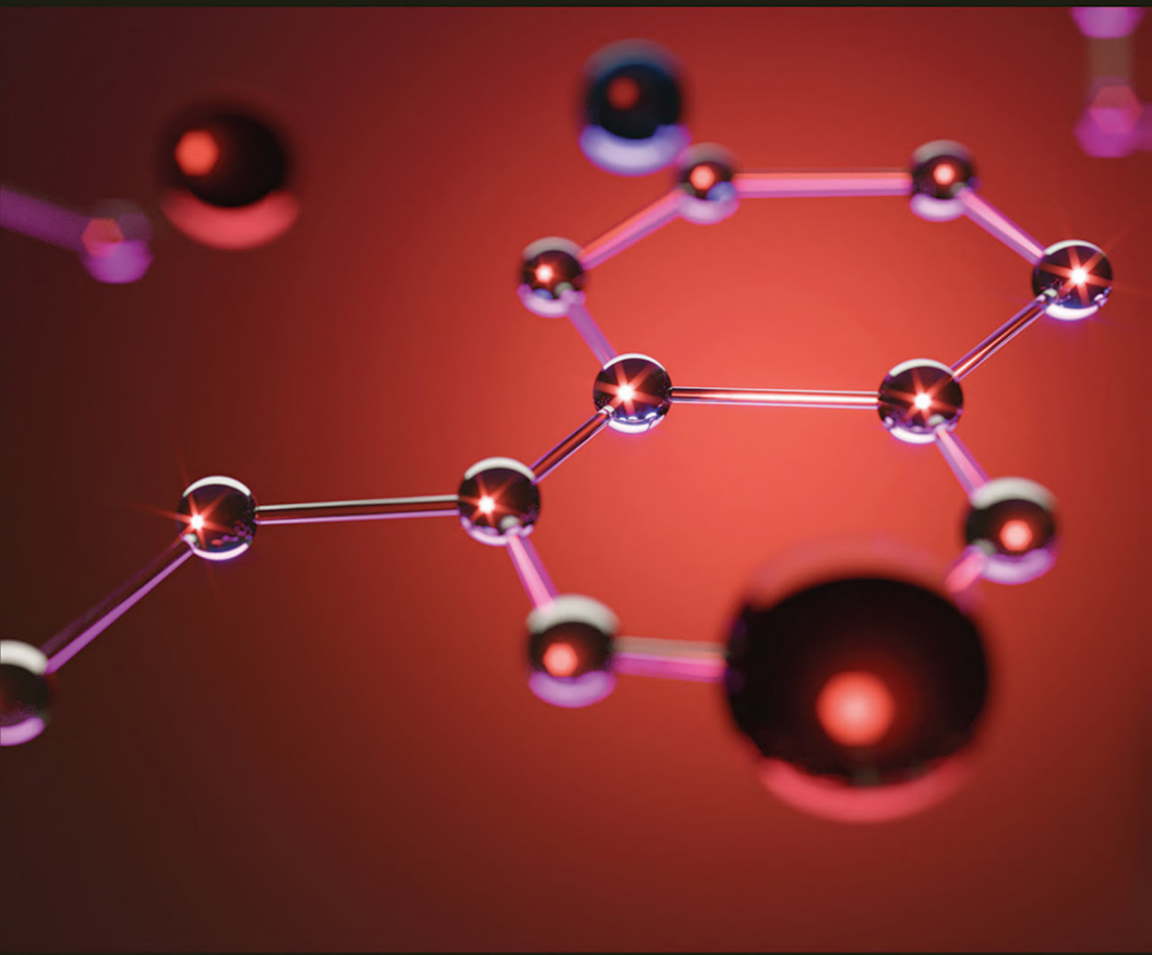
THE SEROTONIN SYSTEM

HISTORY, NEUROPHARMACOLOGY, AND PATHOLOGY



Edited by
Mark D. Tricklebank and Eileen Daly

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ACADEMIC PRESS

An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-813323-1

Publisher: Nikki Levy
Acquisition Editor: Joslyn Chaiprasert-Paguio
Editorial Project Manager: Tracy I. Tufaga
Production Project Manager: Bharatwaj Varatharajan
Cover Designer: Mark Rogers

Typeset by MPS Limited, Chennai, India

Dedication

I would like to dedicate this volume to Dr John Fozard and Professor Gerald Curzon, both of whom had unexpected confidence in me at an early stage without whose influence I would not have had the privilege of having a scientific career. I am deeply grateful to both of them.

Mark D. Tricklebank
October 31, 2018

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Editors' Biography

Dr Mark D. Tricklebank, BSc, MSc, PhD, DSc, FBphS, earned his PhD from the University of Manchester, and after completing postdoctoral training at the Institute of Neurology, he joined the pharmaceutical company Merrell Dow in Strasbourg, where he was instrumental in the identification of the functional relevance of the newly identified 5-HT1A recognition site. He then moved to Merck at Terlings Park, where he worked with Susan Iversen to identify the behavioral effects of the NMDA receptor antagonist MK-801 and showed them to be identical to those of phencyclidine and ketamine. Thereafter serving as head of the Mental Health Unit at Sandoz Pharma in Basel, he was appointed director of In Vivo Pharmacology at the Lilly Research Centre, Windlesham, where he conceived and founded the Lilly Centre for Cognitive Neuroscience, one of the first industrial-academic partnerships in the United Kingdom. After a varied career of several decades in the pharmaceutical industry, he now serves as a Wellcome Trust Fellow in the Institute of Psychiatry, Psychology and Neuroscience at King's College London, and has published more than 160 papers.

Dr Eileen Daly, PhD, is a Senior Lecturer in the Department of Forensic and Neurodevelopmental Sciences at King's College London. When at the National Institute of Aging, National Institutes of Health, USA, she measured neurotransmitter metabolites in human CSF and rodent brain. Relocating to the Institute of Psychiatry in London, she completed her PhD in Developmental Neuroscience with a project using Acute Tryptophan Depletion to modulate serotonin in Autism Spectrum Disorder, and then looking at the brain with fMRI. She continues to study the role of neurotransmitters in developmental disorders and is the author of more than 100 papers.

Preface

Serotonin first became my mistress whilst studying for a masters in neurochemistry at the Institute of Psychiatry when I visited the lab of Gerald Curzon at the Institute of Neurology looking for a student project with him. As a graduate in psychology and biochemistry I was somewhat disappointed that the emphasis on understanding the biochemistry of mental disorder that I had hoped for was missing from the course. There seemed to be much more interest in the control of respiration cycles, that is, the brain was treated as an extension of the liver. But Gerald introduced me to the metabolism of serotonin. When he opened a store cupboard to reveal trays of little brown bottles which on careful examination I found to contain lysergic acid diethylamide, my heart leapt with excitement. The project was exactly what I was looking for: this was 1971 after all. It felt somewhat uncanny that serotonin had been discovered in the year of my birth. And even more uncanny that I would one day work for the Company Sandoz where Hoffman had first extracted LSD from rye mold in a city where each year on April 19th they celebrate Hoffman's 1943 finding with Bicycle day. My Mentor at Sandoz was the cardiovascular pharmacologist John Fozard with whom I had worked a few years earlier at Merrell-Dow in Strasbourgand in whose group I met my second mistress, 8-OHDPAT and enjoyed a menage a trois with the 5-Ht1A receptor and serotonin in a relationship remarkable for its longevity and scientific relevance. I thank the Wellcome Trust and Professor Steven Williams for supporting my return to the Institute of Psychiatry, where I am focused on understanding the role of 5-HT1A receptors in the control of the release of oxytocin in the hope that they might be repurposed for the treatment of disorders associated with impaired social cognition such as Autism Spectrum disorders.

Mark D. Tricklebank,
August 2018

CHAPTER ONE

The metabolism of indoleamines

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Introduction

The history of the discovery of serotonin is described in a detailed and quite excellent way by *Patricia Whitaker Azmitia* [1–4] in an article published in *Neuropsychopharmacology* in 1999. Recognizing that cardiovascular disease was the overriding cause of premature death in the 1930s and 1940s, biological and pharmaceutical research was heavily concentrated on the discovery of the causes of hypertension and heart disease. The serotonin story begins with the Italian Vittorio Erspamer who focused his life's work on the isolation of drugs from natural sources. He particularly concentrated on nitrogenous substances causing the contraction of smooth muscle that can be found in the skin and intestines of a variety of small animals. In the process, he found one substance in the enterochromaffin cells of mammalian gut which he called *enteramine* [5]. Its potential functional importance was amplified to him when he discovered the same compound in the salivary glands of the octopus. In 1952, enteramine was established as the same muscle contractant that *Twarog and Page* [6] had identified in clotted blood and that they called serotonin (*tonic* substance in *serum*, hence serotonin). Serotonin was later identified chemically as 5-hydroxytryptamine by *Rapport, Green, and Page* [6]. Serotonin was then found by *Betty Twarog* to be present in both rodent and human brains. However, the detailed architecture of its cell bodies and projection fields had to wait for *Falk and Hillarp* to develop their fluorescence histochemical technique in order to probe and demonstrate its extensive distribution in brain tissue [7]. It was immediately seen to be concentrated very selectively within the large multipolar neurons of the midline raphe cells first noticed by Ramon and Cajal [8]. Once its chemical structure was determined as 5-hydroxytryptamine (Fig. 1.1), a major

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DOI: https://doi.org/10.1016/B978-0-12-813323-1.00001-3

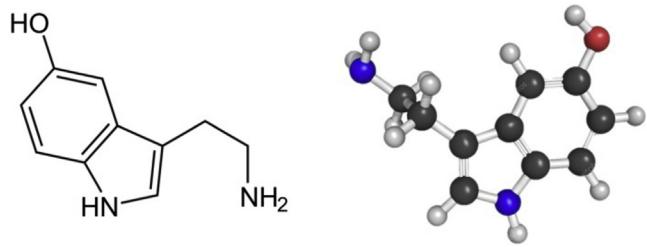

Figure 1.1 Serotonin (5-HT) molecular structure.

role in psychosis was hypothesized. This hypothesis stemmed from the fact that serotonin's actions on smooth muscle in the periphery could be antagonized by the powerful psychotomimetic agent accidentally discovered by the medicinal chemist Albert Hofmann [9] while working on alkaline extracts of the ergot fungus growing on rye [10] to identify circulatory and respiratory stimulants. The compound was structurally related to serotonin and later identified to be lysergic acid diethylamide (LSD). This work led *Brodie and Shore* [11] proposed that serotonin and norepinephrine might act as opposing neurochemical systems analogous to adrenaline and noradrenaline in the sympathetic and parasympathetic systems, respectively. Nevertheless, it was not until 1943 that *Hofmann* took the step to experiment with his own compound and surely was blissfully unaware of the massive impact that his experiment, accidental or purposeful, would have on the world. After falling from his bicycle, *Hofmann* sank into a not unpleasant intoxicated state and perceived an uninterrupted stream of fantastic images with intense kaleidoscopic colors. *Hofmann* had discovered a psychoactive substance of extraordinary potency whose threshold dose turned out to be only 20 μg. As Hofmann's images gradually subsided, they gave way to anxiety and the belief that his neighbor was a malevolent witch. Many similar folk stories of paranoia have endured: the story of the man who convinced that he had become an orange and who was immobilized by the fear of being plunged into a liquidizer to make juice. The electrophysiologist *George Aghajanian* [12] was the first to lower microelectrodes into the midline of the rodent brain to discover that the cells there displayed a slow and regular discharge pattern that was immediately blocked by iontophoretic administration of serotonin. LSD was given the name *Delysid* and sold by *Sandoz* in 1947 for clinical applications in psychiatry—first as a means of modeling psychosis. *Sidney Cohen*, a psychoanalyst who worked with *Aldous Huxley* together, thought that LSD would have a beneficial facilitating effect in

psychotherapy allowing people to access their deep unconscious thoughts and feelings, curing alcoholism, and enhancing creativity. LSD as an investigative and potential therapeutic agent's cause was championed by *Timothy Leary*, an American psychologist who very successfully tapped into the post-war youth culture of discontent with authority and wanted to explore the beneficial effects of LSD on psychiatric patients in controlled settings. For the emerging generation of biological psychiatrists wanting to throw off the shackles and unscientific principles of Freudian psychiatry and engage with the exciting developments in biology and medicine, LSD demonstrated with beautiful imagery the fact that consciousness had a biochemical basis. If a few micrograms of a compound could induce such profound alterations of perception and belief, surely all mental illness could ultimately be explained by altered brain biochemistry. Such revolutionary thoughts captured the culture of the times and LSD rapidly became the recreational drug of choice, giving freedom to experience the hitherto inaccessible unconscious spaces and so allow spiritual enlightenment, giving the individual the strength and courage to fight post-war authoritarianism, violence, repression, and injustice. Governments were uncharacteristically quick to spot what they considered to be an immoral use of a substance and both the United States and the United Kingdom declared possession and use of LSD prohibited in 1966 in the United States and 1970 in the United Kingdom. This did not happen without first taking the opportunity to test for themselves its potential use as a military weapon. Indeed, in 2006, the British Guardian newspaper reported that MI6 paid out thousands of pounds in compensation to servicemen who were fed LSD without their consent in clandestine experiments designed to demonstrate the drug's ability to control the mind for military advantage. They thought it could act as a truth drug and force any captured enemy forces to confess the crimes of their leaders. The experiments were unsuccessful but brought on by fear that communist states had such substances. MI6 was not alone. In 1975, United States President Gerald Ford personally apologized to the family of a CIA operative who had been given surreptitiously a dose of LSD that led to him jumping to his death from the roof of a 10th story hotel room in 1953. Despite these abuses, others were more scientifically prudent and realized that the potent psychoactive compound had important properties that should not be ignored. In 2012, *Krebs and Johansen* published a meta-analysis of trials investigating its success into helping individuals overcome addiction to alcohol [13]. It is difficult to imagine that such impressive

results would be as ignored today as these have been. The benefits are profound and although times have changed, the use of psychoactive drugs in a clinical setting is still severely limited by legislation and bureaucracy [14]. But, the quest goes on with [15]. The psychedelic society (http://psychedlicsociety.org.uk) is committed to the rational investigation of psychedelics because they are rightly convinced that we still have much to learn from them.

Synthesis of serotonin

Serotonin is synthesized from the dietary amino acid L-tryptophan by the action of the enzyme tryptophan-5-hydroxylase (21EC1.14.16.4) (Fig. 1.2). This enzyme is the rate limiting step in the synthesis of serotonin and can be found in brain and the enterochromaffin cells of the gut,

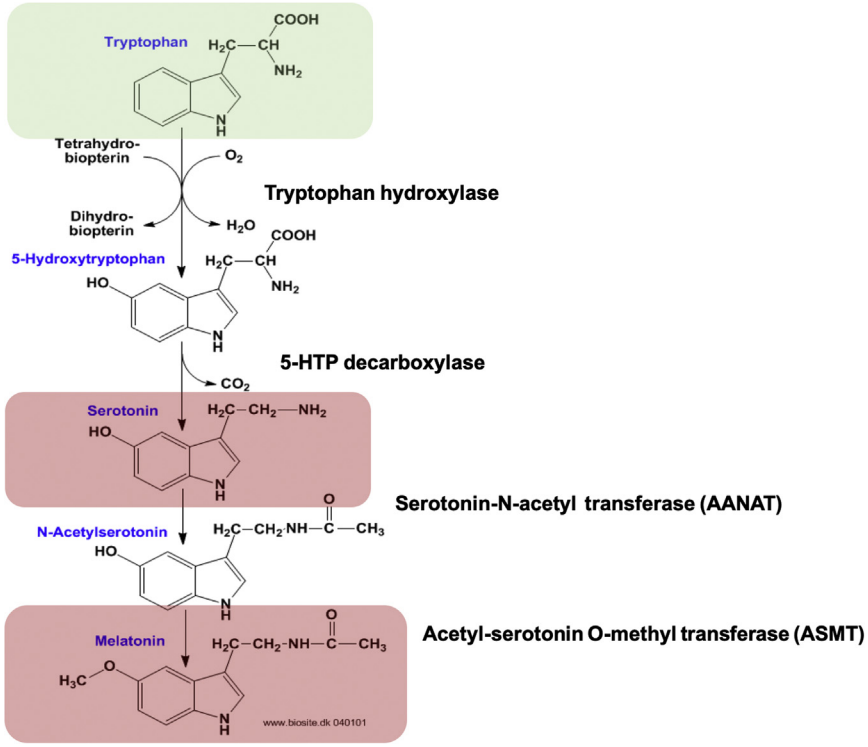

Figure 1.2 The serotonin pathway.

but not in blood platelets which accumulate the amine there after release from the gut [16]. The K_m of the enzyme is higher than the concentration of tryptophan in brain, hence the supply of tryptophan across the blood–brain barrier is crucial for maintaining an adequate supply of serotonin. Tryptophan hydroxylase is activated by phosphorylation and catalyzed by a calcium-activated protein kinase, thus influx of calcium during neuronal firing ensures that serotonin synthesis is paced to neuronal activity [4]. This was elegantly shown in studies of spinal transection in which it was clear that synthetic rate was markedly diminished in the neurons rostral to the cut [4]. Walther et al. [17] genetically deleted tryptophan hydroxylase in mice. The resultant animals were deficient in serotonin in the periphery, while serotonin was close to normal levels in the brainstem. This led to the identification of a second gene for the enzyme designated TPH1 and TPH2 [18]. TPH2 is preferentially expressed in brain, whereas TPH1 is more widely distributed throughout the body.

With fluorescence histochemistry, the extensive ramification of the serotonergic projections throughout the brain became evident. Serotonin cell bodies have both ascending and descending projections and are organized in clusters identified as B1–B9, as summarized in Table 1.1 (Fig. 1.3). The largest group of serotonergic cells is B_7, which is typically described as continuous with a smaller group of serotonergic cells. B_6 and B_7 often are considered together as the dorsal raphe nucleus, with B_6 being its caudal extension. B_8, which corresponds to the median raphe nucleus, is also termed the nucleus central superior. Group B_9, part of the

Table 1.1 Classification of serotonergic cell body groups according to *Dahlstrom and Fuxe* and corresponding anatomical structure.

Groups of serotonin-containing cell bodies	Anatomical structure
B_1	Raphe pallidus nucleus and caudal ventrolateral medulla
B_2	Raphe obscurus nucleus
B_3	Raphe magnus nucleus, rostral ventrolateral medulla, and lateral paragigantocellular reticular nucleus
B_4	Raphe obscurus nucleus and dorsolateral part
B_5	Median raphe nucleus and caudal part
B_6	Dorsal raphe nucleus and caudal part
B_7	Dorsal raphe nucleus principal and rostral part
B_8	Median raphe nucleus, rostral main part; caudal linear nucleus; and nucleus pontis oralis
B_9	Nucleus pontis oralis and supralemniscal region

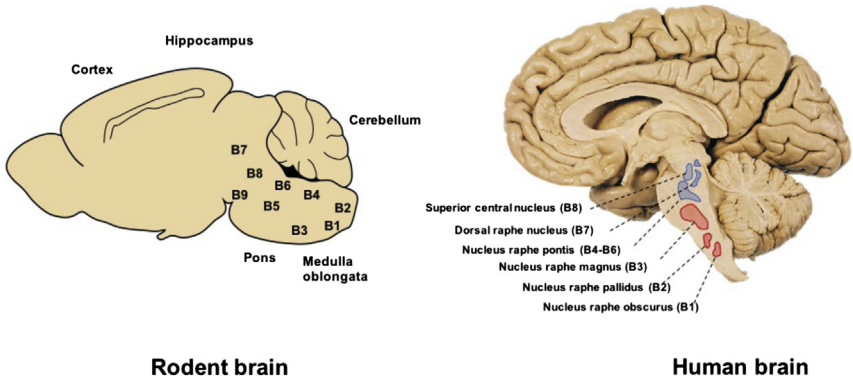

Figure 1.3 Distribution of serotoninergic cells nuclei in the rodent and human brain.

ventrolateral tegmentum of the pons and midbrain, forms a lateral extension of the median raphe and, therefore, is not considered one of the midline raphe nuclei. Ascending serotonergic projections innervating the cerebral cortex and other regions of the forebrain arise primarily from the dorsal raphe, median raphe, and B_9 cell group. B7 raphe dorsalis projects to the caudate/putamen. Descending projections to the spinal cord could be identified as well. The nucleus raphe obscurus innervates the ventral horn of the spinal cord using the posterior fasciculus of the spinal cord while the nucleus raphe magnus innervates the dorsal horn. This differential pattern extends into the medulla, where the nociceptive subnuclei of the trigeminal nuclei receive a very dense infiltration by 5-HT fibers. The last major input into the spinal cord comes from the ventral lateral medullary 5-HT neurons. These fibers use the lateral fasciculus of the spinal cord to innervate the lateral horn. The 5-HT fibers innervate both the sensory and motor nuclei of the autonomic system located at every spinal level [19].

The supply of tryptophan across the blood–brain barrier is crucial for maintaining synthetic rate. The hydroxylase utilizes the substrates L-tryptophan and molecular oxygen under the influence of a reduced pterin cofactor, L-erythro-5,6,7,8-tetrahydropteridine which serves as an electron donor. The product is L-5-hydroxytryptophan which is very readily decarboxylated to 5-hydroxytyptamine by the high capacity enzyme L-aromatic amino acid decarboxylase (EC4.11.28), a soluble pyridoxal-5′phospate dependent enzyme [4]. 5-Hydroxytryptophan decarboxylase, which unlike tryptophan hydroxylase is not confined to serotonin-containing neurons, is found in many different types of neurons that are involved in the

decarboxylation of other aromatic amino acids, notably phenylalanine which gives rise to the catecholamines, dopamine, and noradrenaline:

$$L\text{-tryptophan} = Bh_4 + O_2 \Rightarrow 5 - HTPH2 \;\; \text{biopterin} + H_2O.$$

L-5-HTP is found in brain only in trace amounts but its accumulation over time following inhibition with compounds such as benserazide or carbidopa provides a ready means of estimating synthetic rate [20]. As with other neurotransmitters serotonin is sequestered in storage vesicles. Descending serotonin-containing neurons are distinct from rostrally projecting systems in that they co-store peptides such as substance P and thyrotropin-releasing hormone [4]. Serotonin and norepinephrine are preferentially oxidized by monoamine oxidase (MAO) A, whereas MAO B preferentially oxidizes phenylethylamine [21]. Deletion of the MAOA gene, but not MAOB, is associated with aggression in both rodents and man [22].

Following the release of serotonin, the amine is rapidly taken back into the nerve ending via the specific serotonin transporter or metabolized to 5-hydroxyecetaldehyde, via the action of the mitochondrial monoamine oxidase, and thence to 5-hydroxyindoleacetic acid, via aldehyde dehydrogenase. Again, tracking the accumulation of the amine over time following inhibition of monoamine oxidase with tranylcypromine affords a ready means of estimating serotonin synthesis and utilization. It is the only measure with biological significance. However, unfortunately, such pharmacologically dependent measures may distort or ignore any feedback inhibition [23]. Many manipulations hypothesized to alter serotonin release and metabolism often reveal the tissue concentration of serotonin to be static, even in the presence of large increases in 5-HIAA. Estimates of synthetic rate and metabolism by blocking secretion from the brain by the inhibitor of acid reflux, probenecid [24], readily demonstrate the tight controls exerted on the serotonin metabolic pathway [25]. Although the systemic administration of L-tryptophan leads to increases in both 5-HT and 5-HIAA it was not immediately obvious that these increases reflected serotonergic activity. In vivo brain dialysis [26] has demonstrated that the newly synthesized compound is present in the extracellular space and appears to be linked to neuronal firing. The newly released amine also serves to limit its release via activation of somatodendritic terminal 5-HT1A autoreceptors, which also serve to inhibit neuronal firing. With the notable exception of the 5-HT3 receptor, which is an ionotropic receptor, postsynaptic 5-HT receptors are G-protein-coupled and are,

therefore, metabotropic receptors. Others have resorted to following the passage of radiolabeled tryptophan through the 5-HT metabolic pathway. Some studies gave rise to the notion of pools of amine with differing turnover estimates and others suggested specific pools might be preferentially correlated with neuronal release and therefore function. Indeed, it was initially thought that there was little point using isolated point measurements of 5-HT to indicate serotonergic involvement with ongoing physiology because they were so static. Changes in 5-HIAA content were much more variable. Then the extreme sensitivity in synthetic rate to variations in brain tryptophan content led to considerable debate and experimentation about the factors influencing L-tryptophan transport into the brain [27,28]. The work of *Curzon* and others [29] demonstrated that brain tryptophan and 5-HIAA increased in the brain in response to a physical stress such as immobilization. Stress [30] alters the plasma disposition via activation of hepatic tryptophan oxygenase, forming kynurenic and nicotinic acids [28,31] (Fig. 1.3). Furthermore, L-tryptophan circulates in plasma in close association with plasma albumin—less than 10% is free and readily able to enter the brain [29]. Factors decreasing the binding without changing total plasma concentration such as clofibrate or stress-induced lipolysis will increase brain tryptophan and serotonin synthesis. A major demonstration that all these factors interact was demonstrated by use of the Oldendorf technique. In this technique, [$^{14}C^{1}$] labeled tryptophan is mixed in a known ratio with a solution containing [^{3}H] water. The resultant mixture (which can be supplemented with competing aminoacids or albumin together with clofibrate to inhibit tryptophan albumin binding) is injected rapidly into the carotid artery of an anesthetized rat. The rat is then decapitated within 4 s and the ratio of ^{3}H to ^{14}Cin the solubilized brain determined by liquid scintillation counting. Since ^{3}H is freely diffusible across the blood–brain barrier while the entry of ^{14}C tryptophan is dependent on the amino acid transporter, the concentration of competing amino acids (tyrosine methionine, leucine, and isoleucine) in the injectate and the size of the albumin free fraction tryptophan uptake is reflected in the change in the $^{[3]}Ht:^{[14]}C$ ratio. The size of the pool of free tryptophan is determined by the concentration of unesterified fatty acids which also increase following stress. A specific transport mechanism for L-tryptophan is shared by other neutral aromatic amino acids. An occasionally heated debate about the predominant factor determining brain tryptophan content then ensued with one side favoring plasma free tryptophan and a second favoring the ratio to competing amino acids of

the Oldendorf experiment. These experiments clearly demonstrated how all these factors interact to determine brain tryptophan concentration and, ultimately, serotonin synthesis [27] (Table 1.2).

Table 1.2 Commonly used serotoninergic drugs.

Compound	Action	Reference
Reserpine	Release and depletion of all monoamines	[32]
Para-chloro-phenylalanine	Selective depletion of 5-HT—inhibitor of tryptophan hydroxylase	[33]
Benserazide	5-HTP decarboxylase inhibitor	[34]
Carbidopa	5-HTP decarboxylase inhibitor	[35]
Tranylcypromine	Monoamine oxidase inhibitor	[35]
Pargyline	Monoamine oxidase inhibitor	[36]
N,*N*-dimethyltryptamine	Hallucinogenic	[37]
5-Methoxytrytptamine	5-HT nonselective agonist	[38]
Methysergide	Antagonist 5-HT2; partial agonist 5HT1AR	[39]
Methiothepin	Nonselective antagonist on serotonin, dopamine, and adrenergic receptors	[40]
Ketanserin	Nonselective antagonist 5-HT2R	[41]
Lysergic acid diethylamide	Hallucinogen	[10]
Spiperone	Nonselective D2 antagonist	[40]
L-Pindolol	Stereoselective beta adrenoceptor antagonist and 5-HT1Aantagonist	[42]
L-Propranolol	Stereoselective beta adrenoceptor antagonist and 5-HT1A antagonist	[43]
Haloperidol	D2- and alpha-a1 adrenoceptor antagonist 5-HT2 antagonist	[44]
Clozapine	Atypical neuroleptic	[45]
Loxapine	Clozapine atypical neuroleptic analog typical neuroleptic	[46]
Clofibrate	Lipid lowering agent can increase concentration of unbound tryptophan	[47]
M100907	Selective 5-HT2 receptor antagonist	[48]
WAY-100635	Selective 5-HT1A receptor antagonist	[49]
Imipramine	Reuptake inhibitor	[50]
Iproniazid	Monoamine oxidase inhibitor	[51]
Para-chloro-amphetamine	5-HT releaser and depletory	[51]
5,6-Dihydroxytryptamine	Serotoninergic neurotoxin	[52]
5,7-Dihydroxytryptamine	Serotoninergic neurotoxin	[53]
Quipazine	Moderately selective serotonin receptor agonist, particularly to 5-HT2A and 5-HT3	[54]

Manipulation of dietary tryptophan intake both in animals and in man demonstrated the importance of tryptophan transport into brain, reducing intake being capable of inducing recurrence of depressive symptoms in patients in remission [55]. Given the structural similarities between serotonin storage and reuptake mechanisms, many have considered platelets to be mirrors of synaptic function and have duly sought to gain insight into brain serotonin metabolism through measuring blood levels. Differences have been seen in, for example, cerebral palsy with and without intellectual impairment [56]. In the acute tryptophan depletion method, subjects ingest an amino acid mixture containing all essential amino acids save tryptophan. Since tryptophan is utilized for protein synthesis, its level in plasma falls dramatically, an effect that can be magnified by withholding all food for 24 h—this leads to a lowering of mood 68 and increase in aggression. *Nishizawa* found that tryptophan depletion lowered serotonin synthesis by almost 90% in men and somewhat more in women. It is likely that absolute differences may be dependent on the phase of the menstrual cycle but this has not been examined as far as the author is aware. In a small-scale study of borderline personality disorder characterized by lowered mood and impulsivity, *Okazawa* [57] found decreased synthetic rate in the anterior cingulate, left temporal gyrus, and left putamen. Synthetic rate appears to be relatively uniform throughout the brain but this could reflect the low spatial resolution of the cameras available for the positron emission tomography (PET) studies. Mean rates of synthesis have been estimated to be between 66 and 85 pmol/g/min. Synthetic rate can be overlaid on the distribution and density of serotonin reuptake sites measured by ^{11}CDASB [58]. It is both surprising and disappointing that ^{11}CaMT has not been more utilized in investigating serotonin synthesis in psychiatric disorders, especially given the frequency with which abnormalities in peripheral serotonin metabolism has been found in neurodevelopmental disorders and intellectual disability. Perhaps the best study conducted to date involved the analysis of autistic children serotonin synthetic rate was related to handedness and language impairment [59]. Given the properties of the radioligand and similarities to 2-deoxyglucose, it would be very interesting to use ^{14}CaMT. Following hydroxylation the compound cannot be further metabolized as the product is no longer a substrate for monoamine oxidase a radioligand to probe regional differences in serotonin synthetic rate with histochemical level spatial resolution.

The Kynurenine pathway

The Kynurenine pathway [60] (Fig. 1.4) is initiated by conversion of L-tryptophan, by either of the enzymes tryptophan-2,3-dioxygenase or indoleamine 2,3-dioxygenase each forming formyl-kynurenine, which is then further degraded to kynurenine, the precursor of a number of bioactive compounds, including kynurenic acid, quinolinic acid, picolinic acid, and 3-hydroxyanthranilic acid. The pathway is responsible for over 90% of tryptophan metabolism in the periphery. Many organs and cell types express these enzymes, including the brain and some immune cells. Proinflammatory cytokines, including interferon-ϒ, interleukin-1β, and IL-6 can influence enzyme expression and activate this pathway. L-Tryptophan is required for the synthesis of nicotinamide and its

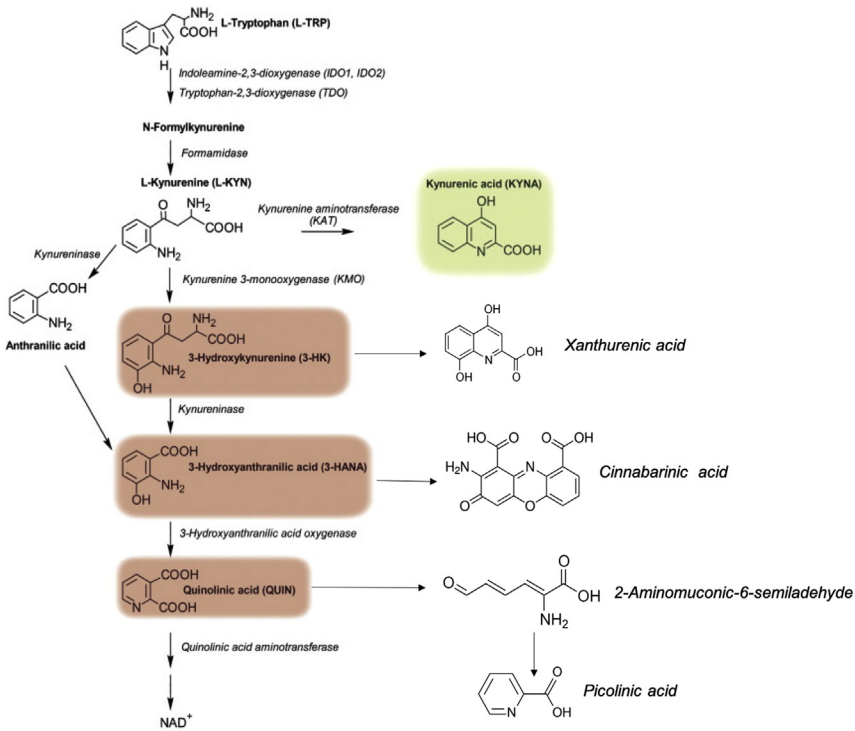

Figure 1.4 The kynurenine pathway. *Amaral M, Outeiro TF, Scrutton NS, Giorgini F. The causative role and therapeutic potential of the kynurenine pathway in neurodegenerative disease, J Mol Med 2013;91(6):705–713.*

metabolites, crucial for energy metabolism. Like kynurenine itself, it acts as an antagonist at the glycine modulatory site on the NMDA receptors, while quinolinic acid is an NMDA-receptor agonist. Glycine and NMDA-receptor antagonists have anticonvulsant and neuroprotective properties but are devoid of the stimulant effects of NMDA ion channel antagonists such as ketamine, phencyclidine, and MK-801. Indeed, some behaviors induced by stimulant-like compounds can be attenuated by glycine site antagonists, one such compound GLYX-13 [61] was suggested to be antidepressant in a small clinical [62] trial but its anti-PCP effects have not yet been tested. It seems somewhat implausible that a compound whose functional effects reflect additional blockade of NMDA receptors, but this interaction was tested across a number of different experimental scenarios [63,64] with both behavioral and neurochemical endpoints. One possible mechanism might be that exposure to a glycine NMDA-receptor antagonist might cause receptor internalization and so the failure of the chain of events giving rise to psychotomimetic effects. It is interesting that using a phencyclidine drug discrimination paradigm, the discriminative stimulus properties of PCP can be robustly antagonized by prior treatment with LSD in the absence of any drug-induced change in response rate [65]. PCP stimulates both dopamine and serotonin release in the medial prefrontal cortex and this release may be activating a number of 5-HT receptor subtypes that could be inhibitory on the expression of PCP-induced behavior. Although glycine NMDA antagonists have not been shown to stimulate serotonin release in vivo, the antidepressant-like effects of L701,324 in the forced swim test are antagonized by depletion of serotonin achieved after prior treatment with para-chloro-phenyl-alanine.

Removal of L-tryptophan from the diet can precipitate depression in patients in remission following treatment with selective serotonin reuptake inhibitors. Although there is evidence of a relationship between plasma tryptophan concentration and suicide attempts, it is interesting that the kynurenine metabolite quinolinic acid appears to accumulate in plasma and brain of suicide victims [66]. Interestingly, quinolinic acid is an agonist at *N*-methyl-D-aspartate receptors (NMDA), while the NMDA antagonist ketamine has robust antidepressant effects particularly against suicidal ideation. Unfortunately, there are no PET ligands that would enable visualization of quinolinic acid in vivo. Tryptophan pyrrolase is an inducible enzyme. The enzyme accumulates in the liver of hydrocortisone-treated animals which is then activated by tryptophan. The rate of inactivation of tryptophan is proportional to the amount of

reduced holoenzyme in the liver by conjugation with hematin, permitting its conversion to the active reduced holoenzyme. This mechanism allows for tryptophan to regulate its own metabolism and also that of serotonin and represents a pathway for psychological stress, that is, any stimulus activating the pituitary-adrenal axis to influence brain serotonin metabolism. Quinolinic acid is perhaps the most important kynurenine pathway metabolite in terms of its biological activity. It is nonenzymically produced by spontaneous conversion of 2-amino-3-carboxymuconic semialdehyde. In the brain, quinolinic acid is primarily produced in the microglia and infiltrating macrophages [67]. The compound is neurotoxic. It is an agonist at the *N*-methyl-D-aspartic acid receptor, specifically those containing NR1 + NR2A and NR2B subunits, which are primarily expressed in the forebrain. Consequently, neurons of the hippocampus, striatum, and neocortex are most vulnerable to quinolinic acid toxicity. It has been found that quinolinic acid in the CSF of suicide attempters are about 300% of the levels of healthy controls [66]. It is interesting the rapid-acting antidepressant ketamine is most effective against suicidal ideation. Might this be because ketamine as an NMDA-receptor antagonist can block the actions of quinolinic acid in brain. These results also suggest that monitoring quinolinic acid levels might reveal those at risk of committing suicide. Were it to be readily detectable in saliva, for example, a simple noninvasive means of detecting individuals at risk would then be available. This test might be particularly useful in individuals who found it difficult to talk to others about emotional problems children of school age, for example, alerting those able to provide help and support. It would also be worthwhile considering the role of quinolinic acid in the antidepressant actions of ketamine. CSF levels of kynurenic acid are significantly lower in schizophrenia patients [68] with a history of suicide attempts than in those without. Of course, attempts to link neurotransmitter metabolism with psychiatric states is limited by the need for invasive surgical procedures to access the areas in direct contact with serotonin and its metabolites. This can partly be overcome by various types of computerized tomographic approaches. PET is perhaps the most relevant to discuss here, an approach championed by *Mirko Dicsic* and *Ted Sourkes* using ^{11}C methyl L-tryptophan at the Montreal Neurological Institute [69]. ^{11}C has a relatively short half-life of 20,334 min compared to ^{14}C 5730 years. Its synthesis requires access to a cyclotron and a speedy synthetic route if sufficient specific activity is to be achieved. It is likely that this has been the greatest impediment to PET studies of serotonin metabolism since the

ligand first became known in 1988. Unlike, the compound ^{11}C-α-methyltryptophan is a suitable ligand as the presence of the methyl group renders the compound resistant to monoamine oxidase and so the radiolabel accumulates in areas of high brain serotonin synthesis. Also, ^{11}C-α-MT cannot be incorporated into protein thus avoiding a potential complicating pool of radioactivity. ^{11}C-α-MT is a substrate for tryptophan hydroxylase; however, its end product is a substrate for aromatic amino acid decarboxylase and thus is converted into α-m-serotonin which is not metabolized by MAOA. The technique has been questioned because when used in rhesus monkeys, no correlation with CSF 5-HIAA was found. Changes in synthetic rate have been shown in depression, autism, and obsessive-compulsive disorders. In suicide victim's brain, synthetic rate is highest in the pineal, the thalamus, and hypothalamus and values correspond well with rates achieved using classical pharmacological approaches measuring metabolite levels at steady state. Using the PET ligand revealed higher rates of synthesis in men than in women, but this difference is likely to be explained by the lower plasma tryptophan levels observed in women compared to men, with values ranging from 1107 to 41 nmoles/g/min.

A fascinating example of the relationship between serotonin synthesis and behavior is the role the monoamine plays in controlling gregarious behavior in locusts. The Desert locust *sschistocerca gregaria* transforms rapidly between a solitary phase and a swarming gregarious phase dependent on population density [70]. It was noticed that swarming was initiated by increasing group density and repeated leg contact seemed an essential part of the process. Modulation of muscle activity was then investigated and serotonin was found to profoundly influence the electrophysiological activity in the leg muscles. 5-HT consistently increased the duration of the fast extensor and flexor, which was associated with an increase in the amplitude of the fast extensor stimulation. It also increased the membrane resistance of the fast extensor and the flexor tibial motor neurons. The effects were mimicked by the 5-HT reuptake inhibitor, imipramine and blocked by the 5-HT receptor antagonist ketanserin. The next logical step was to examine endogenous serotonin in the leg muscles. Solitary locusts acquire full gregarious behavior within 2 h of forced overcrowding which coincides with a substantial but transient increase in serotonin content in the thoracic ganglia but not in the brain. Solitarious locusts crowded for different periods of time demonstrated a clear relationship between thoracic ganglia serotonin content and degree of gregariousness.

The behavior could be induced by stroking the hind femur or electrically stimulating the metathoracic nerve or by mechanosensory stimulation of the hind legs achieved as the insects jostle each other—a cephalic pathway in which combined sight and smell of other locusts is the necessary stimulus. Whatever pathway utilized the same relationship with serotonin endured, in each case the increase in serotonin content correlated with the extent of gregarious behavior. Gregarious locusts had approximately three times more serotonin than solitarious insects. Treatment of gregarious locusts with a mix of methiothepin and ketanserin 5-HT receptor antagonists blocked gregariousness. In turn gregariousness was similarly induced by the 5-HT receptor agonist 5-carboxoamidotryptamine. The behavior was lost after treatment with the 5-HT synthesis inhibitor α-methyl-serotonin.

The melatonin pathway

The synthesis of melatonin [71] represents an additional branch of the indoleamine pathway (Fig. 1.2). Melatonin was discovered in 1958 by Alfred Lerner a dermatologist. He found that extracts of frog skin could lighten skin color [72]. There is a marked circadian rhythm in the pineal content of melatonin which is synthesized from L-tryptophan [71], with its production being stimulated in the absence of light and inhibited by light onset photoreceptor cells in the retina sending signals via the suprachiasmatic nucleus in the hypothalamus to the pineal gland. But melatonin does much more than control the aggregation of melanocytes: It acts as a chemical mediator of photoperiod in regulating seasonal reproduction in a number of mammalian species. Once ^{125}I-melotonin became available, melatonin receptors were located in many tissues. This radioligand is superior to ^{14}C-melatonin because of its higher specific activity and its emission of β- and ϒ-radiation, increasing absolute sensitivity.

Melatonin is formed from serotonin via methylation of *N*-acetyl-serotonin by the action of hydroxyindole *N*-methltransferase [71]. This story represents an interesting episode in the link of serotonin to schizophrenia since many methylated tryptamines are psychotomimetic (see Psychedelics Today, https://psychedelics—an Introduction to psychedelic tryptamine chemistry [73]). Aberrant indole methylation was proposed as a pathophysiological pathway and analysts searched for evidence of their presence

in blood and urine, a particularly difficult task, given the dietary inconsistencies in hospitalized schizophrenics. In species showing a seasonal pattern of breeding, photoperiod, or day length tightly regulates reproduction so that birth occurs at the most favorable time of the year. A distinct photo-neuroendocrine circuit controls this process via melatonin metabolism. Short photoperiods stimulate melatonin production. Photoperiod roles have been ascribed to a number of genes. First, the type 2 and type 3 deiodinases and RFamide-related peptides which involve different hypothalamic nuclei, suggesting that several brain loci may be essential for modulating melatonin to effect reproduction. Like melatonin, pineal levels are highest during daylight hours and fall markedly and rapidly on the onset of light in line with the activity of hydroxyl-indoleo methyltransferase. If darkness is delayed by 12 h, it usually takes 3–4 days for the rhythms to adapt to the new lighting regime. That *N*-methyl-transferase is the key regulatory enzyme is indicated by the highly coherent increase in the concentration of 5-hydroxytryptophol with melatonin. The protein synthesis inhibitor cycloheximide markedly reduced night-time enzyme activity. In contrast, the phosphodiesterase inhibitor aminophylline in light-exposed chicks increased enzyme activity and melatonin levels, simultaneously depleting methoxytryptophol. Two receptor subtypes have been identified for melatonin in rat brain, MT1 and MT2. MT1 receptors are localized on neuronal cell bodies. Both receptors are G-protein-coupled. High levels of binding are localized over the suprachiasmatic nucleus, area postrema, and the spinal tract of the trigeminal nerve. Lower densities are found over the medial preoptic area, the septal hypothalamic nuclei and the medial region of the lateral habenular, the medial amygdaloid nuclei, the ventromedial nuclei, the arcuate nuclei, the subiculum of the hippocampus, and the lateral mammillary nuclei. High levels of binding were also present over the *pars tuberalis* of the pituitary gland and the anterior and posterior pineal. With respect to all aspects of the control of serotonin metabolism, the standard ex vivo methodologies clearly give limited information when trying to relate metabolic change to functional effects. This is where the technology of fast scan cyclic voltammetry is required. Primarily developed for the detection of dopamine, voltammetry exploits the ready oxidation of catechol and indoleamine neurotransmitters and their respective metabolites. The Wightman group in North Carolina have extended this technique to serotonin. Because serotonin can be oxidized electrochemically, carbon fiber electrodes can serve as in vivo sensors. When a potential is applied

between the sensor and a reference electrode and the potential varied until a peak current is achieved using a voltage ramp of 100 V/s, a 1 μM solution of 5-HT would generate a current of 1 nA, with a half rise time of less than 200 ms. Although many other brain neurochemicals are electroactive and are potential sources of contamination, coating the electrode surface with naflon can decrease contamination and improve sensitivity. Potential contaminants can be discriminated by evaluation of respective oxidative and reductive peak potentials. Indeed, cyclic voltammetry with rise times of 300 V/s is fast enough to prevent extensive follow on reactions, avoiding the formation of an insulating film on the electrode's surface. It would be interesting to see which of these is sensitive to cycloheximide. A recent application of cyclic voltammetry [1] to human brain has been reported in parkinsonian patients undergoing surgery for the implantation of electrodes for deep brain stimulation at an anesthetic level enabling the subject to perform a gambling task [1]. With electrodes placed in the striatum, recordings were made while the subjects were performing a decision-making game in a simulated stock market scenario. Participants were first endowed with 100 points and on each trial had to decide an amount to invest with amounts ranging from 0% to 100% of their points. In the beginning, participants were shown a record of actual stock market valuations, so being able to make their investment on actual market movements before making their investment decisions following a delay during which the market movement was revealed and the participant could appreciate lost or gained points in accordance with market returns. Because of the very high temporal resolution it was possible to correlate the reward prediction errors (RPE) with changes in serotonin signals, specifically those occurring within 700 ms of the revelation of the market yield. Cyclic voltammetry has the added advantage of simultaneously detecting the oxidation current generated by dopamine, allowing to inspect the simultaneous contribution of both serotonin and dopamine to decision making. Serotonin displayed across all betting levels an upward fluctuation to negative RPEs and a downward fluctuation to positive RPEs. However, this relationship was reversed when high bets were considered separately from low bet. They then tested whether fluctuations in serotonin could predict the bet level of the next trial. The area under the serotonin curve for all the transient serotonin fluctuations was found to have a highly significant predictive power for the upcoming decision. For large serotonin fluctuations and large bets, participants tended to decrease their bets, while with large serotonin responses and small bets, participants

increased their bets. When the authors tested the relationship between serotonin responses following a negative RPE, there was a strong positive correlation between current serotonin transient and betting levels. The results are consistent with a role for serotonin in decreasing exposure to loss, promoting the persistence of strategies that mitigate risk. These results align with many studies have shown that reduced levels of serotonin promote impulsive behavior. Consistent with the human cyclic voltammetry studies, serotonin neurons in the rat dorsal raphe increase their firing while rats perform a task that involves waiting for a delayed reward. This relationship appears to be causal, since inhibition of raphe firing by application of the 5-HT1A receptor agonist, 8-OH-DPAT decreases tolerance for delayed reward.

Conclusion

Serotonin investigations have come a long way since its identification in mammalian gut as *enteramine*. Without yet being able to pinpoint the exact mechanisms, it is clearly a neurotransmitter with profound implications for mental health and wellbeing. An intriguing finding linking platelet and neuronal serotonin is the fact that an individual's platelet capacity to accumulate serotonin can determine connectivity in the default mode network [74]. This raises intriguing questions about the extent to which could brain activity be modulated by the infusion of additional platelets? What would then be the consequences for behavior? Could such treatment mitigate the consequences of reduced transporter expression seen in individuals carrying the short form of the transporter polymorphism? Surely, the next 70 years of investigations will yield yet more fascinating insights into serotonin metabolism and function. The following chapters will indicate the directions to be taken and I have no doubts that the effort will be worthwhile.

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CHAPTER TWO

Neurodevelopmental roles and the serotonin hypothesis of autism spectrum disorder

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Introduction

In addition to its role as a neurotransmitter, the indolamine serotonin (5-hydroxytrptamine (5-HT)) also acts as a signaling molecule for 5-HT neuron growth in the developing brain. Detected in the first trimester of the human central nervous system, 5-HT acts as a trophic factor — a regulator of neuronal growth, differentiation, migration and survival [1]. This is long before synapse formation and the need for 5-HT to perform as a neurotransmitter. The trophic role of 5-HT continues throughout prenatal and early postnatal development. Disruption, even transient deviations, of the 5-HT system during development, can lead to permanent alterations in brain function and behavior [2]. One neurodevelopmental condition with growing evidence of 5-HT involvement is Autism Spectrum Disorder (ASD) [3].

Development of serotonin neurons

Maturation, then migration to raphe, development of neurites, acquisition of adult synaptic firing characteristics, and wiring of connections are well described in the overview of 5-hydroxytryptamine

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00002-5

(5-HT) neuron development by Deneris and Gaspar (See chapter 1 for a detailed description of the raphe nuclei) [4]. Briefly, neurons destined to transmit 5-HT originate in the rhombencephalon, an area of the hindbrain that develops into the parts of the brain stem containing raphe nuclei. Three proteins acting within this region of the neural tube and specifying 5-HT neuron development are sonic hedgehog (shh), fibroblast growth factors 4 and 8 (Fgf4 and Fgf8) [5]. In the hindbrain, 5-HT neurons appear first, and in the dorsal raphe nuclei (DRN) about 25%–50% are 5-HT neurons, and in the medial raphe nuclei (MRN) about 20%–30% are 5-HT neurons. A 5-HT neuron's specific molecular, structural, and regional identity leads to functioning neurons in a wired network and the specificity is dependent on transcription factors available to the neurons at specific times. Common regulatory factors for all 5-HT neurons involve the synthesis and transport of 5-HT including tryptophan hydroxylase 2 (Tph2), aromatic amino acid decarboxylase (Aadc), 5-HT transporter (Sert), and the vesicular monoamine transporter (Vmat). A gene regulatory network contributing to heterogeneous neuron identities includes additional reuptake and vesicular transport genes (Gata2, Gata3, Insm1, Lmx1B, and Pet1) [6–10]. The growth and branching of 5-HT axons are influenced by growth factors such as Bdnf, S100β, and Gap43 [11–13]. In addition to these factors, the molecule 5-HT also has a role in the development of 5-HT neurons.

Neurodevelopmental role of serotonin

The appearance of 5-HT in the developing human brain occurs much earlier than other monoamine neurotransmitters such as dopamine and noradrenaline. In the first trimester, by the fifth week of gestation, neuroblasts are identified and their 5-HT levels can be measured using immunohistochemical techniques [14]. These neurons grow and proliferate rapidly through gestation week 10 [15] and by the early second trimester, around 15 weeks, the neurons become highly distributed throughout the raphe nuclei of the brain stem, the site of 5-HT production in the mature brain, with caudal (B1-B5) cell bodies descending neurites to brain stem and spinal cord and rostral (B6–B9) groups ascending into the midbrain, forebrain, and raphe nuclei themselves [16]. One ascending, unbranched neuron can have a cell body in the raphe nuclei

and extends all the way to the frontal pole of the cortex. Occurring long before synapse formation and need for neurotransmission, 5-HT contributes "trophic" influences on the regulatory mechanisms of 5-HT neuronal development during embryogenesis and morphogenesis [17] and synaptogenesis and apoptosis [18]. Developmental processes regulated by 5-HT, from oocyte to mature neuron, include proliferation, differentiation, migration, maturation, maintenance, and survival [4,18,19]. An evolutionary curiosity is the role played by indoleacetic acid as a growth hormone in plants which enhances root arborization fungal invasion and filamentation [20].

The discovery of 5-HT in mammalian brain tissue was first reported by Twarog and Page in 1953 [21] with localization of neurons to the lower brain stem by Dahlstrom and Fuxe in 1964 [22]. The presence of 5-HT in the very early stages of embryogenesis was reviewed by Baker and Quay in 1969 [23]. By investigating early and late prenatal ontogeny of 5-HT neurons in the rat with fluorescence histochemistry, Olson and Seiger in 1973 identified the nine 5-HT neuron complexes (B1–B9) that arise bilaterally from prenatal brain stem raphe nuclei at E17-18 [24,25] and Levitt and Moore in 1978 observed the midline fusion or pairing of B1–B2, B4–B5, and B7–B8 by birth [26].

The entire process of ontogenesis of 5-HT neurons is influenced by the 5-HT molecule itself [19]. Much of the earliest work on 5-HT neurogenesis and the hypothesis that 5-HT is a trophic factor was led by Jean Lauder and colleagues [27]. They investigated the onset of monoamine neuron cell differentiation and synaptogenesis in rats [28,29]. Using H^3-thymidine autoradiography to date cell differentiation to 5-HT neurons, they observed neurogenesis in the DRN at day E11, much earlier than in other regions such as the cerebellum and hippocampus [28]. The 5-HT neurons were "born" several days before the neurons; they will eventually innervate. Synaptogenesis was dated by ethanolic phosphotungstic acid (E-PTA) fluorescence at days E19 through to birth [29]. Description of 5-HT as a "differentiation signal" during neurogenesis and before neurotransmission was furthered by Lauder and colleagues' experiments involving the injection of the 5-HT-depleting drug parachlorophenylalanine (pCPA) into rat dams followed by ^{3}H-thymidine autoradiography to identify the end of cell division in the fetal brains. The loss of maternal 5-HT leads to ongoing cell proliferation without differentiation to neuronal cells. This lack of differentiation occurred

in brain regions that would have matured into 5-HT target cells [30]. As biosynthesis of 5-HT is dependent on its amino acid precursor L-tryptophan and the rate-limiting enzyme-tryptophan hydroxylase (tph), Lauder and colleagues then injected rat dams with L-tryptophan and they reported a 34% increase of tph enzyme activity in the head of the embryo, indicating a synthesis of 5-HT. Conversely, when the dams are treated with pCPA as an inhibitor of tph activity, a resulting 91% decrease of tph activity in the head is described indicating a delay of cell differentiation [31]. With immune-titration methods, they reported a 50% depletion of 5-HT within the neurons of the embryonic raphe (B6—B9) complex [32]. Their further research treating rat dams with pCPA and using immunocytochemistry-audioradiographic techniques observed close associations between the developing 5-HT neurons and proliferating neuroepithelial cells in embryonic brains and proliferating glioblasts in postnatal pups [33]. Combining 5-HT immunocytochemical method with peroxidase antiperoxidase staining techniques allowed Lauder and colleagues to examine 5-HT organization and ultimately migration patterns of ascending and descending axons. Within the raphe nuclei, neurons from the (B6—B9) complex begin to synthesize 5-HT early and this contributes to their ascending axonal migration toward the forebrain [32,34]. In 2016, Lauder discussed her original 1978 paper proclaiming 5-HT as a "differentiation signal" and presented supporting literature describing 5-HT's role as a "growth, trophic, and morphogenetic signal" [27].

An additional role of 5-HT on brain development is that it regulates the architecture and connectivity of neurons, synaptogenesis, and corticogenesis [35]. The motile growth cones of migrating axons are rich in 5-HT [24,36] and the molecule has an inhibitory effect on neurite outgrowth for some, such as the snail B19 buccal ganglion neurons. The immobilization of neurite elongation, and ultimately synaptogenesis, was reported to be modulated by 5-HT's effect on the intracellular calcium-cyclic AMP relationship [35,37,38]. The cellular mechanisms 5-HT acts upon to affect neuronal cytoarchitecture are electrical ionic channels and second messenger systems such as calcium (Ca^{2+}), cyclic AMP (cAMP), and protein kinase C (PKC) [39]. The signaling function of 5-HT also influences the topographical organization of thalamocortical axons that can result in altered circuit formation [40]. Further effects on synaptogenesis were studied with pCPA treatment of rat dams, causing 5-HT deficiencies and delays in synaptogenesis both pre- and postnatally in the DRN [41]. Ultimately, 5-HT has a role in corticogenesis or cortex

formation. In a monoamine oxidase A (MAOA) transgenic mouse, excessively high 5-HT levels lead to failure of barrel cells to form therefore disrupting the development of the somatosensory cortex [42]. Knockout (KO) mice models have highlighted the resultant alterations to 5-HT innervation to certain brain regions, such as the Gap43 and Pet1 KOs showing decreased 5-HT innervation to the cortex, as well as the hippocampus [11,43].

Supply of serotonin to embryo

Once a 5-HT neuron matures, it can provide an endogenous source of 5-HT; however, during the early developmental period, the embryo requires an exogenous source of the chemical agent to facilitate the signaling for development of the neuron [44]. A possible source of 5-HT to the embryo is provided by the mother. The contribution of maternal 5-HT can be directly measured by studying knockout mice lacking the tph1 gene, who consequently cannot produce peripheral 5-HT. Compared to wild type mice, the tph1 knockout mice dams' embryos express reduced mitotic activity in regions that would have developed into the cerebral cortex, therefore highlighting the importance of the maternal supply of 5-HT [45].

Recently, the literature has identified a transient source of 5-HT available to the embryo that is synthesized by the placenta [46]. Using Pet1 KO mouse dams lacking 5-HT, the resultant embryos still had a source of 5-HT. The Pet1 KO dams still provided the amino acid precursor tryptophan, which was then synthesized to 5-HT within the placenta and then delivered to the fetus [46]. The exogenous 5-HT sources (maternal or fetal) signal the earliest differentiation of hindbrain (i.e., raphe nuclei) cells to neurons that project posteriorly toward the cerebellum and the raphe itself. An endogenous 5-HT source is available when early DRN neurons start synthesizing 5-HT and for the next 5 weeks, the number of neurons increases rapidly with the completion of raphe nuclei organization by week 15 for human embryo [16,47]. The neurogenesis of anterior projections to the forebrain is delayed, and this area will continue to depend on the placental produced 5-HT for cell proliferation, differentiation, and maturation [46].

Serotonin receptors contribute to neurodevelopment

Not only is the 5-HT molecule involved in development of neurons, but various 5-HT receptors also modulate a variety of developmental stages—for instance, cell division, cell differentiation (e.g., neurogenesis, dendrite formation, and synaptogenesis), axon branching, neuronal migration, and programmed cell death (i.e., apoptosis) [18]. Early in development, immature receptors form [50] to open ion channels and regulate metabolism through regulating cAMP (chemical) and Ca^{2+} (electrical) levels. Later, various 5-HT receptors regulate neuronal morphology and apoptosis. 5-HT receptors appear in very high amounts between 16 and 22 weeks' gestation. At this time, they are involved in cellular mechanisms affecting early metabolism, enzyme action, and genetic transcription factors to differentiate, proliferate, and mature target cells [18]. For example, 5-HT_{1A} receptors inhibit metabolic enzymes such as cAMP and protein kinase A (PKA), as well as cell transcription factors such as pCREB. In addition, 5-HT_{2A} receptors modulate postsynaptic cellular activity through enhancing cAMP, Ca^{2+}, PKC, and other transcription factors. The 5-HT system is also crucially involved in the next two major steps in neuron development (differentiation of cell types and then the proliferation of cells)—with 5-HT_{1A} receptors being more involved in the former, and 5-HT_{2A} receptors influencing the latter. Axon guidance cues occurring in thalamocortical neurons are reported to be modulated by 5-$HT_{1B/1D}$ receptors [40]. Last, a critical part of neuronal development is apoptosis or cell death—and 5-HT_{1A} receptors are antiapoptotic. The effects of 5-HT that I have described so far occur in neurons; however, 5-HT receptors can also act on other targets within the CNS. For instance, 5-HT_{1A} receptors act on astrocytes to stimulate the release of S-100β (a growth factor involved in cell maturation), while the 5-HT_{2A} receptors promote astrocytic metabolism and cell proliferation. A 5-HT neuron-astroglial receptor interaction also exists, with regulation by Ca^{2+} and other growth factors [51]. Thus there is significant evidence that 5-HT receptor systems influence brain development, and this occurs before the 5-HT system is required for neurotransmission.

Disruption of serotonin homeostasis in early development

Healthy 5-HT development leads to neurons that innervate cortical circuits of a typically functioning brain. Disruptions of neurodevelopment, even transient alterations in 5-HT homeostasis, can result in permanent disruption of brain connections and resultant changes in lifelong behaviors [2]. A review by Brummelte et al. presents the consequences of developmental 5-HT signaling disruptions on the postnatal brain [50], some of which we will present here. Some of the topics they address are genetic (G, i.e., allelic variations of SERT) and environmental (E, i.e., selective serotonin reuptake inhibitors (SSRI) exposure) factors and the need to understand G × E interactions. The authors focus on in utero exposure to the most commonly prescribed antidepressants for prenatal maternal depression and anxiety—SSRIs that block SERT from the presynaptic neuron leading to increased extracellular 5-HT levels. These SSRIs can enter the fetus by way of the placenta; however, no major neuroteratogenic effects have been identified in humans [50].

In summary, there is overwhelming evidence that 5-HT has a dual nature—it is not simply a neurotransmitter, but is also a critical modulator of many factors involved in the development, maintenance, and death of neurons. The genesis of many psychiatric disorders needs further study as developmental5-HT homeostasis might possibly be implicated. One such psychiatric condition with overtones of 5-HT issues is autism spectrum disorder (ASD) [2,51]. The rest of this chapter will discuss the role of 5-HT in ASD.

The serotonin hypothesis of autism spectrum disorder

ASD is a heterogeneous, highly genetic, and neurodevelopmental condition that affects approximately 1:40 individuals [52]. The core symptoms that characterize ASD are impairments in communication and social interaction, and restricted, stereotypic, and repetitive behaviors [53]. In the United States, ASD is a costly medical condition, which is estimated

at $268 billion per year and is predicted to reach $461 billion in 2025 which would exceed the costs of stroke and diabetes [54]. One-third of people with autism have an intellectually disability [55] and certain medical issues such as gastrointestinal disorders (2%−90%), [56] epilepsy (9%−24%) [57], sleep disturbance (50%−80%), [58] attention deficit hyperactivity disorder (30%) [59], phobias (20%), anxiety (54%), and depression (54%) [60].

Unfortunately, there are currently no effective pharmacological treatments available for core symptoms of ASD. In addition, co-occurring psychiatric conditions such as anxiety, disorders, and depression are more prevalent in ASD than in the general population [61] and further increase the impact on functional impairment and carer's burden [62].

The biological basis of ASD is not fully understood till today and presently, a combination of genetics and environmental factors are considered to underpin the etiology of ASD. Many neurochemical systems are implicated in the pathophysiology of ASD including monoamines (i.e., 5-HT), glutamate/γ-aminobutyric acid, and neuropeptides [63]. There is mounting evidence supporting the hypothesis that 5-HT system dysfunction (e.g., 5-HT synthesis, transport, and metabolism) plays an important role in early development and it has been suggested that altered 5-HT levels during early development can lead to abnormal brain circuitry and autistic symptoms [64]. The rest of this chapter is dedicated to the literature supporting the 5-HT hypothesis of ASD.

Autism spectrum disorder and peripheral serotonin biochemistry: hyperserotonemia

The presence of hyperserotonemia in ASD has, since first reported in 1961 [65], been replicated on numerous occasions [66]. In a recent meta-analysis, that included 23 studies of autistic individuals, elevated 5-HT blood levels were reported in 28% from whole blood and 23% from platelet-rich plasma samples [67]. Hyperserotonemia has also been noted in mental retardation associated with cerebral palsy [68]. However, hyperserotonemia has not yet proven to be a reliable diagnostic biomarker of ASD. This may be explained by the observation of hyperserotonemia in relatives of ASD individuals [69]. Potentially, blood 5-HT levels may be useful in combination with other blood markers. For example, a recent

study, which combined 5-HT levels with *N*-acetylserotonin (NAS) and melatonin, could distinguish between individuals with ASD and controls with a sensitivity of 80% and a specificity of 85% [70]. Whether such a multimarker approach would be clinically applicable, still needs to be determined. Another possible clinical application of peripheral 5-HT levels may be as an indicator of symptom severity. However, no consistent patterns have yet been observed. For example, a study in children with ASD found no association between core ASD symptoms and 5-HT levels. However, a significant inverse relationship between 5-HT levels and self-injurious behavior was observed [71]. Another study in children with ASD reported a negative association between 5-HT levels and consistency of intellectual response and general impression, as measured by the child autism rating scale (CARS) [72]. Furthermore, a study that consisted mainly of adults with ASD observed higher 5-HT levels in individuals with poorer speech development [73]. The inconsistency in associations may be explained by different sample characteristics (e.g., age and IQ) as well as differently used behavioral measures.

During early development, blood hyperserotonemia can enter the fetal brain and the resultant exposure to high levels leads to a negative feedback effect with the loss of 5-HT innervation [64] that continues through the development and leads to symptoms of ASD. Studies of the 5-HT agonist 5-methoxytryptamine (5-MT) result in hyperserotonemia levels similar to those seen in ASD [74]. Pregnant rat dams injected with 5-MT produce pups that display "autistic-like" behaviors such as decreased bonding with the dam, sensory hypersensitivity, seizures, and motor stereotypies. The 5-MT treatment also modulates dorsal raphe nuclei (DRN) cells. The DRN cells innervate oxytocin (OXT) cells in the paraventricular nucleus of the hypothalamus (PVN), OXT projections to the amygdala, and calcitonin gene-related peptides (CGRP) in the amygdala [64]. Both the PVN and amygdala are brain regions associated with abnormal socialization and emotion processing. Both OXT and CGRP are influenced by 5-HT and the 5-MT leads to decrease of the former and increase of the latter.

Therefore differences in blood 5-HT levels are an intriguing observation in ASD research but have not been proven to serve (alone) as a diagnostic marker or to explain autistic behavior. It is possible, however, that variation in blood 5-HT levels may be associated with individual differences in response to serotonergic medications.

Autism spectrum disorder and serotonin homeostasis

The role of 5-HT homeostasis and ASD is reviewed by Garbarino et al. who posit that both enhancement and/or depletion of 5-HT during development can have deleterious effects on 5-HT neurodevelopment and this might underpin ASD [75]. A consequence of the early 5-HT disruptions may lead to lifelong abnormalities seen in ASD. For example, neuroimaging studies of children with ASD report unilateral increases in serotonin synthesis in the dentothalamocortical pathway using the PET ligand ^{11}Cα-methyl-L-tryptophan [76] and reduced SERT binding capacity using SPECT ligand [^{123}I] nor-β-CIT [77].

In addition to maternal 5-HT, SSRI can cross the placenta, the fetal blood–brain barrier, and modulate 5-HT metabolism [78]. Meta-analyses on the link between prenatal SSRI exposure and an increased risk of ASD report contradictory results. Earlier papers report an increased risk of ASD from exposure to SSRIs during pregnancy [79], while more recent and larger reviews reported no evidence for an association [80,81]. Thus causality (as opposed to an association) cannot yet be determined and needs to be confirmed. However, maternal SSRI use during pregnancy, together with low birth weight, has been used to identify homogenous subgroups within ASD were reported to be associated with greater sleep disturbances and a greater number of gastrointestinal complaints in children with ASD [82]. This approach could potentially lead to a feasible method for creating homogenous subgroups within ASD, which could help to inform future clinical trials.

Autism spectrum disorder and neuroimaging using serotonin modulators

The modulatory role of serotonergic medications on brain function in ASD has been assessed in several studies. For instance, the mediating role of 5-HT on inhibitory brain function in ASD has been investigated during a Go/No-Go task of response inhibition. After acute tryptophan depletion (ATD) —thus reducing 5-HT—within autistic individuals, fronto-thalamic activations were upregulated and striato-cerebellar

activations were downregulated toward control sham levels, "normalizing" the fronto-cerebellar dysfunctions [83]. Furthermore, in ASD, ATD has been shown to differentially modulate processing of facial expressions of emotions in the putamen, medial and middle frontal, and lingual gyri [83]. These regions have previously been reported to have relatively high 5-HT synthesis/receptor or transporter density [76,84–86]. Taken together, these findings suggest that an ATD related decrease in extracellular 5-HT can modify brain functional differences in ASD—a key question, however, is whether it also modifies behavior. Unfortunately, an earlier report documented an increase in repetitive and irritability in ASD after ATD [87], but not in all participants. Also, this study used a heterogeneous sample of mixed genders and IQ-scores. Furthermore, in boys with ASD, overactivation of the inferior frontal cortex was observed during a motor inhibition task, which was downregulated after increasing extracellular 5-HT by a single dose of an SSRI [88]. Upregulating brain function in ASD to control levels after an SSRI has also been observed in the medial prefrontal cortex during a reversal task, which assesses cognitive flexibility [89].

Autism spectrum disorder and the serotonin transporter

Multiple mechanisms may account for hyperserotonemia including gut-production, 5-HT receptors/5-HT transporter (SERT or 5-HTT) function, and platelet 5-HT uptake, which is regulated by the SERT. A recent study reported no differences between ASD and controls in platelet poor plasma 5-HT, which suggests that hyperserotonemia in ASD is probably not caused by an increased exposure of the platelet to 5-HT but by the platelet's handling of 5-HT by SERT [90]. In ASD children, a significant increase in platelet SERT density has been observed in a [^{3}H]-Paroxetine binding study [91]. Throughout the brain, a decrease in SERT binding in adults with ASD has been reported in a positron emission tomography (PET) study [92]. In this study, a reduction of SERT binding in the anterior cingulate cortex and posterior cingulate cortex was associated with impairment in social cognition and a reduction of SERT binding in the thalamus correlated with repetitive behaviors [92]. Adult mice expressing the SERT Ala56 variant have hyperserotonemia and present with ASD-like social, communication, and repetitive behavior

symptoms [93]. SERT Ala56 genotype dams produce embryos with decreased placental and forebrain 5-HT embryos [94].

Although 5-HT cannot cross the mature blood–brain barrier, an intriguing relationship between serotonin-containing platelets and brain activity has recently been found [95] in which maximal 5-HT uptake was assessed in subjects who then underwent resting-state fMRI. Platelet Vmax for serotonin reuptake by SERT significantly predicted brain default-mode network suppression [95]. Further understanding of these 5-HT related mechanisms is important as it could help to explain differences in response to SERT binding medications.

Autism spectrum disorder and serotonin receptors

In addition to abnormalities in presynaptic reuptake of 5-HT, there have also been differences reported in postsynaptic signal transduction in 5-HT receptors in ASD. For example, decreased binding of the platelet 5-HT_{2A} receptor has been observed in young adults with ASD [73], and this is consistent with the results of neuroimaging studies. A single-photon emission computed tomography (SPECT) reported decreased cortical 5-HT_{2A} receptor binding in cingulate, frontal, temporal, and parietal cortices in adults with Asperger's syndrome that was associated with abnormal social communication [86]. A PET study observed decreased 5-HT2 binding throughout the cortex in parents of children with ASD [96]. In addition, a postmortem study of ASD cases demonstrated significant reductions in the density of 5-HT_{2A} receptors in superficial layers of the posterior cingulate cortex and fusiform gyrus and of 5-HT_{1A} receptors in superficial and deep layers of the posterior cingulate cortex and fusiform gyrus [97]. Furthermore, the 5-HT_{2A} receptor gene (HTR2A) has been proposed as a candidate gene for ASD [98].

Another promising biological hypothesis for ASD is based on the neuropeptide OXT. Deficits in oxytocin secretion and polymorphisms in the oxytocin receptor gene are associated with ASD [99] and there is evidence that OXT and 5-HT work together in social situations [100,101]. Rat pups treated during gestation with 5-MT exhibit loss of 5-HT, ASD traits, and also the loss of OXT in the PVN and amygdala [64]. The release of OXT is modulated by activation of 5-HT_{1A} (inhibitory) and 5-HT_{2A}

(excitatory) receptors [101,102] on the OXT cells. Williams syndrome is an autosomal dominant neurodevelopmental disorder in which there is mild to moderate mental retardation and behavioral symptoms that appear to be the antithesis of ASD. Characterized by the friendliness and high levels of trust in strangers, individuals harbor a small chromosomal deletion on 7q11.23 and high basal levels of plasma OXT [103]. In transgenic mice exhibiting the Williams syndrome candidate gene GTF2IRD1 deletion, the mice show less aggression and engage in more social interaction [104]. Levels of 5-HIAA are significantly elevated in the amygdala and in frontal and parietal cortices. It can be hypothesized that dysregulated OXT expression is a consequence of increased activity at 5-HT receptors. Two possible treatment options suggest themselves (1) attenuation of excessive OXT exposure by administration of a 5-HT_{1A} receptor antagonist such as WAAY100635 maleate or (2) a brain-penetrating OXT receptor antagonist such as L-368,899.

Evidence from peripheral, transporter, and receptor literature suggests that there are widespread biochemical differences in the serotonergic system in ASD. It is therefore crucial to understand more about the impact of serotonergic agents on brain function, especially as drugs that target the serotonergic system are frequently prescribed in ASD populations [105].

Autism spectrum disorder and genetics of serotonin

The heritability of ASD is high, estimated at 80% [106]. Individuals with monogenetic syndromes including Phelan-McDermid syndrome, Tuberous Sclerosis, and Fragile X syndrome often meet criteria for ASD, but only account for 10%–15% of all cases [107]. More frequently, the genetic risk is polygenic and includes numerous single nucleotide polymorphisms (SNPs) which are common genetic variations that normally occur throughout a person's DNA [108,109]. Also, de novo mutations and copy number variations (CNVs) have been reported to increase the risk of ASD [110]. De novo mutations are new mutations that are present in an affected individual, but not in their parents. CNVs are structural variations in genes where a section of a chromosome is either duplicated or deleted. Specific abnormalities in genes coding for serotonergic neurotransmission are common in ASD, evidenced from both rodent and human studies.

Rodent studies

In rodents, abnormalities in genes can be examined by studying transgenic mice, both by "knocking out" or "knocking in" a gene by disrupting it with an artificial DNA fragment. In KO mice of the 5-HT transporter gene (SLC6A4: which is located on the 17q11−q21 region), increased stress reactivity, decreased aggression, and disruption of brain architecture in sensory cortex and amygdala have been reported [111]. Changes in cortical structure and loss in somatosensory whisker barrel fields have been observed in a study that also used SLC6A4 KO mice [112]. Another study that used SLC6A4 KO mice observed exacerbation of macrocephaly and decreased sociability [113]. In the knock-in (KI) mouse SERT Ala56, enhanced 5-HT clearance rates and hyperserotonemia were reported. These effects were accompanied by the altered basal firing of raphe 5-HT neurons, as well as 5-HT_{1A} and 5-HT_{2A} receptor hypersensitivity. Furthermore, SERT Ala56 KI mice displayed alterations in social function, communication, and repetitive behavior [93]. This suggests an involvement of the transporter gene SLC6A4 in brain development, autistic-like behavior, and abnormalities in 5-HT levels.

Human studies

In ASD individuals, genes that are linked to serotonergic neurotransmission (especially SLC6A4) have been associated with symptomatology, peripheral 5-HT, neuroimaging findings, and treatment response to SSRIs. Repeated polymorphic regions of SLCA64 (5-HTTLPR) consist of short (S) and long (L) alleles, which are associated with reduced and increased SERT expression, respectively [114]. Increased SERT expression has, within ASD, been associated with increased repetitive behaviors [115,116] as well as with obsessions [117]. In contrast, decreased SERT expression has been correlated with impaired social behavior [115]. However, a recent meta-analysis failed to find an association between ASD and 5-HTTLPR [118], which can possibly be explained by the ethnical heterogeneity of the samples. This suggests that abnormalities of SLC6A4 may not be specific for ASD but could partially explain behavioral heterogeneity within ASD.

Genetic studies have also focused on the link between 5-HTTLPR and abnormalities in neuroimaging findings in ASD. For instance, it has been reported that functional activation of the amygdala (as measured with fMRI) during observation of sad faces, depended on low or high

expression of 5-HTTLPR in ASD [119] —low expressing genotypes did not display habituation whereas high expressing genotypes did. Another study that used structural MRI observed an association between short allele 5-HTTLPR and increased cortical gray matter in 2 to 4-year-old children with ASD [119]. This suggests a link between variation in 5-HTTLPR and abnormal brain function and development.

5-HTTLPR variations may also affect psychotropic treatments that modulate 5-HT. For instance, in one study in children with ASD, an association was reported between low expression of 5-HTTLPR and less response to escitalopram (as measured with the Aberrant Behavior Checklist Irritability Subscale), as compared to medium and higher expression of 5-HTTLPR [120]. The direction of these findings reveals similarities with pharmacogenetic studies investigating major depressive disorder in neurotypical individuals [121]. However, a more recent study did not observe associations between 5-HTTLPR and SSRI response in children and adults with ASD, as measured with the Aberrant Behavioral Checklist-Community Version (ABC-CV) and the Repetitive Behavior Scale-Revised (RBS-R) [122]. The discrepancy in findings is possibly explained by different ethnic backgrounds and age ranges that were used in the studies.

Other genetic studies have implicated alleles that encode for the integrin beta 3 (ITGB3), a protein that is found on platelets. It has been reported that ITGB3 interacts with SERT activity [123] and an association of functional polymorphisms of ITGB3 and hyperserotonemia has been observed in ASD [124].

Taken together, the animal and human literature supports the genetic influence on 5-HT in neurodevelopment neurotransmission and is associated with ASD and further research on these genes involved in 5-HT may help to stratify behavioral or biological subgroups within ASD.

Autism spectrum disorder and serotonin psychopharmacology

Currently, the standard treatment for depression and anxiety in ASD includes targeting the 5-HT system with SSRIs. Research studies on SSRIs in treating core symptoms in individuals with ASD have been done; however, results remain inconsistent. One study, not a randomized

control trial (RCT), of the SSRI fluoxetine showed a significant reduction in repetitive behaviors [125], whereas an unpublished RCT trial of fluoxetine reported no effect [126]. Additionally, a large trial of citalopram in children with ASD reported no improvement in repetitive behaviors [127]. In contrast, three small studies in adults reported positive effects of fluoxetine and fluvoxamine in reducing repetitive behaviors [128–130]. Due to the inconsistency in the literature on clinical trials and the continuing unmet need for treatments for core symptoms in some people with ASD, further investigations on the effectiveness of SSRIs in ASD is required. However, conducting clinical trials has become increasingly expensive and most trials in neuropsychiatric disorder fail [131]. The ability to stratify patients based on 5-HT features such as hyperserotonemia or transporter polymorphisms may influence the planning of future RCTs using serotonergic agents that may alleviate some of the core (or comorbid) symptoms of ASD.

Conclusion

In this chapter, we hoped to highlight the role 5-HT has on the development of the brain, including embryogenesis, neurogenesis, morphogenesis, synaptogenesis, and apoptosis. From oocyte to mature neuron, 5-HT regulates proliferation, differentiation, migration, maturation, maintenance, and survival. Understanding these early roles and mechanisms, that occur at critical developmental stages, may lead to a better understanding of the biological basis of neurodevelopmental disorder such as ASD. Understanding these basic underpinnings may lead to treatments of the core and comorbid symptoms of ASD by the development or repurposing 5-HT related medications.

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CHAPTER THREE

The role of serotonin receptors in the control of cardiovascular function

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Introduction

As compared to other endogenous monoamines, serotonin (5-hydroxytryptamine, 5-HT) is undoubtedly unique for its complex effects that are mediated by a wide variety of receptors (i.e., 5-HT_1–5-HT_7). In mammals, 5-HT is predominantly found in platelets, enterochromaffin cells, and in the central nervous system (CNS), where it plays a role as a neurotransmitter [1,2].

The last two decades have witnessed a remarkable progress in the characterization and classification of 5-HT receptors due to the adoption of structural, transductional, and operational (pharmacological) criteria [2–7], as well as the discovery of agonists and antagonists acting selectively at 5-HT receptors (Table 3.1). On the basis of these essential criteria, there are at least seven 5-HT receptor types that can be grouped into (1) six metabotropic (G-protein-coupled) receptors, namely the 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptor types and (2) one ligand-gated ion channel(s) represented by the ionotropic 5-HT_3 receptor type (Table 3.1). Some of these 5-HT receptor types are heterogeneous and consist of several subtypes. These subtypes share similar structural and transductional properties, but display very different operational (pharmacological) profiles (Table 3.1), namely (1) the 5-HT_1 (encompassing the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E}, and 5-HT_{1F} receptor subtypes); (2) the 5-HT_2 (comprising the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes); and (3) the 5-HT_5 which includes the 5-HT_{5A} (preferentially coupled to $G_{i/o}$ proteins) and 5-ht_{5B} (transductional system unknown)

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00014-1

Table 3.1 Classification of 5-hydroxytryptamine receptors.

Receptor	Agonists	Antagonists	Transduction	Distribution	Function
5-HT_{1A}	8-OH-DPAT	WAY 100635	$G_{i/o}$	Raphe nucleus	Central hypotension
5-HT_{1B}	Sumatriptan CP-93,129 (rodents)	SB224289	$G_{i/o}$	Cranial blood vessels	Vasoconstriction, sympatho-inhibition
5-HT_{1D}	PNU-109291, PNU-142633	BRL15572	$G_{i/o}$	Presynaptic neurons	Autoreceptor, sympatho-inhibition
5-ht_{1E}	5-HT ≫ 5-CT BRL54443	Methiothepin (nonselective)	$G_{i/o}$	Cortex	Unknown
5-HT_{1F}	LY344864, lasmiditan, LY334370	Methysergide (nonselective)	$G_{i/o}$	CNS, trigeminal ganglia	(−) Trigeminal system
5-HT_{2A}	DOI, DOB α-methyl-5-HT	MDL100907 Ketanserin	G_q	Smooth muscle, platelets	Vasoconstriction, platelet aggregation
5-HT_{2B}	DOI, BW723C86 α-methyl-5-HT	SB204741 RS-127445	G_q	Rat fundus, endothelium	Vasoconstriction, release of NO
5-HT_{2C}	DOI, Ro 60-0175 α-methyl-5-HT	SB242084 RS-102221	G_q	Choroid plexus	CSF production
5-HT_3	Phenylbiguanide 2-methyl-5-HT	Tropisetron Granisetron MDL 72222	Na^+/K^+ channel	Peripheral nerves, vagal afferents	(+) Neuronal activity, reflex bradycardia
5-HT_4	Renzapride, BIMU8 ML10302, SC53116	GR 113808 SB204070	G_s	Gastrointestinal tract, pig and human atrium	(+) Neuronal activity, vasodilatation, tachycardia in pigs and humans
5-HT_{5A}	5-HT, ergotamine	SB 699551	$G_{i/o}$	CNS?	Cardiac sympatho-inhibition in rats
5-ht_{5B}	5-CT (nonselective)	Unknown	Unknown	Unknown	Unknown
5-HT_6	5-MeO-T ≥ 5-HT SB357134, SB271046	Ro 630563	G_s	CNS, absent in the cardiovascular system	Memory, not involved in cardiovascular regulation by 5-HT
5-HT_7	5-CT ≫ 5-HT AS-19	SB 269970 SB258719	G_s	CNS, smooth muscle, cat atrium	Circadian rhythm, vasodilatation, tachycardia in cats

5-CT, 5-carboxamidotryptamine; 5-MeOT, 5-methoxytryptamine; AS-19, (2*S*)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin; *CNS*, central nervous system; *CSF*, cerebrospinal fluid; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; *LSD*, lysergic acid diethylamide; NO, nitric oxide; (−), inhibits; and (+), stimulates.

Source: Modified from Villalón CM, Centurión D. Cardiovascular responses produced by 5-hydroxytryptamine: a pharmacological update on the receptors/mechanisms involved and therapeutic implications. Naunyn-Schmiedeberg's Arch Pharmacol 2007;376:45−63.

subtypes. This progress, in addition to supporting the role of 5-HT receptors in various pathophysiological conditions (e.g., anxiety, depression, schizophrenia, drug addiction, obesity, aggression, migraine, cardiovascular pathologies, etc.), has led to the development of 5-HT receptor agonists and antagonists for the therapeutic treatment of many of these diseases [2–3,5–7].

General effects of 5-hydroxytryptamine in the cardiovascular system

5-HT elicits complex responses in the cardiovascular system comprising bradycardia or tachycardia, hypotension or hypertension, and vasodilatation or vasoconstriction [2,8,9]. These responses involve multifaceted interactions of 5-HT with different receptors at several levels. These levels include the CNS; autonomic ganglia and postganglionic neurons; dorsal root ganglia and primary sensory nerves; and the heart and blood vessels [2,8–10]. Depending on the species and the experimental conditions, these responses are mainly mediated by serotonin 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_{5A}, and 5-HT_7 receptors, as well as by a tyramine-like action or unidentified mechanisms [2,8,9]. Curiously, the 5-HT_6 receptor is not involved (neither centrally nor peripherally) in cardiovascular regulation [9,11].

Specific actions of 5-hydroxytryptamine at different central and peripheral levels to induce cardiovascular responses

In order to avoid lengthy findings' descriptions on the full pharmacological identification of the receptors/mechanisms involved in the cardiovascular responses to 5-HT, we will only consider (1) the effect of a given 5-HT receptor agonist mimicking the actions of 5-HT and its blockade by the corresponding antagonists, omitting the effects of agonists and antagonists for the other 5-HT receptor (sub)types (listed in Table 3.1) and (2) the effects of agonists/antagonists for receptors unrelated to 5-HT (when tested).

Sensory afferents

In general, 5-HT given intravenously (i.v.) in intact or anesthetized animals elicits cardiovascular reflex responses (mainly bradycardia and hypotension) by acting on vagal sensory (and other) afferents [1,2]. These reflex responses are mainly mediated by 5-HT_3 receptors as they are (1) mimicked by 2-methyl-5-HT or phenylbiguanide and (2) antagonized by MDL 72222 or tropisetron (Table 3.1).

Sympathetic ganglia

5-HT (i.v.) produces stimulation and/or inhibition at sympathetic ganglia which, in turn, may result in sympatho-excitation and/or sympatho-inhibition and, consequently, in vasopressor, vasodepressor, tachycardic, and/or bradycardic responses [1,2]. Some studies suggest that 5-HT elicits hyperpolarization of sympathetic ganglia by activation of ganglionic 5-HT_{1A} receptors in rats [12] or 5-$HT_{1B/1D}$ receptors in cats [13] by using their respective agonists and antagonists (Table 3.1).

Heart rate responses to 5-hydroxytryptamine

Intravenous or central administration of 5-HT can elicit bradycardia and/or tachycardia. The pharmacological profile of the 5-HT receptors involved has been identified in most species [1,2,8], as follows.

Bradycardia

In most mammals, i.v. 5-HT produces a transient bradycardia that is abolished by ganglion blockade, vagotomy, atropine, or spinal section [1]. Thus, this bradycardia involves a von Bezold–Jarisch-like reflex originating from depolarization of afferent cardiac neurons but, as described below, this response may also be elicited by a central or a prejunctional action on sympathetic (inhibition) and/or cholinergic (stimulation) neurons [14].

von Bezold–Jarisch-like reflex

As previously reviewed [1,2], i.v. administration of 5-HT, phenylbiguanide, and/or 2-methyl-5-HT in most experimental mammals results in a bradycardia that can be abolished by (1) atropine or bilateral vagotomy and (2) MDL 72222 or tropisetron (Table 3.1). Accordingly, this reflex bradycardia is mediated by 5-HT_3 receptors on cardiac sensory neurons.

Prejunctional inhibition of the cardiac sympathetic outflow

In vagotomized-pithed rats, preganglionic (C_7-T_1) electrical stimulation of the sympathetic cardioaccelerator outflow results in tachycardic responses that are inhibited by i.v. 5-HT [15]. Subsequent pharmacological analysis [16–18] showed that this cardiac sympatho-inhibition was (1) unaffected after the antagonists GR 127935 (5-$HT_{1B/1D}$), the combination of WAY 100635 (5-HT_{1A}) plus GR 127935, ritanserin (5-HT_2), tropisetron (5-$HT_{3/4}$), LY215840 (5-HT_7), or a cocktail of antagonists/inhibitors consisting of yohimbine (α_2), prazosin (α_1), ritanserin, GR 127935, WAY 100635, and indomethacin (cyclooxygenase); (2) abolished by methiothepin (5-$HT_{1/2/6/7}$ and recombinant 5-$ht_{5A/5B}$); and (3) mimicked by the agonists 5-CT (5-$HT_{1/7}$ and recombinant 5-$ht_{5A/5B}$), CP-93,129 (*r*5-HT_{1B}), sumatriptan (5-$HT_{1B/1D}$), PNU-142633 (5-HT_{1D}), and ergotamine (5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$), but not by indorenate (5-HT_{1A}) or LY344864 (5-HT_{1F}). Moreover, 5-CT-induced cardiac sympatho-inhibition was abolished by (1) methiothepin, the above cocktail of antagonists/inhibitors, GR 127935 or the combination of SB224289 (5-HT_{1B}) plus BRL15572 (5-HT_{1D}) [16,17]; and (2) GR 127935 plus SB 699551 (5-HT_{5A} receptor antagonist) [18]. Thus, this cardiac sympatho-inhibition (1) does not involve 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_6, 5-HT_7 receptors, $\alpha_{1/2}$-adrenoceptors, or prostaglandins synthesis and (2) involves the activation of 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{5A} receptors.

Stimulation of cholinergic neurons

In isolated perfused hearts obtained from reserpine-treated rabbits, 5-HT elicits a MDL 72222-susceptible bradycardia, which involves activation of 5-HT_3 receptors on parasympathetic ganglia/nerves [1]. Moreover, in pithed rats (pretreated with atenolol, ketanserin, and methiothepin), 5-HT and *m*-chlorophenyl-biguanide enhanced the bradycardia induced by vagal stimulation. Since this effect was blocked by MDL 72222 [19], 5-HT_3 receptors enhanced the parasympathetic outflow.

Tachycardia

The tachycardia produced by i.v. 5-HT in vagotomized animals is notoriously species-dependent and may be mediated by a wide variety of receptors/mechanisms as follows.

Tyramine-like action

In spinal guinea pigs, i.v. 5-HT elicits a tachycardic response (1) not blocked by methiothepin, ketanserin, or MDL 72222; (2) blocked by ß-adrenoceptor blockers (propranolol) or by the 5-HT reuptake inhibitor indalpine; and (3) attenuated after reserpine [20]. Accordingly, this involves the displacement of catecholamines by a mechanism similar, but not identical, to that of tyramine [20].

5-HT_{2A} receptor stimulation

In reserpinized pithed rats, i.v. 5-HT elicits a tachycardia that, in addition to remaining unaffected by propranolol, was (1) blocked by the 5-HT_{2A} receptor antagonists ketanserin or spiperone [21]; (2) unaffected by the antagonists rauwolscine (5-HT_{2B}), SB204741 (5-$HT_{2B/2C}$), or Ro 04-6790 (5-HT_6) [21]. Accordingly, 5-HT_{2A} receptors are involved [21].

5-HT_3 receptor stimulation

1. In rabbit perfused hearts, the tachycardia to 5-HT is attenuated by reserpine, propranolol, cocaine, or MDL 72222, but not by desipramine or methiothepin [1]. Hence, activation of 5-HT_3 receptors on cardiac sympathetic neurons is involved, resulting in noradrenaline release and cardiac stimulation.
2. In conscious dogs, i.v. 5-HT and 2-methyl-5-HT produce a tachycardia blocked by 5-HT_3 receptor antagonists including tropisetron, but not by propranolol (which excludes indirect effects by stimulation of cardiac β-adrenoceptors). Thus, 5-HT_3 receptors are involved [22].
3. In isolated guinea pig atrium, 5-HT produced positive inotropic and chronotropic responses which were (1) unaffected by propranolol or imipramine (which excludes indirect mechanisms) and (2) blocked by 5-HT_3 receptor antagonists including tropisetron and granisetron. Therefore, 5-HT_3 receptors are involved [23].

5-HT_4 receptor stimulation

1. In anesthetized pigs, i.v. 5-HT elicits tachycardic responses which are (1) unaffected after the antagonists methiothepin (5-$HT_{1/2}$), ketanserin (5-HT_2), and/or MDL 72222 (5-HT_3) [1] and (2) blocked by zacopride, cisapride, or high doses of tropisetron [24,25]. Thus, 5-HT_4 receptors are involved.
2. In human atria, the positive inotropic action to 5-HT also involves 5-HT_4 receptors as this response is blocked by high concentrations

(2 μM) of tropisetron [26] or by the selective 5-HT_4 receptor antagonist GR 113808 [27], but not by other antagonists.

3. In rats with chronic heart failure, i.v. 5-HT produced a positive inotropic response blocked by the 5-HT_4 receptor antagonist, GR 113808 [28].

5-HT_7 receptor stimulation

In spinal cats, 5-HT-induced tachycardia was (1) unaffected by guanethidine, propranolol, burimamide, 5-HT_2 receptor antagonists (ketanserin, ritanserin, etc.), or adrenalectomy [1] (2) mimicked by several compounds with a rank order of agonist potency of 5-CT ≫ 5-HT > 5-methoxytryptamine; and (3) blocked by several antagonists including mesulergine (5-$HT_{2/7}$), clozapine (5-$HT_{2/6/7}$), and lisuride (5-HT_7), but not by GR 127935 (5-$HT_{1B/1D}$) [29]. Thus, 5-HT_7 receptors are involved [29].

Activation of CGRPergic sensory neurons

As previously discussed, 5-HT_3 receptors mediate the tachycardia to 5-HT in isolated guinea pig atria [23]. Furthermore, among other findings, this response was (1) attenuated by capsaicin and (2) blocked by the CGRP receptor antagonist, $CGRP_{8-37}$ (which also blocked the tachycardia to CGRP). Hence, activation of 5-HT_3 receptors induces CGRP release from cardiac sensory neurons which, in turn, activates cardiac CGRP receptors [30,31].

Unidentified mechanisms

The hearts of certain lamellibranch and gastropod species (including *Mercenaria mercenaria*, *Patella vulgata*, *Helix aspersa*, *Aplysia*, etc.) are extremely sensitive to 5-HT [1,2]. However, the receptors/mechanisms involved in the positive inotropic and chronotropic effects of 5-HT in these species have not been fully identified ("atypical receptors/mechanisms").

Bradycardia and tachycardia by central mechanisms

In general, central 5-HT pathways regulating cardiovascular function involve two main receptors, namely 5-HT_{1A} receptors (mediating sympatho-inhibition) and 5-HT_2 receptors (inducing sympatho-excitation) [11,32]. Furthermore, central administration of 5-HT elicits complex and apparently contradictory responses which depend, among other factors, on the species, the exact site of central application, and dose employed.

For instance, in anesthetized rats, intracerebroventricular (i.c.v.) application of 5-HT elicits a brief bradycardia mediated by 5-HT_2 receptors followed by tachycardia mediated by 5-HT_{1A} receptors [33].

In anesthetized cats, fourth ventricle application of 5-HT and several 5-HT_{1A} receptor agonists decreased sympathetic nerve activity and blood pressure without affecting heart rate [34]; however, in the presence of cinanserin (a 5-HT_2 receptor antagonist), these compounds produced bradycardia. In contrast, i.c.v. application of 5-HT increased blood pressure, heart rate as well as cardiac and splanchnic sympathetic nerve activity [35]. These effects are mainly mediated by 5-HT_2 receptors since they were (1) mimicked by the 5-HT_2 receptor agonist, DOI and (2) blocked by the 5-HT_2 receptor antagonist cinanserin [35].

Interestingly, in rats, a possible role of 5-HT_7 receptors in cardiovascular regulation has also been suggested since SB 269970, a 5-HT_7 receptor antagonist, blocked the bradycardia evoked by cardiopulmonary reflex, baroreflexes, and chemoreflexes [36,37] (see Table 3.1).

Blood pressure responses to 5-hydroxytryptamine

As described by Page and McCubbin [38], i.v. 5-HT results in a triphasic response on blood pressure as follows.

Initial transient vasodepressor response

It is the result of an abrupt bradycardia (and the consequent decrease in cardiac output) following stimulation of 5-HT_3 receptors on cardiac vagal afferents (see earlier). This response is observed in intact rats, rabbits, cats, and dogs [1,2].

Vasopressor response

Being blocked by 5-HT_2 receptor antagonists (ketanserin, cyproheptadine, and pizotifen), this response is due to activation of vascular 5-HT_2 receptors (resulting in peripheral vasoconstriction) in several species including rats, cats, dogs, monkeys, and humans [1,2,8,9,39]. In dogs, a release of catecholamines by adrenomedullary 5-HT_2 receptors is also involved [40].

However, in cranial blood vessels of humans, pigs, and dogs, as well as in the saphenous vein and external/internal carotid arterial bed of dogs, 5-HT_1 receptors mediate vasoconstriction. This response, being blocked by the antagonists GR 127935 (5-$HT_{1B/1D}$) [2,39,41] or SB224289 (5-HT_{1B}), but not by BRL15572 (5-HT_{1D}) or ketanserin (5-HT_2) (see Table 3.1), involves 5-HT_{1B} receptors [2,5,42,43].

In some cases, both 5-HT_{1B} and 5-HT_2 receptors mediate vasoconstriction in the same blood vessel of several species [2,9,39] (e.g., the canine internal carotid arterial bed [43,44]). In rarer instances, 5-HT may act directly on α-adrenoceptors in isolated rabbit ear and external carotid arteries [45,46].

Late long-lasting vasodepressor response

It has been shown to be mainly, but not exclusively, mediated (see below) by musculotropic 5-HT_7 receptors [8,9,47,48], using selective agonists and antagonists (see Table 3.1). In addition to being resistant to blockade by antagonists at 5-HT_{1A} (WAY 100635), 5-$HT_{1B/1D}$ (GR 127935), 5-HT_2 (ketanserin or ritanserin), 5-HT_3 (MDL 72222), and 5-$HT_{3/4}$ (tropisetron) receptors, the pharmacological profile of these 5-HT_7 receptors mediating vasodepressor/direct vasodilator responses includes, at least, the following three characteristics:

1. Mimicked by 5-CT and 5-methoxytryptamine, with a rank order of agonist potency of 5-CT ≫ 5-HT ≥ 5-methoxytryptamine (with sumatriptan inactive). This is a pharmacological fingerprint for the 5-HT_7 receptor type [3], an order which is reversed for the other 5-HT receptors (see Table 3.1).
2. Blocked by the nonselective antagonists such as methiothepin, lisuride, clozapine, and/or mesulergine [2,39]. It is noteworthy that methiothepin, lisuride, and clozapine have a high affinity for both 5-HT_6 and 5-HT_7 receptors [3]; however, mesulergine displays an almost 300-fold selectivity for 5-HT_7 receptors ($pK_D = 8.15$) over 5-HT_6 receptors ($pK_D = 5.76$) and does not interact with the 5-HT_1 receptor family [3].
3. Blocked by the selective 5-HT_7 receptor antagonist, SB-269970 [47].

Indeed, there is a good correlation between the vasodepressor responses induced by the above tryptamines and their affinity for the 5-HT_7 receptor in anesthetized vagotomized cats [48] and rats [49]. Notwithstanding, several vascular mechanisms may contribute to different degrees in different experimental conditions and species. These mechanisms contributing to the late long-lasting vasodepressor response are as follows.

Direct vascular relaxation

The direct vasorelaxation to 5-HT is also mediated by 5-HT_7 receptors [2] (see earlier). For example, (1) in vivo in the dog external/internal carotid vascular beds [50,51] and (2) in vitro in the cat saphenous vein,

rabbit jugular vein, rabbit femoral vein, sheep pulmonary vein, canine coronary artery, canine femoral vein, and neonatal pig vena cava [2,3,5,39].

Moreover, in blood vessels where both vasodilator 5-HT_7 and vasoconstrictor 5-HT_2 receptors are present, the ultimate response to 5-HT would depend on the preexisting vascular tone, the dose employed, and the proportions in which the two receptors are distributed. For example, in vivo studies have shown that intracarotid infusions of 5-HT in anesthetized dogs produce (1) external carotid vasodilatation [52] by both postjunctional 5-HT_7 [50] and prejunctional sympatho-inhibitory 5-HT_{1B} [53] receptors and (2) external carotid vasoconstriction by postjunctional 5-HT_{1B} receptors [42]. These—and other [1]—studies indicate that the density of 5-HT_7 and 5-HT_2 receptors varies in different segments of a given vascular bed. The vasodilator 5-HT_7 receptor is located primarily on resistance blood vessels [54], the vasoconstrictor 5-HT_{1B} receptor on nonnutrient blood vessels (arteriovenous anastomoses) [55], while the vasoconstrictor 5-HT_2 receptor is mainly present on large conducting blood vessels [1]. Interestingly, in the sheep pulmonary vein, 5-HT_4 receptors mediate vasorelaxation [56].

Prejunctional inhibition of vascular sympathetic neurons

The prejunctional inhibition elicited by 5-HT and related agonists on noradrenaline release from sympathetic neurons has been confirmed in many blood vessels including, among many (1) the isolated canine [57] and human [58] saphenous veins and the porcine coronary artery [59] and (2) the canine external carotid circulation [52,53] as well as the rat cardiac [15] and vasopressor [60] sympathetic outflow.

These vascular sympatho-inhibitory responses are, in most cases, mainly mediated by 5-HT_1 receptors. For example, 5-HT-induced inhibition of the rat vasopressor sympathetic outflow is mainly mediated by sympatho-inhibitory 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptor subtypes [61], as this response was (1) mimicked by 8-OH-DPAT, indorenate, and sumatriptan and (2) blocked by WAY 100635 and GR 127935. Interestingly, sympatho-inhibitory 5-HT_7 receptors could also be involved when rats are chronically pretreated with the 5-HT_2 receptor antagonist sarpogrelate [62] as the sympatho-inhibition to 5-HT was (1) mimicked by AS-19 (a 5-HT_7 receptor agonist) and (2) blocked by SB258719 (a 5-HT_7 receptor antagonist) (see Table 3.1).

Endothelium-dependent vasorelaxation

In the absence of endothelium, the relaxant effect of 5-HT is attenuated while the contractions are augmented in isolated blood vessels of the pig, dog, chick, horse, and rabbit [1,39,63,64]. These findings indicate that 5-HT can release endothelial nitric oxide, and this effect is mainly mediated by 5-HT_1 receptors [2]. Interestingly, in porcine isolated blood vessels, the 5-HT-induced endothelium-dependent vasorelaxation is mediated by (1) 5-$HT_{1B/1D}$ receptors (stimulated by sumatriptan) in coronary arteries [65] or (2) 5-HT_{2B} receptors in pulmonary arteries [66,67].

5-Hydroxytryptamine-induced vasodepressor and vasopressor responses by actions in the central nervous system

Injection of 5-HT into the CNS may result in depressor, pressor, or biphasic responses depending on the exact site of application, dose employed, the species used, etc. [1,60]. This discrepancy may be due to the fact that 5-HT neurons in (1) dorsal and median raphe, anterior hypothalamus, and ventrolateral medullary raphe areas produce pressor effects and (2) midline medullary raphe nuclei produce either pressor or depressor effects [68]. Basically, cardiovascular regulation by central 5-HT neurons involves two receptors [11,32], namely (1) 5-HT_{1A} receptors (associated with sympatho-inhibition, hypotension, and bradycardia) and (2) 5-HT_2 receptors (associated with sympatho-excitation and hypertension).

Receptor-independent actions of 5-hydroxytryptamine

Recent studies suggest that 5-HT can also play cardiovascular (patho)physiological roles independent of 5-HT receptor activation. For example, (1) rats pretreated with fluoxetine (a selective 5-HT reuptake inhibitor) were protected from monocrotaline-induced pulmonary hypertension [69]; (2) transgenic mice overexpressing the 5-HT transporter gene in smooth muscle developed pulmonary hypertension [70]; and (3) 5-HT taken up by its transporter can modify intracellular proteins (i.e., "serotonylation" of proteins by transglutaminase-2) [71], a mechanism that mediates the mitogenic and profibrotic effects of 5-HT without receptor activation [72].

Current and prospective use of 5-hydroxytryptamine receptor agonists and antagonists in cardiovascular pathologies

As considered in detail in other reviews [1,2,8,9,73], the cardiovascular pharmacology of 5-HT has already led to the development of some 5-HT receptor agonists and antagonists with current and prospective therapeutic usefulness in the treatment of several (cardio)vascular diseases/disorders including, among others: migraine; systemic, pulmonary, and portal hypertension; cardiac disorders; some peripheral vascular diseases; and cerebral ischemia.

Implications for future research

The conjunction of structural, transductional, and operational (pharmacological) criteria [2–7] has enabled the characterization and nomenclature of 5-HT receptors in a more meaningful way in many biomedical areas, including cardiovascular research. Notwithstanding this remarkable progress, further research is required to identify the specific mechanisms involved in some cardiovascular actions of 5-HT, for example (to name but a few):

1. The capability of 5-HT to amplify the vasoconstrictor responses to adrenergic and nonadrenergic neurohumoral mediators [46,74]. This is an important issue, as free plasma concentrations of 5-HT are rather low, 5-HT displays a low affinity for 5-HT_{2A} receptors [3] and, accordingly, its plasma concentrations are apparently insufficient to activate vascular 5-HT_{2A} receptors. Nevertheless, with its aforementioned amplifying action, which is mediated by ketanserin-sensitive 5-HT_2 receptors [46], 5-HT plasma concentrations could indirectly affect vascular tone.
2. The (patho)physiological relevance of the receptor-independent intracellular actions of 5-HT depends on the activity of the 5-HT transporter and, thus, affected by selective 5-HT reuptake inhibitors [69–72].

Acknowledgments

I am indebted to Consejo Nacional de Ciencia y Tecnología (CONACyT, Project No. 219707; Mexico City) for their financial support and Belinda Villanueva-Castillo (B. Pharm.) and Eduardo Rivera-Mancilla (M.Sc.) for formatting the references.

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CHAPTER FOUR

Serotonin receptors nomenclature

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) was identified initially in the gut (as enteramine) [1] and blood (as serotonin) [2,3]; 5-HT produces its effects through a variety of membrane-bound receptors and transporters [4,5]. There are seven distinct classes 5-HT receptors (5-HT_1 to 5-HT_7) according to structural, transductional, and pharmacological features. 5-HT receptors belong to either the G protein-coupled receptor (GPCR) or the ligand-gated ion channel superfamilies of receptors. All known 5-HT receptors have been cloned, and at least for some of them, there is structural knowledge as determined from X-ray crystallography [6–8]. Receptor subtype-selective ligands have become available for most receptors, although the notion of selectivity is blurred by the existence of multiple receptor splice- and even editing variants and other complicating factors, such as biased signaling, which affects both apparent affinity and efficacy, since a single compound may act as partial or full agonist or inverse agonist or behave as neutral antagonist, depending on receptor–effector coupling and expression. In addition, species differences in pharmacology have been reported for a number of receptors, for example, 5-HT_{1B}, 5-ht_{1E}, 5-ht_{5B}, 5-HT_3, or various members of the 5-HT_2 receptor family. In some cases, there may be no human equivalent of a rodent receptor (e.g., 5-ht_{5b}), or the rodent version may not exist (e.g., 5-ht_{1e}). Thus, in spite of numerous advances in molecular biology, pharmacology, and medicinal chemistry, the complexity of the 5-HT system is still remarkable, due to the

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00004-9

existence of multiple receptor splice variants (e.g., for 5-HT_4 and 5-HT_7 receptors), ligand-biased signaling, species differences in pharmacology, distribution and function, multiple RNA edited versions (5-HT_{2C}), the numerous GIPs (GPCR interacting proteins) affecting the function of many 5-HT receptors, and the potential for homo- or heteromer formation not only for the various 5-HT GPCRs, but also for 5-HT_3 receptors.

5-Hydroxytryptamine receptor nomenclature

After a rather long period of confusion and debate following the first description of 5-HT receptors in the gut [9], all kinds of nomenclatures were proposed/used to describe effects believed to be mediated by putative 5-HT receptors subtypes: Bradley and colleagues [10] proposed a pragmatic approach to the classification of 5-HT receptors. In addition, there had been a fundamentally strong opposition toward the concept of multiple 5-HT receptors [4]. Thus, Bradley and colleagues proposed to recognize the existence of three families of 5-HT receptors, namely 5-$HT_{1\text{-like}}$, 5-HT_2, and 5-HT_3. The new scheme was based primarily on functional criteria, that is, the rank order of potency of agonists and antagonists as determined in "functional" assays, primarily in organ bath experiments or in vivo. It proved to be a robust and useful framework for several years. However, 1986 was also the year of the cloning and sequencing of the $beta_2$ adrenoceptor by the group of Bob Lefkowitz [11]; subsequently, molecular biology of 5-HT receptors acquired a fast pace (the second GPCR to be cloned) and sequenced was the 5-HT_{1A} receptor [12]. Over the subsequent 8 years or so, a multitude of both known and unknown 5-HT receptors, was cloned, sequenced, expressed, and characterized in recombinant systems [4,5,13,14]. Thus, the vast amount of data generated over a short time period resulted in a game changer, which required at least an update and extension of the Bradley classification. Bradley and colleagues in 1984–96 did not take into account more biochemical aspects of receptor features, such as second messenger or simply radioligand binding, which had already hinted at the existence of multiple 5-HT receptor subtypes (e.g., 5-$HT_{1A,1B,1C,1D,1E}$ or 5-$HT_{2A,\ 2B,\ 2C}$) and additional receptors [15–29]: for instance, the 5-HT_4

receptor identified in both the gastrointestinal and central nervous systems, was already known in the late 1970s but not recognized as a separate entity. In addition, it became rapidly evident from their pharmacological profile, second messenger, and structural features that 5-HT_{1C} receptors as they were designated initially [20–23,30] would be better incorporated into the 5-HT_2 receptor family. On the other hand, ongoing and earlier second messenger studies suggested differences and specific features separating 5-HT_1 and 5-HT_2 receptors and their subtypes [31–38]. However, the most compelling case to be made for a new nomenclature came from the intense receptor gene cloning efforts (see below) which led to the identification/confirmation of most of the known or recognized receptors, but also to the discovery of "new" receptor genes and proteins, tentatively called 5-ht_{1E}, 5-HT_{1F}, 5-ht_{5A}, 5-ht_{5B}, 5-HT_6, or 5-HT_7. The cloning a 5-HT_3 receptor [39] and of potentially up to five subunits (5-$HT_{3A,3B,3C,3D,3E}$) was not entirely surprising, given the complexity of the nicotinic receptor family, but was unsuspected based on the in vitro and in vivo data collected so far. In each case, receptors were expressed and characterized in recombinant systems, with radioligand binding, second messenger studies, and their mRNA and protein distribution studied in animal and human tissues using various techniques, including in situ hybridization and immunohistochemistry; keeping in mind that in most cases, antibodies raised against GPCRs are of poor quality and do not allow proper slice distribution work. Nevertheless, the accumulating evidence suggested the existence of multiple families of 5-HT receptors and receptor subtypes for GPCRs or subunits for the ligand-gated family (5-$HT_{3A,3B,3C,3D,3E}$). All these receptors could be studied using various second messenger and electrophysiologic tools; thus, new criteria for receptor classification were developed, proposed, and eventually adopted for the 5-HT receptor family and more generally for hormone and transmitter receptors at large. The massive and overwhelming evidence accumulated in various academic and pharma industry research centers justified a profound rethinking and implementation of a new classification scheme for 5-HT receptors and by extension, for the whole receptor field [40]. The proposed new principles were rather simple: structural, transductional, and pharmacological information. At the time, we were referring to sequence information (gene sequence), second messenger and effector coupling information (cAMP, PI, Calcium, kinases, and channels), and rank orders of potency for agonists/antagonists as determined in isolated tissue or organ bath assays and recombinant systems, keeping in mind

that the two latter criteria can be very much dependent on cell type and species. Clearly, real structural information is still to come as X-ray or solution NMR information on the structure is still in its very early days [4,6,7,13,14].

The three basic classification principles (structural, transductional, and pharmacological criteria) were subsequently adopted for other receptors by the Receptor Nomenclature Subcommittees and Committee of the International Union of Pharmacology (NC-IUPHAR), the main body in charge of receptor nomenclature. The current classification [4] has been progressively adapted to accommodate new information obtained with both recombinant and native receptors. From the start, it favored an alignment of nomenclature with the human genome, to avoid confusions due to species differences which had been revealed over the course of in vitro and in vivo investigations, as receptors from various species were cloned and extensively characterized and compared [14,41–45]; for regular updates, check the Guide to Receptor which is published every second year in the British Journal of Pharmacology (BJP), as a joint venture between the British Pharmacological Society (BPS) and the International Union for Pharmacology (IUPHAR) [46]. (Note that the lower case designates receptors have not been established definitively in functional tests, see Tables 4.1 and 4.2).

Most 5-HT receptors, except 5-HT_3 which are ligand-gated channels with a structural analogy to nicotinic or $GABA_A$ receptors, belong to the GPCR family A. Lower case means that the receptor is not functionally defined in native in vitro or in vivo systems. Note that 5-HT_{1C} is missing, as it was renamed 5-HT_{2C} in order to avoid confusion with previous literature. No lower case for 5-HT_3 receptor subunits as they may form heteromers for which in vivo proof is not established although fairly possible.

Table 4.1 5-hydroxytryptamine receptors families and receptor subtypes.

5-HT_1	5-HT_2	5-HT_3	5-HT_4	5-ht_5	5-HT_6	5-HT_7
5-HT_{1A}	5-HT_{2A}	5-HT_{3A}	5-HT_4	5-ht_{5A}	5-HT_6	5-HT_7
5-HT_{1B}	5-HT_{2B}	5-HT_{3B}		5-ht_{5B}		
5-HT_{1D}	5-HT_{2C}	5-HT_{3C}				
5-ht_{1E}		5-HT_{3D}				
5-HT_{1F}		5-HT_{3E}				

Table 4.2 Classification of 5-hydroxytryptamine receptor nomenclature as proposed by the NC-IUPHAR Subcommittee on 5-hydroxytryptamine receptors.

5-HT$_1$					
Nomenclature	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{1D}$	5-HT$_{1E}$	5-HT$_{1F}$
Previous names	–	5-HT$_{1D\beta}$	5-HT$_{1D\alpha}$	–	5-ht$_{1E\beta}$, 5-HT$_6$
Selective agonists	8-OH-DPAT (R)-UH301 U92016A	Sumatriptan L 6194247	Sumatriptan PNU 109291	–	LY 334370 LY 334864
Selective antagonists (pK$_B$)	(±)WAY 100635 (8.7)	GR55562 (7.4) SB 224289 (8.5) SB236057 (8.9)	BRL 15572 (7.9)	–	–
Radioligands	[^{3}H]WAY100635 [^{3}H]8-OH-DPAT	[^{125}I]GTI [^{125}I]CYP (rodent) [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{125}I]GTI [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]LY 334370
G protein effector	G$_{i/o}$	G$_{i/o}$	G$_{i/o}$	G$_{i/o}$	G$_{i/o}$
Gene/Chromosomal localization	HTR1A/5q11.2-q13	HTR1B/6q13	HTR1D/1p34.3-36.3	HTR1E/6q14-15	HTR1F/3p11-p14.1
Structural information	h421 P8908 m421 Q64264 r422 P19327	h390 P28222 m386 P28334 r386 P28564	h377 P28221 m374 Q61224 r374 P28565	h365 P28566	h366 P30939 m366 Q02284 r366 P30940
5-HT$_{2,\ 3,\ 4}$					
Nomenclature	5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$[b]	5-HT$_3$	5-HT$_4$
Previous names	D/5-HT$_2$	5-HT$_{2F}$	5-HT$_{1C}$	M	–
Selective agonists	DOI[a]	BW723C86 Ro 600175, DOI	Ro 600175, DOI	SR 57227 m-chlorophenylbiguanide	BIMU 8 RS 67506 ML 10302
Selective antagonists (pK$_B$)	Ketanserin (8.5-9.5) MDL 100907 (9.4)	SB 200646 (7.5)[c] SB 204741 (7.8)	Mesulergine (9.1) SB 242084 (9.0) RS 102221 (8.4)	granisetron (10) ondansetron (8-10) tropisetron (10-11)	GR 113808 (9-9.5) SB 204070 (10.8) RS 100235 (11.2)

(*Continued*)

Table 4.2 (Continued)

Radioligands	[^{125}I]DOI [^{3}H]Ketanserin [^{3}H]MDL 100907	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]Mesulergine	[^{3}H](S)-zacopride [^{3}H]tropisetron [^{3}H]granisetron [^{3}H]GR 65630 [^{3}H]LY 278584	[^{125}I]SB 207710 [^{3}H]GR 113808 [^{3}H]RS 57639
G protein effector	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	[d]	G_s
Gene/Chromosomal Localization	HTR2A/13q14-q21	HTR2B/2q36.3-q37.1	HTR2C/Xq24	HTR3/11q23.1-q23.2	HTR4/5q31-33
Structural information	h471 P28223 m471 P35362 r471 P14842	h481 P41595 m504 Q02152 r479 P30994	h458 P28335 m459 P34968 r460 P08909	Multisubunit[e] 5-HT$_{3A}$, 5-HT$_{3B}$, 5-HT$_{3C}$	h387 Y09756^{AS} m387 Y09587^{AS} r387 U20906^{AS}

5-HT$_{5,\ 6,\ 7}$

Nomenclature	5-ht$_{5A}$	5-ht$_{5B}$	5-HT$_6$	5-HT$_7$
Previous names	5-HT$_{5\alpha}$	–	–	5-HT$_X$ 5-HT$_1$-like
Selective agonists	–	–	–	–
Selective antagonists (pK$_B$)	SB-699551 (8.0)	–	Ro630563 (7.9) SB 271046 (7.8) SB 357134 (8.5)	SB 258719 (7.9) SB 269970 (9.0)
Radioligands	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]SB 258585 [^{125}I]LSD [^{3}H]5-HT	[^{125}I]LSD [^{3}H]SB 269970 [^{3}H]5-CT [^{3}H]5-HT
G protein effector	$G_{i/o}$	None identified	G_s	G_s
Gene/Chromosomal localization	HTR5A/7q36.1	htr5b/2q11-q13	HTR6/1p35-36	HTR7/10q23.3-24.3
Structural information	h357 P47898 m357 P30966 r357 P35364	m370 P31387 r370 P35365	h440 P50406 m440 NP_067333 r438 P31388	h445 P34969^{AS} m448 P32304 r448 P32305^{AS}

[a]Also activates the 5-HT$_{2C}$ receptor.
[b]Multiple isoforms of the 5-HT$_{2C}$ receptor are produced by RNA editing.
[c]Nonselective blockade.
[d]The 5-HT$_3$ receptor is a transmitter-gated cation channel that exists as a pentamer of 4TM subunits.
[e]Human, rat, mouse, guinea pig, and ferret homologues of the 5-HT$_{3A}$ receptor have been cloned that exhibit interspecies variation in pharmacology. A second 5-HT$_3$ receptor subunit, 5-HT$_{3B}$, imparts distinctive biophysical properties upon hetero-oligomeric (5-HT$_{3A}$/5-HT$_{3B}$) versus homo-oligomeric (5-HT$_{3A}$) recombinant receptors. The function of the 5-HT$_{3C}$ subunit and other putative members of the family requires further investigations (see text).
NC = nomenclature committee.

5-HT$_1$ receptors

The 5-HT$_1$ receptor class comprises five different receptors (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht$_{1E}$, and 5-HT$_{1F}$) which share 41%–63% overall sequence identity and couple preferentially to $G_{i/o}$ proteins to inhibit cyclic adenosine monophosphate (cAMP) formation. The putative 5-ht$_{1E}$ receptor keeps a lower case appellation to denote that corresponding endogenous receptors with a physiological role have not yet been found.

5-HT$_{1A}$ receptors

The 5-HT$_{1A}$ receptor was the first 5-HT receptor to be cloned [12] and actually only the second cloned GPCR immediately after the beta$_2$ adrenoceptor. The 5-HT$_{1A}$ receptor clone was initially known as G21 and had an orphan status, as the endogenous ligand was not identified. It also emphasizes the close similarity between these receptors and explains that beta receptor antagonist has an affinity for some 5-HT$_1$ receptors. The 5-HT$_{1A}$ gene sequence is intronless and codes for a 422, 421, and 422 amino acid product in human, rat, and mouse, respectively, with all the attributes of a GPCR. It is known as P08908 in the human protein database and HTR1A on the HUGO database. The human 5-HT$_{1A}$ receptor is located on chromosome 5q11.2-q13 and has no splice variants. 5-HT$_{1A}$ receptors are largely distributed in the brain. In the raphe nuclei they are somatodendritic and act as autoreceptors to inhibit cell firing; postsynaptic 5-HT$_{1A}$ receptors are present in a number of limbic structures, especially the hippocampus. 5-HT$_{1A}$ receptors are present in limbic brain areas, hippocampus, lateral septum, cortical areas (particularly cingulate and entorhinal cortex), and also in the mesencephalic raphe nuclei (both dorsal and median raphe nuclei) whereas 5-HT$_{1A}$ binding sites in the basal ganglia and cerebellum are barely detectable. Activation of central 5-HT$_{1A}$ receptors induces a behavioral syndrome, which is characterized by a flat body posture, reciprocal forepaw treading, and head weaving. In addition, a decrease in blood pressure and heart rate can also be triggered. Gastrointestinal 5-HT$_{1A}$ receptors were identified on the guinea pig myenteric plexus where they function as inhibitory modulators of fast excitatory postsynaptic potentials. 5-HT$_{1A}$ agonism may have a positive effect in anxiety and depressive states, and reverse memory impairment.

There are numerous high affinity and selectivity 5-HT_{1A} ligands. 8-OH-DPAT, DP-5-CT, and U92016A are excellent tools. Buspirone (5-HT_{1A} partial agonist) is used to treat generalized anxiety disorders, as well as depression. Thus, 5-HT_{1A} receptor agonists, for example, PRX-00023, gepirone, are/were developed for similar indications. 5-HT_{1A} receptor antagonists, lecozotan or agonists, for example, xaliproden are developed as cognitive enhancers in Alzheimer's disease. Some atypical antipsychotic drugs with partial agonism at 5-HT_{1A} receptors (e.g., aripiprazole, bifeprunox, ziprasidone, or SSR 181507) may reverse memory deficits in contrast to typical antipsychotics. Tandospirone (a 5-HT_{1A} receptor partial agonist) may augment verbal memory in schizophrenic patients. Finally, WAY-211612, a dual 5-HT_{1A} receptor antagonist/selective 5-HT reuptake inhibitor may represent a novel class of antidepressants, in reproducing the improved onset of action in depression seen after combining pindolol with SSRIs. Amongst antagonists, WAY100,635, UH301, and NAD299 (robalzotan) are very good tools. In addition, derivatives of WAY100,635 and MPPF are excellent PET ligands used to determine in vivo receptor distribution and receptor occupancy by 5-HT_{1A} antagonists.

5-HT_{1B} receptors

The 5-HT_{1B} receptor has 390, 386, and 386 amino acids in human, rat, and mouse, respectively [47–51]. There are no splice variants, and the human 5-HT_{1B} gene is located on chr. 6q13. It is known as P28222 in the human protein database and HTR1B on the HUGO database. Although similarities in transductional features, function, and brain distribution led to the suspicion that the rodent "5-HT_{1B}" and nonrodent "5-HT_{1D}" receptors were species homologues [24,25,41,52,53], this was demonstrated unequivocally only after the receptors were cloned [42]. It was first shown that the pharmacologically defined human 5-HT_{1D} receptor was, in fact, a composite of two subtypes, encoded by distinct genes, which were called 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. This notation reflected the fact that the operational profiles of these two receptors were almost indistinguishable when using the ligands available at that time. It was subsequently shown that in spite of overt differences in their pharmacological profiles, the 5-HT_{1B} and 5-$HT_{1D\beta}$ receptors are, respectively, rodent and nonrodent species homologues with high overall (97%) sequence homology. Identification of the 5-$HT_{1D\alpha}$ gene in rats confirmed that 5-$HT_{1B/1D}$ receptors represent only two different classes, prompting the need to revise the receptor notation

according to the classification principles. Accordingly, the 5-$HT_{1D\beta}$ receptor was named 5-HT_{1B}, consistent with the fact that it is the human homologue of the original rodent 5-HT_{1B} receptor. However, it is important to remember that, because the human receptor assumes preeminence, the operational characteristics of the 5-HT_{1B} class are those defined for the human receptor. 5-HT_{1B} receptors are expressed in the central nervous system and are also found on cerebral arteries and other vascular tissues. Central 5-HT_{1B} receptors are thought to serve as terminal autoreceptors [52,53]. Thus, a high density of 5-HT_{1B} sites in the basal ganglia, especially the substantia nigra, globus pallidus, ventral pallidum, and entopeduncular nucleus, but also many other regions and a number of vessels [54,55]. Peripheral effects have been described, such as inhibition of noradrenaline (norepinephrine) release in vena cava and inhibition of plasma extravasation produced by trigeminal ganglion stimulation in guinea pigs and rats. 5-HT_{1B} receptors mediate contraction of rat caudal arteries. In nonrodents, they exhibit the 5-HT_{1D} "pharmacology." 5-HT_{1B} receptor agonists have been developed for the treatment of migraine attack (dihydroergotamine, ergotamine, sumatriptan, rizatriptan, naratriptan, zolmitriptan, eletriptan, and others) [56–59].

5-HT_{1B} receptor ligands do generally bind 5-HT_{1D} receptors as well, especially agonists such as CP 93129, CP 94253, GR 46611, L 694247, or SKF 99101H. Similarly, the triptans, that is, sumatriptan, naratriptan, donitriptan, rizatriptan, zolmitriptan, and eletriptan do not show selectivity for 5-HT_{1B} receptors. On the other hand, SB216641, SB 224289, SB236057, and GR55562 are antagonists with high affinity and selectivity for 5-HT_{1B} receptors, while GR 127935 is a potent dual 5-$HT_{1B/1D}$ receptor antagonist [60–62]. The triptans are 5-$HT_{1B/1D}$ receptor agonists used for the acute treatment of a migraine, and sumatriptan has also an affinity for 5-HT_{1F} receptors.

5-HT_{1D} receptors

The 5-HT_{1D} receptor has 377, 374, and 374 amino acids in human, rat, and mouse, respectively [48]. There are no splice variants, and the human 5-HT_{1D} gene is located on chr. 1p36.3-p34.3. It is known as P28221 in the human protein database and HTR1D on the HUGO database. The receptor was initially reported as RDC4 in dog and then as 5-HT1Dα due to high sequence homologies with 5-$HT_{1D\beta}$ receptors, and uncertainty about the alignment with the known endogenous 5-$HT_{1B/1D}$ receptors

and species differences between human and rodent receptors. The level of expression of 5-HT_{1D} receptors is very low compared to that of 5-HT_{1B} receptors, and to assign a functional role to 5-HT_{1D} receptors has been an ongoing issue until the availability of selective ligands, for example, SB216641 (5-HT_{1B}) or BRL 15572 (5-HT_{1D}) [63] allowed the respective contributions of the two receptors to be better determined. Thus, 5-HT autoreceptors in the brain are apparently exclusively of the 5-HT_{1B} type; on the other hand, 5-HT_{1D} receptors in the human heart appear to modulate 5-HT release. 5-HT_{1B} receptors but not 5-HT_{1D} receptors are largely distributed in vascular beds. Central 5-HT_{1D} receptors are found at low levels in the basal ganglia, for example, the globus pallidus, substantia nigra, and caudate putamen and also the hippocampus and cortex but at low densities. Since absent from vascular beds, it was expected that 5-HT_{1D} receptor agonists may be effective in the treatment of migraines, but without the cardiovascular side effects of the triptans (PNU-14263)[64]. However, this did not materialize in the clinic and such efforts have been stopped. Along the same lines, since sumatriptan has an affinity for the 5-HT_{1F} receptor, that route was also followed with greater success. Interestingly, 5-HT_{1B} and 5-HT_{1D} receptors tend to codistribute [55], and although they may form homomers, there are also indications that they may form heteromers [65].

Many of the antimigraine triptans do not distinguish between 5-HT_{1B} and 5-HT_{1D} receptors. However, PNU 109291 and PNU 142633 are selective agonists, whereas LY 310762, SB714786, and BRL 15572 are selective and high-affinity 5-HT_{1D} receptor antagonists. PNU 142633 was tested in migraine trials, but results were disappointing, suggesting that 5-HT_{1D} receptor agonism is not a viable approach to migraine therapy.

5-ht_{1E} receptors

The 5-ht_{1E} receptor has 365 amino acids in human, whereas no rodents' genes are known. There are no splice variants, and the human 5-ht_{1E} gene is located on chr. 6q15. It is known as P28566 in the human protein database and HTR1E on the HUGO database. The putative 5-ht_{1E} receptor was first identified in [^{3}H]5-HT radioligand binding studies in the human frontal cortex, but it was not possible to determine its overall distribution and pharmacology, since [^{3}H]5-HT labels most other 5-HT GPCRs. 5-ht_{1E} receptor mRNA and binding sites are present in the brain (cortical areas, including entorhinal cortex, and the caudate/putamen, with lower levels in amygdala and hypothalamus), but their function is still

ill-defined [55]. The main difficulty, with 5-ht$_{1E}$, is that it is not expressed in rodents (rats or mice) and selective ligands are largely absent inexistent.

BRL 54443 is a potent agonist at 5-ht$_{1E}$ and 5-HT$_{1F}$ binding receptors; however, 5-ht$_{1E}$ receptor selective compounds have not yet been reported.

5-HT$_{1F}$ receptors

The 5-HT$_{1F}$ receptor has 366 amino acids in human, rat, and mouse [66]. There are no splice variants, and the human 5-HT$_{1F}$ gene is located on chr. 3p11-p14.1. It is known as P30939 in the human protein database and HTR1F on the HUGO database. The 5-HT$_{1F}$ receptor is a pure product of molecular biology. It was cloned by homology to the 5-HT$_{1E}$ receptor as its existence was not suspected earlier on. The mRNA for the human 5-HT$_{1F}$ receptor has been identified in the brain, mesentery, and uterus, but not in kidney, liver, spleen, heart, pancreas, or testis. Brain 5-HT$_{1F}$ binding is found in olfactory bulb and tubercle, cortical layers 4–5, nucleus accumbens, caudate putamen, thalamus, medial mammillary nucleus, hippocampus (CA3), subiculum, and various amygdaloid nuclei. Its distribution may suggest a role as another 5-HT autoreceptor [55].

BRL 54443 is a potent agonist at 5-HT$_{1E}$ and 5-HT$_{1F}$ binding sites. LY 334370 and LY 344864 are selective and potent 5-HT$_{1F}$ receptor agonists. However, there are no selective 5-HT$_{1F}$ receptor antagonists. Interestingly, sumatriptan can label 5-HT$_{1F}$ receptors. LY 334370 inhibits trigeminal stimulation-induced early activated gene (Fos protein) expression in nociceptive neurons in the rat brainstem. This effect combined with the sumatriptan affinity for 5-HT$_{1F}$ receptor suggested that a migraine may be an attractive indication since there is no evidence for vascular 5-HT$_{1F}$ receptors: therefore, a selective 5-HT$_{1F}$ agonist would be devoid of the side effects characteristic of the triptans as eventually confirmed in the clinic with Lasmiditan [67–69].

5-HT$_2$ receptors

Three 5-HT$_2$ receptors have been cloned; they exhibit 46%–50% overall sequence identity [70–72] and couple preferentially to guanine nucleotide binding protein of the G$_{q/11}$ type to increase the hydrolysis of

inositol phosphates and raise cytosolic calcium concentrations. The 5-HT_{2A} receptor refers to the classical 5-HT_2 receptor or 5-HT_D as described originally. The 5-HT_{2B} receptor mediates the contractile action of serotonin in the isolated rat fundus and was considered an oddity for some time. The third 5-HT_2 subtype corresponds to the previously known 5-HT_{1C} receptor, as described initially in the choroid plexus, and which was renamed as 5-HT_{2C} receptor.

5-HT_{2A} receptors

The 5-HT_{2A} receptor gene codes for 471 amino acids in human, rat, and mouse [70–72]. It is known as P28223 in the human protein database and HTR2A on the HUGO database. The human 5-HT_{2A} receptor is located on chromosome 13q14-q21 and has no splice variants. 5-HT_{2A} receptors are widely distributed in peripheral tissues [10], but are also present in the CNS: 5-HT_{2A}-binding is seen in cortical areas, caudate, nucleus accumbens, olfactory tubercule, and hippocampus. The distribution of 5-HT_{2A}-binding, immunoreactivity, and mRNA overlaps reasonably well, suggesting that the receptor is primarily postsynaptic. 5-HT_{2A} receptors mediate contractile responses in many vascular smooth muscle preparations, in bronchial, uterine, and urinary smooth muscle, and part of the contractile effects of 5-HT in the guinea pig ileum (the D receptor) described by Gaddum and Picarelli [9]. In addition, platelet aggregation and increased capillary permeability following exposure to 5-HT can be included as 5-HT_{2A} receptor–mediated actions. The 5-HT_{2A} receptor mediates the hallucinogenic effects of LSD and has been the target of intense research in schizophrenia, although selective 5-HT_{2A} antagonists are less widely effective in the clinic than clozapine and other atypicals.

Agonists with high affinity for 5-HT_{2A} receptors include LSD, DOI, DOB, and DOM but these are mixed 5-$HT_{2A/2B/2C}$ receptor ligands. Potent 5-HT_{2A} receptor antagonists are M 100907, R 96544, and sarpogrelate. Other 5-HT_{2A} receptor antagonists have mixed profiles, for example, cinanserin (DA D_4), ketanserin (5-HT_{2C}, 5-HT_{1D}, α1-adrenoceptor), risperidone (DA D_2), and SR 46349B (5-HT_{2B}) or nefazodone (5-HT/NA uptake inhibitor). Ketanserin was initially developed to treat hypertension but it has not been established whether 5-HT_{2A} receptor antagonism is a valid antihypertensive principle since ketanserin is also an α_1-adrenoceptor antagonist. Sarpogrelate is used as an antiplatelet agent for the treatment of peripheral arterial disease; nefazodone is an

antidepressant. A number of atypical antipsychotics (e.g., clozapine, olanzapine, and risperidone) share 5-HT_{2A} receptor antagonism. M100 907 was developed for schizophrenia, but clinical trials in acute schizophrenia were stopped because of lack of efficacy, although the final word has not been told. Therefore, it seems that it is the combination of dopamine D_2 and 5-HT_{2A} receptor antagonism that may best explain the antipsychotic activity of drugs such as clozapine, olanzapine, Seroquel, and others. Other 5-HT_{2A} receptor antagonists, for example, eplivanserin, pimavanserin, pruvanserin, and volinanserin, are being investigated for improving sleep maintenance and quality in insomnia with mixed results. Behavioral and biochemical evidence demonstrates that LSD and other hallucinogens rely on activation of 5-HT_2 receptors for their effects [73], but selectivity at subtypes of this receptor remains to be established; there is evidence that the 5-HT_{2A} agonist which induces hallucinations may do so by interacting selectively with a 5-HT_{2A}/mGluR$_2$ receptor heterodimer. Finally, analogues of M 100907 and altanserin were developed as PET ligands.

5-HT_{2B} receptors

The 5-HT_{2B} receptor gene codes for 481, 479, and 504 amino acids in human, rat, and mouse, respectively, it was also shortly named as the 5-HT_{2F} (fundus) receptor [74]. It is known as P41595 in the human protein database and HTR2B on the HUGO database. The human 5-HT_{2B} receptor is located on chromosome 2q36.3-2q37.1 and has no splice variants. The 5-HT_{2B} receptor, which mediates very effectively the effects of 5-HT on fundic smooth muscle contraction, has not been easy to characterize pharmacologically because of operational features that are rather similar to those of the other 5-HT_2 receptor subtypes. Eventually, the situation was clarified by the subsequent cloning of the rat, mouse, and human "fundus" receptor, also known as 5-HT_{2F} for a short time. 5-HT_{2B} receptor mRNA is found in fundus, gut, heart, kidney, lung, and brain of rat. 5-HT_{2B} receptor-like immunoreactivity was reported in rat brain but it is restricted to the cerebellum, lateral septum, dorsal hypothalamus, and medial amygdala and a brain function remains to be defined. The mouse homologue is expressed in intestine, heart, kidney, and brain. The 5-HT_{2B} receptor is present on endothelial cells of pig pulmonary arteries, mediating vasorelaxation (via nitric oxide release) upon activation. This is supported by the presence of 5-HT_{2B} receptor mRNA in a

number of blood vessels and the absence of 5-HT_{2C} receptor mRNA. Moreover, 5-HT_{2B} receptors mediate endothelium-dependent relaxation in isolated rat jugular vein and contraction of the longitudinal muscle in the human small intestine. There is suggestive evidence that the 5-HT_{2B} receptor plays a role in pulmonary hypertension. 5-HT_{2B} receptors play a crucial role during development, especially of the heart; their stimulation can have drastic effects and explain the valvulopathies that have been observed with the pen fen combinations used for appetite reduction in the 1970s and 1980s. Eventually, it was discovered that fenfluramine, norfenfluramine, and active metabolites are 5-HT_{2B} receptor agonists which lead to serious valvulopathies. These compounds, as well as others like pergolide, have been withdrawn from the market. 5-HT_{2B} receptor agonism is a clear Tox signal and precludes any further clinical development [75–79].

5-HT_{2B} receptor selective agonists (BW723C86, Ro 600175) and antagonists (EGIS-7625, LY 272015, RS127445, and SB 204741) helped to distinguish 5-HT_{2B} receptor–mediated effects. SB 200646 and SB 221284 are mixed 5-$HT_{2B/2C}$ receptor antagonists. Cyproheptadine and pizotifen, nonselective 5-HT_{2B} receptor antagonists, are used in migraine prophylaxis. There is clear evidence that direct or indirect 5-HT_{2B} agonists such as the 5-HT releasers fenfluramine or MDMA (ecstasy) by activating 5-HT_{2B} receptors will induce valvulopathies; as a result of such findings, it has been strongly recommended to screen for 5-HT_{2B} agonist activity and such compounds should not be developed or should be retired from clinical practice [75–79].

5-HT_{2C} receptors

The 5-HT_{2C} receptor gene codes for 458, 459, and 460 amino acids in human, mouse, and rat, respectively [72,80,81]; it was initially named as the 5-HT_{1C} receptor due to its high affinity for and labeling by [^{3}H]5-HT. It is known as P28335 in the human protein database and HTR2C on the HUGO database. The human 5-HT_{2C} receptor is located on chromosome Xq24. The 5-HT_{2C} receptor gene has a very complex exon–intron structure, with a number of splice variants and except for a GPCR, a large number of editing variants (up to 25 in humans). 5-HT_{2C} receptors are involved in various behaviors related to appetite control, food intake, mood, and possibly addiction and epilepsy. Indeed 5-HT_{2C} KO animals have marked overweight and seizure phenotypes and it is

expected that 5-HT_{2C} antagonist will lead to weight gain as is the case with a number of antipsychotics which act as 5-HT_{2C} antagonists, for example, olanzapine and others. It is also known that fenfluramine and analogues are reducing food intake because they are 5-HT_{2C} receptor agonists. Several 5-HT_{2C} agonists are in development or on the market to treat not only food intake and obesity, but also various types of addiction, including smoking and possibly drug-related addictions. In addition, the antidepressant agomelatine is melatonin receptor agonist and 5-HT_{2C} receptor antagonist: 5-HT_{2C} antagonism may lead to increased central dopamine release and thus have antidepressant effects. There is also evidence for 5-HT_{2C} receptors editing in depression and suicidal ideation. Finally, 5-HT_{2C} editing (i.e., leading to increased receptor constitutive activity) in spinal cord injury may lead to increase spasms especially in the lower limbs, which can be treated with 5-HT_{2C} receptor inverse agonists such as cyproheptadine.

The 5-HT_{2C} receptor is absent outside of the central nervous system and the choroid plexus, where it was discovered. 5-HT_{2C} receptors are expressed in the nucleus accumbens, caudate putamen, olfactory tubercle, claustrum, septum, cingular cortex, amygdala, dentate gyrus, periaqueductal gray, entorhinal cortex, and several brainstem motor nuclei. It has been suggested that the anxiogenic component of m-chlorophenylpiperazine (mCPP) is mediated by 5-HT_{2C} receptor activation, and selective 5-HT_{2C} receptor antagonists such as SB242084-A display anxiolytic properties in various animal models. 5-HT_{2C} receptors have been shown to modulate mesolimbic dopaminergic function, where they exert a tonic inhibitory influence over dopamine neurotransmission. The 5-HT_{2C} receptor knockout mouse suffers from spontaneous convulsions, cognitive impairment, increased food intake, and obesity, but similar effects are not consistently reproduced by selective antagonists, suggesting that these changes may result from neuroadaptation. However, a number of 5-HT_{2C} ligands are in clinical development (see below), and clinical outcome will clear some of these issues.

The 5-HT_{2C} receptor is one of the very few GPCRs to be subjected to RNA editing [82,83]. Deamination of one or more adenine bases present at five specific sites in the receptor pre-mRNA results in the conversion of the edited bases to inosine. Upon translation of the mature mRNA, these inosine bases are read as guanine, resulting in up to three amino acid changes in the second intracellular loop and the formation of nine distinct receptor isoforms (and potentially many more).

As tissue-specific (choroid plexus versus other brain regions) and species-specific differences in 5-HT_{2C} mRNA editing have been documented, it is conceivable that this process represents yet another means of achieving phenotypic specificity of signaling for 5-HT_{2C} receptor-mediated events. It is interesting that PLC activity in the choroid plexus is very sensitive to 5-HT. For the fully edited receptor isoform, coupling efficiency to PLC is reduced at least 10-fold.

Many high-affinity 5-HT_{2C} receptor agonists lack selectivity: for example, MK 212 and Ro 60-0175. More selective agonists are CP809191, WAY 163909, Org 12962, VER-3323, YM 348, or PNU 22394 (a partial agonist). Lorcaserin, an agonist, is on the market for obesity, and in clinical development for various types of addiction. Agomelatine, a 5-HT_{2C} antagonist and melatonin$_{1,2}$ receptor agonist is an antidepressant. Selective and potent 5-HT_{2C} receptor antagonists do exist: for example, RS 102221, FR260010, and SB 242084, whereas SDZ SER-082 is a mixed 5-$HT_{2C/2B}$ receptor antagonist.

5-HT_3 receptors, ligand-gated receptors

5-HT_3 receptors (the M receptors of [9]) belong to the Cys-loop ligand-gated ion channel receptor superfamily, similar to nicotinic acetylcholine or $GABA_A$ receptors (see [84]). They are found on neurons of both central and peripheral origin where they trigger a rapid depolarization due to a transient inward current subsequent to the opening of non-selective cation channels (Na^+, K^+ influx). 5-HT_3 receptors are present in high densities in certain brain regions such as the area postrema and the nucleus tractus solitarius, although they were believed to be only peripheral (but see [85–87]). In the periphery, they are located on preganglionic and postganglionic autonomic neurons and on neurons of the sensory nervous system. Apart from its pronounced effect on the cardiovascular system, serotonin induces diverse effects via 5-HT_3 receptor activation throughout the gastrointestinal tract by regulating motility as well as intestinal secretion. The 5-HT_{3A} receptor was cloned from a neuronally derived cell line and placed within the nicotinic/$GABA_A$ receptor superfamily. Two splice variants have been described in neuroblastoma-glioma cells (NCB-20, NG108-15) and from rat native tissue; they appear to

have similar distributions, pharmacological profiles, and electrophysiological characteristics when expressed as homomers. The native receptor, as revealed by electron microscopy, performed with neuroblastoma-glioma cells, is a pentamer. 5-HT_3 receptor subtypes may exist, but it appears that species differences provide the basis of the pharmacological heterogeneity reported so far. A second subunit, named 5-HT_{3B} was cloned; it results from a duplication in the same locus (11q23.1). It appears that the heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits is needed to provide the full functional features of the 5-HT_3 receptor, as either subunit alone results in receptors with very low conductance and response amplitude as determined in electrophysiological experiments. 5-HT_3 receptor antagonism is used in chemotherapy- and radiotherapy-induced nausea and vomiting, both of which are treated with ondansetron, granisetron, and tropisetron. Since 5-HT_3 receptor activation in the brain leads to dopamine release, and 5-HT_3 receptor antagonists produce central effects in animal models comparable to those of antipsychotics (e.g., dopamine receptor antagonists such as haloperidol) and anxiolytics; schizophrenia and anxiety were considered as potential indications. However, there are no clinical data to substantiate such activities yet. Similarly, the hypothesis that 5-HT_3 antagonists may prove useful in the treatment of a migraine did not materialize in clinical studies. The situation has become more complex recently as more potential 5-HT_3 receptor subunits have been cloned, also resulting from duplications on chromosome 3, named as 5-$HT_{3C,3D,3E}$. However, it remains to be seen whether they encode for functional receptors, or only for partial inactive sequences (less than 300 aa instead of the usual 480–500 aa) [88]. One hypothesis is that these subunits may serve as chaperones. Along these lines, coexpression of 5-HT_{3A} and 5-$HT_{3C,3D,3E}$ subunits has been demonstrated in human colon. As with other 5-HT receptors, there are species differences for 5-HT_3 receptors; many ligands display significantly reduced affinities at the guinea pig 5-HT_3 receptor. As is common with, for example, $GABA_A$ receptors, native and recombinant 5-HT_3 receptors are subject to allosteric modulation by extracellular divalent cations, alcohols, several general anesthetics, and 5-hydroxy- and halide-substituted indoles, in addition to a number of natural plant extracts. A number of drugs also show cross-reactivity with nicotinic cholinergic receptors. Although the structure of the 5-HT3 receptor has not been revealed, much knowledge has been accumulated from the Cys-loop family (see [89,90]) about the pentameric structure and binding sites for 5-HT and allosteric modulators.

5-HT_{3A}

The 5-HT_{3A} receptor gene codes for 478, 487, and 483 amino acids in human, mouse, and rat, respectively. It is known as P46098 in the human protein database and HTR3A on the HUGO database. The human 5-HT_{3A} receptor subunit is located on chromosome 11q23.1-q23.2 and has several splice variants.

5-HT_{3B}

The 5-HT_{3B} receptor gene codes for 441 amino acids in human. It is known as O95264 in the human protein database and HTR3B on the HUGO database. The human 5-HT_{3B} receptor subunit is also located on chromosome 11: 113,904,677-113,946,565 and has several splice variants. The 5-HT_{3B} receptor is not functional as a homopentamer but is functional as a heteropentamer 5-HT_{3A}/5-HT_{3B} whereas 5-HT3A can function as a homopentamer although with reduced functionality.

5-HT_{3C}

The 5-HT_{3C} receptor gene codes for 447 amino acids in human located on chromosome 3: 184,053,047-184,060,671. It is known as Q8WXA8 in the human protein database and HTR3C on the HUGO database. No functional data have been reported.

5-HT_{3D}

The 5-HT_{3D} receptor gene codes for 454 amino acids in human also located on chromosome 3: 184,031,544-184,039,369. It is known as Q8WXA8 in the human protein database and HTR3D on the HUGO database. Several isoforms are much shorter, for example, 404, 279, or 233 aa. No functional data have been reported for either of the putative subunits.

5-HT_{3E}

The 5-HT_{3E} receptor subunit gene codes for 456 amino acids in human also located on chromosome 3: 184,100,179-184,106,995. It is known as A5X5Y0 in the human protein database and HTR3E on the HUGO database. Several isoforms are similar or longer, for example, 441, 456, 471, 483, or 233 aa. No functional data have been reported for either of the putative subunits.

2-Methyl-5-hydroxytryptamine is a 5-HT_3 and 5-HT_6 receptor agonist. m-Chlorophenylbiguanide, SR 57227, and RS 56812 are potent and selective 5-HT_3 receptor agonists. Some of the 5-HT_3 receptor selective antagonists such as alosetron, bemesetron, dolasetron, granisetron, itasetron, ondansetron, Y25130, tropisetron, or zatosetron have reached clinical use/development for the prevention of chemotherapy-induced nausea and vomiting. Alosetron is indicated in the treatment of IBS (irritable bowel syndrome) with diarrhea, but are contraindicated in IBS with constipation and has a black box warning for that reason.

5-hydroxytryptamine receptors positively coupled to adenylyl cyclase, 5-$HT_{4,\ 5,\ 6,\ 7}$ receptors

Although 5-HT_4, 5-HT_6, and 5-HT_7 receptors all couple preferentially to G_s and promote cAMP formation, they are classified as distinct receptor families because each of them exhibits less than 40% overall sequence identity with any other 5-HT receptor. The 5-HT_6 receptor is structurally more closely related to the 5-HT_2 receptor family than to other 5-HT receptors.

5-HT_4 receptors

The 5-HT_4 receptor gene codes for about 387–388 amino acids in human, mouse, and rat. Depending on splice variant, of which there are many, and not always the same in different species, the length ranges from about 360–406 amino acids. It is known as Q13639 in the human protein database and HTR4 on the HUGO database. The human 5-HT_{2c} receptor is located on chromosome 5q31-q33. The 5-HT_4 receptor gene has a complex exon–intron structure, with a great number of splice variants (see below). The 5-HT_4 receptor was first characterized in the late 1980s by Bockaert and colleagues using a mouse and guinea pig brain, although its existence was suspected in rat neonatal colliculi 20 years ago [91,92]. Substituted benzamide derivatives such as cisapride, renzapride, or zacopride, which act as agonists at the "atypical" 5-HT receptor in mouse colliculi, were identified as 5-HT_4 receptor agonists. Interestingly, the potent 5-HT_3 receptor antagonist tropisetron was reported as the first competitive 5-HT_4 receptor antagonist. The 5-HT_4

receptor gene has a very complex structure: it has 14 exons which lead to multiple splice variants, of which at least 10 functional ones have been described so far for the human transcripts, most of which happen at the C terminal domain, but many more combinations are feasible [92,93]. Some of these splice variants show higher levels of constitutive activity whereas others may couple to alternate transduction mechanisms. Apart from adenylate cyclase stimulation, direct coupling to potassium channels and voltage-sensitive calcium channels has also been proposed as being postreceptor events. 5-HT_4 receptor activation triggers acetylcholine release in the guinea pig ileum and contracts the esophagus and colon. In addition to its modulatory function on gastrointestinal motility, the 5-HT_4 receptor is also involved in mediating secretory responses to serotonin in the intestinal mucosa. Electrogenic ion transport is stimulated through 5-HT_4 receptors in the small intestine. 5-HT_4 receptors in piglet heart mediate tachycardia (right atria) and positive inotropic effects (left atria). Similarly, isolated human atrial appendages respond with increased contractile force to 5-HT_4 receptor agonists. 5-HT_4 receptors are expressed in the central nervous system, where they appear to promote neurotransmitter release, enhance synaptic transmission, and favor memory. 5-HT_4 receptors are highly expressed in the basal ganglia (caudate nucleus, putamen, nucleus accumbens, globus pallidus, and substantia nigra), the hippocampal formation (CA1 and subiculum), and in the neocortex (superficial layers and lower levels in deep layers). 5-HT_4 receptors appear to be largely postsynaptic. However, presynaptic localization on terminals of GABAergic (dentate gyrus), dopaminergic, and serotonergic neurons is also likely, since the release of these neurotransmitters is modulated by 5-HT_4 agonists. Exposure of the receptor to agonists results in desensitization in most experimental in vitro 5-HT_4 receptor models which, in tissue preparations of the alimentary tract, is readily reversible upon agonist removal.

5-HT_4 receptor agonists, for example, cisapride, zacopride, BIMU 1, and BIMU 8, may display diverse receptor selectivities. BIMU 1, BIMU 8, and zacopride are also highly potent 5-HT_3 receptor ligands. Selective partial agonists at 5-HT_4 receptors are common: CJ 033466, ML103302, PRX-03140, RS 17017, RS67333, RS 67506, SL65.0155, tegaserod, and VRX-03011. Highly selective and potent 5-HT_4 receptor antagonists such as GR 113808, GR 125487, RS 67532, RS 100235, and SB 204070 have been described. A number of gastroprokinetic drugs share agonism at the 5-HT_4 receptor, which is a target in irritable bowel syndrome

(with constipation); however, cisapride, renzapride, mosapride, and tegaserod had to be stopped or withdrawn for various reasons. On other hand, compounds such as ATI7505 or TD-5108 which are full agonists in the GIT with little cardiac effects are still in development for the treatment of functional bowel disorders (see [94,95]). The 5-HT_4 receptor has central effects and plays a role in memory and depression [92]. Thus, PRX-03140 and SL65.0155 are in clinical trials for Alzheimer's disease. Derivatives of SB 207145 have been reported as PET ligands.

5-ht_5 receptors

5-ht_{5A} receptors

The 5-ht_{5A} receptor gene codes for 357 amino acids in human, mouse, and rat. It is known as P47898 in the human protein database and HTR5A on the HUGO database. The human 5-ht_{5A} receptor is located on chromosome 7q36.1 and has no splice variants.

5-ht_{5B} receptors

The 5-ht_{5B} receptor gene codes for 370 amino acids in mouse and rat, but it is not expressed in humans due to the presence of a stop codon. The human 5-ht_{5A} pseudogene is located on chromosome 2q14.1a. It is known as HTR5BP on the HUGO database.

The putative 5-ht_5 receptors are listed here as they may promote cAMP production, although this has been debated. Since functional evidence in native systems is still sparse, the receptor are described with lower case. Two subtypes of the 5-ht_5 receptor (5-ht_{5A} and 5-ht_{5B}), which share 70% overall sequence identity, have been found in rodents, whereas only the 5-ht_{5A} gene product has been reported in humans (the 5-ht_{5B} gene is a pseudogene, with a stop codon, there is no evidence for being transcribed). Binding was found in the olfactory bulb and medial habenula, with lower densities in the neocortex, hippocampus, and caudate putamen. There are only a few reports found concerning physiological responses. 5-ht_5 receptors may play a role as autoreceptors as described in rodents using the first selective 5-ht_5 receptor antagonist, SB-699551 [96]. A role in schizophrenia/psychosis was suggested in association studies as well as in preclinical studies using further selective 5-ht_5 receptor

antagonist. Finally, influence of 5-ht_{5A} receptors upon circadian rhythms has been suggested, since A843277 inhibited light-induced phase advances in hamster circadian wheel-running rhythms [97] and antipsychotic effects have been suggested with this compound. However, the putative 5-ht_5 receptor keeps its lowercase appellation, until more definitive data are made available.

SB-699551 and A843277 are highly potent and selective 5-ht_5 receptor antagonist; there are no selective agonists.

5-HT_6 receptors

The 5-HT_6 receptor gene codes for about 440, 440, and 436 amino acids in human, mouse, and rat, respectively. It is known as P50406 in the human protein database and HTR6 on the HUGO database. The human 5-HT_6 receptor is located on chromosome 1p36-p35. The 5-HT_6 receptor has no known splice variants. The recombinant receptor promotes intracellular accumulation of cAMP, and a receptor with similar operational characteristics is found in mouse neuroblastoma N18TG2 cells as determined in cAMP formation and binding studies using ^{125}I-labeled lysergic acid diethylamide (LSD). In addition, NCB-20 cells and rat striatal neurons in culture express a receptor that couples positively to adenylyl cyclase and displays an operational profile consistent with the recombinant 5-HT_6 receptor. 5-HT_6 receptor binding is prominent in the caudate putamen, nucleus accumbens, Islands of Calleja, olfactory tubercle, and choroid plexus, whereas moderate levels are present in the hippocampus, cerebral cortex, thalamus, hypothalamus, and substantia nigra and still lower levels are present in the globus pallidus, cerebellum, other mesencephalic regions, and the rhombencephalon.

[^{3}H]clozapine binds with nanomolar affinity to two distinct sites in the rat brain, one of which displays the operational profile of the recombinant 5-HT_6 receptor. A number of antidepressants and antipsychotics show intermediate to high affinity for 5-HT_6 receptors (clomipramine, amitriptyline, clozapine, olanzapine, fluperlapine, and seroquel). A selective 5-HT_6 receptor (Ro 04-6790) antagonist produces a behavioral syndrome involving an increase in acetylcholine neurotransmission. EMD 386088, E-6801, WAY 181187, and WAY 208466 are potent and selective

5-HT_6 receptor agonists. Potent and selective 5-HT6 receptor antagonists include Ro 04-6790, Ro630563, SB 258585, and SB 399886. Several 5-HT_6 receptor antagonists are in clinical development for cognitive dysfunction in Alzheimer's disease, for example, SB 742457, SAM-531, PRX-07034, SYN-114, and SUVN-502 and SGS-518 for schizophrenia. In addition, the 5-HT_6 receptor antagonists BVT 74316, PRX-07034, and SUVN-502 are also being developed for obesity.

5-HT_7 receptors

The 5-HT_7 receptor gene codes for about 479, 448, and 448 amino acids in human, rat, and mouse, respectively. It is known as P34969 in the human protein database and HTR7 on the HUGO database. The human 5-HT_7 receptor is located on chromosome 10q21-q24. The 5-HT_7 receptor has a number of splice variants which vary depending on species and has also been known as 5-HT_X and is most probably the equivalent of the 5-HT1-like receptor that was referred to in vascular tissues by Bradley et al. [10]. 5-HT_7 receptors are expressed in vascular and nonvascular smooth muscle and the central nervous system. Alternate splicing produces at least four functional variants of the 5-HT_7 receptor which are all functional but may vary across species. [^{3}H] 5-carboxamidotryptamine (5-CT) in the presence of (−)-cyanopindolol and sumatriptan labels 5-HT_7 recognition sites in guinea pig cerebral cortex membranes. Autoradiographic distribution is in agreement with that of 5-HT_7 receptor mRNA. High levels of 5-HT_7 binding are found in the anterior thalamus and hippocampus (dentate gyrus). Intermediate levels are present in septum and hypothalamus (suprachiasmatic nucleus), other hippocampal regions (CA1, CA2), anterior cingulate and other cerebral cortical areas, various amygdalar and brainstem nuclei and basal ganglia, and cerebellum (Purkinje cells). 5-HT_7 receptors equate with the originally described "5-HT_1-like" receptor mediating relaxation in the guinea pig isolated ileum and cat saphenous vein, also shown to mediate an increased concentration of cAMP and relaxation in neonatal porcine vena cava. Indeed, the 5-HT_7 receptor has a wide vascular distribution and is responsible for the prominent, persistent vasodilator response to 5-HT in anesthetized animals.

Ritanserin, LSD, and a number of ergolines such as metergoline, methysergide, or mesulergine have a high affinity for 5-HT_7 receptors. High-affinity 5-HT_7 receptor agonists have been reported more recently: AS 19, LP 44, and LP12. Potent 5-HT_7 receptor antagonists are SB656104, SB 258719, and SB 269970.

Orphan receptors

Several functional 5-HT receptors have been described for which corresponding gene products have yet to be identified, such as the putative 5-HT_{1P} receptor in the gastrointestinal system. One of these, the so-called "5-HT_1-like" receptor mediating direct vasorelaxation has been shown to correspond to the 5-HT_7 receptor, but the situation with the remaining orphan receptors (see [4]) has not evolved further and the status quo ante remains. Whether these functional "orphans" relate to single genes, yet to be discovered, species variations in pharmacology, or to the formation of heterodimeric receptors remains to be seen.

Summary

The existence of at least 15 different receptor subtypes and their potentially many more variants explains the wide-ranging features of 5-HT. To assign a specific and unique role to any single 5-HT receptor may be too simplistic and will largely depend on normal physiology or pathology. Indeed, it appears that, to various degrees, each serotonin receptor subtype is able to modulate its own signaling properties, condition cellular responses mediated by apparently independent receptor systems, or produce a spectrum of cellular effects via different signaling cascades, and these features may vary in health and disease. The molecular mechanisms underlying these behaviors are only beginning to be uncovered and there is still a long way to go. The richness and complexity of serotonergic modulation of (patho) physiological processes represent pharmacologically and chemically both great opportunities but also significant challenges. As selective ligands are being discovered, some of which enter clinical development, the

pathophysiological role(s) of a number of 5-HT receptors have been revealed. Thus, the involvement of specific 5-HT receptors in a given process provides an opportunity to pharmacologically target these specific receptors in a related disease state, for example, 5-$HT_{1B/1D}$ receptors in a migraine. However, since each individual 5-HT receptor may be involved in multiple processes certainly presents a challenge, even a selective drug may affect a variety of systems which may only become apparent in disease. Thus, 5-HT_{1B} agonists may target coronary vascular receptors in certain patients at risk, when these receptors are largely silent in a healthy population, and thus it is very difficult to characterize in normal animal models or healthy human tissues. Similarly, both 5-HT_3 antagonists and 5-HT_4 agonists have clinical use in IBS; however, IBS is not a stable condition, and certain patients will fluctuate between IBS with diarrhea (where 5-HT_4 agonism is contraindicated since it worsens the situation) and IBS with constipation (where 5-HT_3 antagonism is contraindicated as it is a constipating principle). For some 5-HT receptors, there is little evidence for a link to disease or even for a physiological function, that should not be surprising, and the 5-HT field is no exception. Some receptors clearly have arisen from gene duplication and may have "lost" function, as the system is probably 800 million years old. Other receptors have clear links to pathology, such 5-HT_{2B} which when stimulated will lead to valvulopathies or 5-HT_{2C} when inhibited may lead to significant weight gain as seen under some antipsychotic treatment. On the other hand, 5-HT_{1B} and 5-HT_{1F} receptor turned out to be good targets for the treatment of a migraine, whereas 5-HT_3 and 5-HT_4 have great potential in gastrointestinal disorders. On the other hand, 5-HT_6 and 5-HT_7 receptors are still at early stages. Complicating factors in drug discovery relate to biased signaling, splice or editing variants, heterodimerization, and the influence of multiple GIPs (GPCR interacting proteins) on the function of 5-HT receptors [98–100] in health and diseases and also depend on cell/tissue expression.

Acknowledgment

Sincere thanks to Jacquie Munro-Smith for editorial help and support.

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CHAPTER FIVE

A perspective: from the serotonin hypothesis to cognitive neuropsychological approaches

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Serotonin and depression

Depression is one of the most pervasive and debilitating conditions affecting over 300 million people worldwide. It is the world's leading cause of disability and a major contributor to the global burden of disease, thus underscoring the need to elucidate its pathophysiological processes and the development of effective treatment therapies to combat this mental illness [1]. For many decades, the serotonin (5-HT) system has played a central role in trying to navigate the pathophysiological processes and management of depression. At one stage, the serotonin hypothesis even achieved some notoriety akin to "conspiracy theory" status, accused of supposedly being misused by the pharmaceutical industry to market selective serotonin reuptake inhibitors (SSRIs) [2]. Nonetheless, increasingly it is becoming apparent that single neurotransmitter theories are unlikely to fully explain the many facets of depression including how the treatments targeting this disorder are working.

The serotonin hypothesis of clinical depression is now 50 years old [3]. In its simplest form, the hypothesis proposes that reduced serotonergic activity plays a causative role in the pathophysiology of depression. This idea was partly based on the finding that tricyclic antidepressants inhibited 5-HT and noradrenaline reuptake and thus presumably increased serotonergic activity in depressed individuals. The arrival of SSRIs further suggested that enhanced serotonergic function may be sufficient to ameliorate depressive symptoms in such affected individuals [4]. Of course, when the serotonin hypothesis of depression was first proposed, direct

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DOI: https://doi.org/10.1016/B978-0-12-813323-1.00005-0

study of the living human brain was still not feasible and as such one relied on indirect biochemical markers for evidence of serotonergic abnormalities with the most reliable findings including lowered uptake of serotonin by blood platelets [5] and diminished plasma levels of the serotonin precursor amino acid tryptophan [6].

The best evidence that serotonin plays a role in the pathophysiology of depression comes from tryptophan depletion studies, where a dietary restriction free of tryptophan leads to a lowering of plasma and brain tryptophan and thus a decrease in serotonergic function [7]. Thus, while it was shown that tryptophan depletion in healthy volunteers did not induce clinically significant changes in mood, tryptophan depletion in recovered depressed patients (free of medication) did produce a transient yet significant return of depressive symptomatology [8,9]. Thus, in some circumstances, reduced serotonergic activity may result in depressive symptomatology but this in itself is not sufficient nor essential. And, further, while diminished serotonergic function may be a factor in the return of depressive symptomatology in people recovering from depression, it does not suggest that it has a central effect in lowering mood in all high-risk individuals such as those with a strong family history of depression [9].

Neurochemical models

Certainly, one of the main reasons in linking serotonin to depression is the fact that antidepressants such as SSRIs have proven to be effective in a significant number of patients. This is further reflected in their adoption as first-line treatments in clinical guidelines in the management of major depression [10]. In addition to SSRIs, other classes of antidepressants are used in the treatment of depression such as the selective norepinephrine reuptake inhibitor (NRI) reboxetine, the norepinephrine, and dopamine reuptake inhibitor bupropion or serotonin and norepinephrine reuptake inhibitors such as venlafaxine. More recently, drugs have been developed, which in addition to blocking the reuptake of 5-HT, have the extra effect of targeting a variety of 5-HT receptor subtypes such as vilazodone with partial agonist activity at 5-HT_{1A} receptors [11]. Although sometimes doubt has been expressed about the efficacy of monoamine

potentiating drugs in depression, a recent meta-analysis of 21 licensed antidepressants in 522 randomized trials involving over 116,000 depressed patients showed unequivocally that antidepressant medications are more effective than placebo in the short-term management of depression [12]. Some of the SSRIs, notably paroxetine and escitalopram were among the most efficacious agents.

With all these antidepressants, a time lag in the appearance of an alleviation of depressive symptomatology is observed. This discrepancy between monoamine increases and the display of clinically significant changes thus led to the exploration of neuroadaptive changes following initiation of antidepressant treatment. The essential notion was that the neurobiological adaptive modifications, which correlate in time with the start of the therapeutic response could represent a more direct antidepressant target than the initial action of preventing norepinephrine and serotonin reuptake. This is especially exemplified in the case of SSRIs and the shift in focus to the role of the 5-HT_{1A} autoreceptors, whose function is to normally prevent the presynaptic release of serotonin. However, repeated administration of an SSRI in both humans and animals attenuated 5-HT_{1A} autoreceptor sensitivity leading to the suggestion that a delay in therapeutic onset of action of SSRIs could represent the time required for autoreceptor desensitization and thus resulting in enhanced serotonin concentration in the synapse [13]. Based on this, one would assume that combining drugs that selectively block 5-HT_{1A} autoreceptors and an SSRI ought to speed up the start of the SSRI therapeutic effect; however, this approach has so far not proved to be clinically beneficial [14].

Neuroplasticity models

Beyond monoamine neurotransmitter receptors, research has more recently explored mechanisms of neuroplasticity: fundamental processes that form the basis for learning and memory plus the capacity of neuronal systems to incorporate and adjust to environmental stimuli and then to create appropriate adaptive responses to related future stimuli. Neuroplasticity is mediated by complex mechanisms [15], which are vulnerable to chronic stress but which may be counteracted with chronic antidepressant treatment [16]. The effects caused by stress may be

counteracted by the chronic treatment with an NRI or SSRI thought to involve increased expression of brain derived neurotrophic factor (BDNF), a major actor that plays a significant role in the growth, differentiation, guidance, and survival of neurons during development as well as synaptic plasticity and survival in the adult brain [17,18]. Additionally, there is evidence that chronic SSRI administration enhances synaptic plasticity in animal models. For example, chronic fluoxetine administration can reinstate ocular dominance neuroplasticity even in adult rats as well as increasing fear extinction training in mice [19,20]. Rapid induction of synaptic plasticity has also been proposed as a mechanism by which the NMDA antagonist ketamine exerts rapid antidepressant effects in treatment resistant depressed patients [21] though direct evidence for this in the human brain is difficult to test.

Cognitive neuropsychological models

In addition to the molecular and cellular pathways described, the effects of serotonergic manipulation on core psychological processes have also become a growing interest of study. As previously discussed, acute tryptophan depletion can cause a transient re-emergence of depressive symptomatology in recovered depressed patients. In this context, and on a neuropsychological level, an acute low-dose dietary depletion of tryptophan has been shown to induce changes in cognitive function and emotional processing, demonstrating reduced recognition of happy facial expressions in recovered patients but not in healthy controls in the absence of a change in subjective mood [22]. Further, on a neural level, this type of serotonergic modulation on facial expression processing was shown to vary with emotion type and intensity [23].

Stressful life events have a propensity to increase the incidence of depression [24] and individual disparities in the perception, experience, and remembering of negative events can worsen these effects. Individuals with depression have the tendency of perceiving social cues more negatively, are more prone to attend to negative stimuli and preferentially recall negative rather than positive information about themselves [25,26]. Focusing on and recalling negative social and affective information and discounting positive information is thought to reinforce negative beliefs, thoughts, and feelings observed in depression.

Negative affective biases during remission are thought to be associated with a greater risk of relapse [25] and enhanced positive emotional processing has been seen to precede changes in depressive symptomatology [27]. These findings demonstrate that negative bias may not be just a by-product of low mood but actually play a part in determining response to everyday emotional and social situations, stressors, and life events as well as the evolution of depressive symptomatology. Moreover, studies have now highlighted negative bias as a notable target for psychological and pharmacological treatments in clinical depression [26,28,29].

Antidepressant drug administration has been shown, very early on in treatment to increase positive affective processing in both healthy volunteers and depressed patients [28]. For example, a single dose of reboxetine enhanced the recognition of positive facial expressions and the recall of positive self-referent memory in depressed patients compared to those receiving placebo and despite an absence of changes in subjective mood ratings [30]. Further, single, as well as repeated, antidepressant administration from a variety of pharmacological classes including SSRIs such as citalopram in heathy volunteers has been shown to enhance the recognition of positive versus negative social cues in a facial expression recognition task [28,31]. Early effects of antidepressants on negative affective bias may act to attenuate the influence of this central maintaining factor and set the stage for an amelioration of symptoms with time [32,33]. In addition to pharmacotherapy, early modifications in affective processing have been observed following treatment with other modalities such as negative ion treatment [34], transcranial direct current stimulation [35] and cognitive behavioral therapy in panic disorder [36]. Interestingly, vagal nerve stimulation in patients with resistant depression might also selectively inhibit memory for negative emotional information [37].

From neuroimaging studies, depression has been shown to be associated with an enhanced response in limbic areas to negative stimuli, significant for the detection and response to emotionally salient stimuli, and, further, this has been coupled with an attenuated engagement of areas essential in the regulation and inhibition of these responses such as the dorsolateral and medial prefrontal cortex (PFC) [29]. Treatment with antidepressants reverses this neural response pattern to affective stimuli in patients with depression as well as demonstrating a similar trend in healthy volunteers [38]. This has been shown, for instance, with acute clinical doses of SSRIs decreasing the response of the amygdala to negative affective faces [39,40] with this effect also observed following 7 days of

administration in depressed patients [41] and healthy volunteers [42]. Notably, these effects occur despite an absence of any significant changes in depressive symptomatology suggesting that they may in fact be an early mechanism of change. Importantly, these early changes in affective processing are maintained with long-term treatment as well such as in a cohort of depressed patients with a 6-week SSRI treatment course, in which they displayed attenuated responses in the anterior cingulate, amygdala, and fusiform face area to negative facial expressions, whilst demonstrating enhanced responses to happy faces [38,43].

Are early changes in affective processing predictive of clinical response over time? Indeed, it has been demonstrated that early change in the perception and neural response to positive facial expressions has been accompanied with an ensuing improvement in depression severity [32,33,44]. Further, one classification based approach of data proposed that if an early change in positive processing is not observed with antidepressant treatment, patients would be unlikely to respond to this same treatment at a later date [32]. Similarly, in a group of older patients with depression on citalopram, a lack of improvement in the recognition of positive faces at 1 week was subsequently followed by a lack of clinical response after 8 weeks of treatment [33]. An early change in neural response to emotional stimuli has also been associated with a later clinical outcome as demonstrated with the clinical response to escitalopram after 6 weeks of treatment following an early change during affective processing in the thalamus, insula, cingulate, and amygdala [44]. Overall, all these findings challenge the perception that antidepressants do not exert clinically relevant effects until they have been given for a period of several weeks and suggest that there are swift changes in nonconscious mechanisms involved in how stressors and interactions with others are processed and recalled.

Why do the clinical effects of pharmacotherapy experience a delay especially in light of antidepressants having rapid effects on emotional processing? It may be argued that such nonconscious changes only become apparent to the individual after they become aware of the products of having a more positive bias, that is, through experience of exposure to a stressor. In line with this, experimentally inducing a negative affective bias in healthy participants does not directly affect subjective state but does impair the mood response following exposure to a stressor [45]. The role of negative bias in mood response is demonstrated by a positive correlation between the effects of a course of SSRIs on negative affective bias and resistance to a negative mood induction in healthy individuals [46].

Translating a change of negative bias into a clinical response might thus involve relearning a series of emotional associations, that is, where ambiguous cues are perceived more positively while taking a course of antidepressant medication.

The observation that rapid antidepressant effects can be induced by drugs like ketamine in treatment resistant depressed patients suggests that this delay is not always inevitable for treatment success. The neuropsychological mechanisms underpinning fast acting antidepressant action are not known but preclinical data suggest that while SSRI treatments require new information to be processed to detect changes in negative bias, drugs like ketamine can modulate existing negative biases and memories [47]. Such a difference could lead to a fast onset of action since environmental input becomes less critical. However, this interesting hypothesis still needs to be tested in humans.

Conclusion

Serotonin has long been implicated in depression and mechanisms of antidepressant drug treatment. However, there are a number of complexities in this picture, such as the lack of depressive symptom induction following tryptophan depletion in healthy individuals and the delay in symptom remission with SSRI treatment. A role for changes in learning, memory and plasticity has been provided by work in animal models. Human neuropsychological studies suggest that antidepressants have early effects on emotional processing, which may affect vulnerability and maintenance to symptoms rather than negative affect directly. The challenge lies in translating this information into the clinical context including early prediction of treatment response and as a framework for rational drug development and screening.

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CHAPTER SIX

Endocrine and genetic moderation of serotonin systems and the psychopathology of affective disorders

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The development of our ability to identify errors in an individual's genetic code brought the not unreasonable hope that psychiatric disorders could be both explained and ultimately cured by the identification of errors in the translation or transcription of the genetic code that would advertise the molecular target affected and thence reveal novel targets for drug discovery so that the psychopharmacological revolution in the approach to psychiatric treatment could continue at the rate first seen in the1950s when serendipity ruled drug discovery strategies. It quickly became apparent that the idea of a single gene deletion being responsible for a disorder as heterogeneous as major depression, schizophrenia, or autism was highly unlikely. Geneticists did not quell the nature or nurture debate. Indeed they added to it by demonstrating the power of life experiences to effectively modulate gene expression in a heritable fashion [1]. There are many lessons from other clinical disciplines that urge caution. For example, genetic deletion of the gene for the NaV1.7 ion channel leads to loss of painful sensation [2], so immediately began the search for small molecule inhibitors of the ion channel with clinical validation assured by the geneticists. Such compounds would be effective analgesics. It did not take long for medicinal chemists to reach their goal, but, sadly these compounds were devoid of analgesic properties. But pain sensitivity could be restored in those with the channelopathy by treatment with the opioid receptor antagonist naloxone. Then it was revealed that in animals and in man NaVav1.7 gene deletion led to the upregulation of endorphin peptide

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DOI: https://doi.org/10.1016/B978-0-12-813323-1.00006-2

expression: loss of pain sensitivity was thus explained not by gene *loss per se* but by the pleiotropic response to gene loss [3]. The body is more devious than the man who thinks he can control the body.

The serotonin transporter is the drug target for most current antidepressant drugs. The gene for the transporter, SLC6A4, has been cloned and found to be identical across species. Although now strongly questioned by much larger study cohorts, evidence from twin studies suggested that stressful life events vary in their ability to induce depression. Stressful life events precede the onset of major depression more frequently than expected by chance but individuals display wide variation in susceptibility with some showing great resilience in response to even severe stress. In both adolescent and adult twins, stressful life events clearly influence sensitivity to depression. Caspi et al. [4] first reported that a functional polymorphism in the promoter of the serotonin transporter moderated the influence of stressful life events, finding that those with 1 or 2 short alleles SS were more sensitive than those with long alleles LL. Also females were more sensitive than males. Susceptibility markedly increases with the level of threat. The greatest risk occurs within 3 months of exposure to threat in susceptible individuals. Interestingly, in healthy individuals, transporter polymorphisms mediate inhibitory control as measured by the stop signal reaction time [5]. Some but not all studies suggest that the S allele is associated with risk for major depression. Carriers have a 2−3 fold increase in transcription rate compared to the L allele. A study by Gorwood et al. [6] addressed a possible link with alcohol use disorder [7] and found that the S allele was not related to the incidence of alcoholism but was associated with risk for suicide attempts [8]. A metaanalysis of studies of the serotonin transporter allowed the analysis of several thousand subjects. A significant association between the incidence of major depressive disorder with a frequency of the S or L allele was found with a higher frequency for the short allele with 21% of depressed subjects compared with 17% of controls being homozygous for the S allele ($P < .05$) [9]. Similar results were obtained for bipolar disorder [9]. The relationship is statistically very strong in some cohorts. As with all psychiatric disorders, there will always be confounds related to diagnosis, gender, and ethnicity. Recent evidence indicates that transient currents of serotonin release impact prediction errors in man [10]. It would be extremely interesting to determine the effect of transporter polymorphisms on the temporal availability of serotonin in such behavioral situations. Neuroimaging approaches have allowed the assessment of how the transporter polymorphisms influence the engagement of specific neural systems

which may be more reliably measured than behavioral syndromes using event-related fMRI. Five independent functional imaging studies have reported a similar association between amygdala hyper-reactivity and the S allele carriers in response to angry or fearful facial expressions in healthy volunteers [11] and in patients with social phobia [11]. Such quantitatively robust relationships obtained with data from a total of only 14 subjects. It clearly demonstrates the statistical power added by a more precise definition of the hypothesis being tested and allowed by knowledge of the circuitry underlying the pathological behavior. However, more conventional analysis on a large sample size has also revealed an impact of the transporter polymorphism on morphometric variables using voxel-based morphometric analysis of structural MRI data [11]. In a sample of 114 healthy adults, S allele carriers had significantly reduced gray matter volume of the perigenual anterior cingulate cortex and amygdala. Independent of polymorphism status, the amygdala and subgenual anterior cingulate cortex are significantly connected as indicated by significant covariance between regional volumes of the amygdala that covaries with that of the perigenual anterior cingulate cortex. When observations were restricted to S allele carriers, the coherence btween the two regions was heightened, but the reactivity of the amygdala was ignificantly reduced compared to L-allele carriers. The authors suggest that this reduced coherence could underlie the heightened reactivity of the amygdala during the processing of negative stimuli, that is, the distinct zones of connectivity possibly reflect a feedback loop on amygdaloid activity. The activity level may be developmentally determined has been shown by exposure of neonatal mice to acute exposure to the selective serotonin reuptake inhibitor (SSRI) fluoxetine in comparison [12] with adult mice carrying the S allele of the transporter which leads to reduced transporter expression in adulthood. Drug treatments were administered from P4 until P21 days of age. At 9 weeks postnatal age mice were tested for exploratory activity in an open field and elevated plus maze which was reduced in drug treated mice, as it was in the genetically modified mice. In a shock avoidance paradigm in which both experimental groups were deficient. Overall the findings suggest that interference with 5-HT function in early life predisposes the organism to heightened emotionality in adulthood and maladaptive responses to stress [12]. However, in another behavioral analysis using transgenic animals overexpressing the transporter studying responses to both rewarding and aversive cues: the insertion or deletion of a repetitive sequence located in the proxima 5' regulatory region of the gene promoter gives rise to long and short alleles, respectively. As a consequence

the L allele displays greater transporter expression than the S allele. Overexpressing mice showed reduced sensitivity to both positive and negative reinforcers [13]. The field has become yet more complicated since more detailed analysis of the transporter gene has found additional polymorphisms [14] with 14, 15, 16, 19 and 22 repeat elements. In total 10 previously unknown single nucleotide polymorphism have been identified, additionally, there are significant ethnic differences in allelic frequency between Japanese and Caucasian subjects [14] the impact that the discovery of S and L alleles had. Much needs to be done to first identify the functional consequences for serotonergic neurotransmission of each of the polymorphisms before their relative importance for psychiatric disorders can start to be investigated. This is in addition to the potential moderation of genetic effects by environmental impact. Heritability estimates range from 30% to 40% and yet the two largest genomewide studies of major depressive disorder incorporating 3,000 subjects, the loci identified had only small effects with odds ratios <1. The failure to detect robust effects of environmental impact linked to the presence of the polymorphisms was specifically analyzed in a metaanalysis, looking specifically for the impact of childhood trauma [15]. Data from nine cohorts give a sample size of 3,024 depressed and 2,741 control subjects. A polygenic risk score with73,576 single nucleotide polymorphisms was poorly correlated with depression. Childhood trauma was defined as self-reported history of physical or sexual abuse. Overall, there was no evidence of a gene $\times$ environment interaction [16]. Other serotonin receptors have been investigated for their potential involvement in psychiatric genetics reaction time [5].

Given the strong link between stress and depressive disorders, it is important to determine the role, if any, that serotonergic systems might play in the control of the endocrine function. An actual or perceived threat activates numerous counter regulatory processes, primarily in the hypothalamic-pituitary HPA [17] axis. This cascade culminates in the synthesis and secretion of adrenal steroids. Signals conveying the threat are relayed to the mediobasal hypothalamus which elicits the release of multiple adrenocorticotropin (ACTH) secretagogues into the hypophysical portal circulation, one of which is corticotropin-releasing factor (CRF) elaborated by parvocellular perikarya in the paraventricular nucleus. Glucocorticoids exert many effects on metabolism, inflammation, and immunity. The HPA is self-regulating mediated by the cytosolic Type 1 mineralocorticoid receptors expressed in septal and hippocampal regions and by Type II glucocorticoid receptors in the anterior pituitary gland.

Multiple aspects of the HPA appear to be dysregulated in major depressive disorder [17]. This is reflected in basal circulating cortisol, pituitary gland enlargement, urinary or salivary cortisol, adrenal gland hyperplasia and hypercortisolemia in patients with major depression. Cortisol secretion is subject to feedback inhibition. In order to test whether defective feedback inhibition is responsible for hypercortisolemia, dexamethasone and CRH have been used in depressed patients in order to estimate the efficacy of feedback inhibition. In the treatment response to serotonin reuptake inhibitors (SSIs), that is, enhancing serotonergic function not only decreases the symptoms of depression, it also normalizes impaired HPA signaling. The combination of dexamethasone with CRH is thought to maximize the sensitivity of detection of an elevated endocrine response in depressed individuals. This has led to the proposal that the ability of known or novel antidepressants can be used to [18] predict antidepressant activity suggesting that normalization of HPA regulation is central to the mechanism of action of serotonergic antidepressants. Interestingly, another mechanism thought to be central to antidepressant action is hippocampal neurogenesis. Using transgenic mice, Brandon and McCay [5,7] have demonstrated that serotonergic antidepressants stimulate neurogenesis via activation of the 5-HT1A receptor [19,20]. 5-HT1A receptors somewhat paradoxically given the antidepressant effects of selective 5-HT1Areceptor agonists or partial agonists also stimulate the release of CRH and ACTH in the rat [21]. Activation of 5-HT1A receptors also stimulates the release of the neuropeptide oxytocin [22]. Interestingly, in healthy controls, oxytocin decreases cortisol release and reduces anxiety in response to social stress, and also reduces amygdala reactivity to threatening fearful images [23]. It may also be significant that vagal stimulation, an approved treatment for treatment-resistant depression [24] also releases oxytocin in the rat [25]. A major function of oxytocin is the stimulation of milk let down in nursing mothers. There is evidence that there is a serotonergic modulation of prolactin release. There is a simultaneous afternoon increase of serotonin and prolactin in brain and blood. Some psychedelic drugs stimulate prolactin release and ecstasy stimulates the release of both oxytocin and prolactin [26,27]. However, overall, serotonin, unlike dopamine, does not have any major role in the control of prolactin release [26]. This chapter discusses these aspects.

Genetics A promoter polymorphism in the human monoamine oxidase A gene responsible for the metabolism of 5-HT to 5-HTIAA has been associated with altered transcriptional activity and heightened levels

of aggression and impulsivity [28]. Similarly a polymorphism in the human gene for aromatic amino acid decarboxylase has been associated with bipolar disorder [29]. While a polymorphism in the tryptophan hydroxylase gene is associated with increased risk for suicide, impulsivity aggression, and alcoholism, an SNP in the upstream regulatory region of the tryptophan hydroxylase isozyme TPH2 is associated with increased amygdala reactivity [30] to facial expressions. Much attention has also been placed on the 5-HT2A receptor. The high clinical efficacy and atypical antipsychotic profile of clozapine have spurred many companies to develop selective 5-HT2A receptor antagonists as putative clozapine-like antipsychotics with little clinical success. The lesson of these findings is not to put weight on any gene/behavior association study unless the number of subjects is in the multiple thousands. The ramifications for serotonin research are major, particularly in the realm of the development of animal models of depression where exposure to stress usually plays a central role. Indeed, instead of searching for genes influencing depression symptoms, perhaps the whole investigation should be turned through 360 degrees [15] and we should be looking for genes that convey resilience to trauma. Atypicality with respect to antipsychotics is here defined as a relative inability to induce extrapyramidal side effects thought to reflect the blockade of striatal dopamine D2 receptors. However, clozapine also has appreciable D2 antagonist affinity as well as an affinity for other 5-HT receptor subtypes and α2-adrenoceptor activity [31].A number of possible explanations have been proposed to explain clozapine's atypicality ranging from being a function its low receptor off rate of given its moderate D2 receptor affinity compared to those with very high affinity, for example, haloperidol, to a differential disposition within the striatal system. One hypothesis that has been carefully tested is that clozapine might be self-inhibiting its cataleptogenic response. Using the regional stereoisomers of clozapine, loxapine and isoclozapine have been found to be cataleptogenic and able to block catalepsy, respectively [31]. Unlike clozapine, loxapine induces catalepsy in the rat which can be blocked by clozapine. Initial thoughts were that the high affinity of clozapine for 5-HT2A receptors was responsible for the high clinical efficacy and low propensity to induce extrapyramidal side effects. This hypothesis led to the search for highly selective 5-HT2A receptor antagonists in the hope that they would more closely resemble clozapine. Indeed the prototypical selective 5-HT2 receptor antagonist M100907 was found to possess some preclinical similarities to clozapine [32]. However, clinical trials did not reveal

clozapine-like antipsychotic efficacy, though some benefit on negative symptoms was detected 5-HT2A receptor antagonists have not yet made an impact on the management of psychiatric disorders [33,34].

As indicated in Chapter 11, the hallucinogenic effects of lysergic acid diethylamide are likely mediated by 5-HT2A receptors. However, LSD as a model psychosis has been largely dismissed mainly because the nature of drug-induced hallucinations differs markedly from those experienced by schizophrenics. Others have searched for an association between 5-HT2A polymorphisms and schizophrenia as well as in association with response to clozapine [35]. The 5-HT2A receptor is most heavily expressed in cortical brain areas most importantly in the frontal lobes. Fortunately, there are a number of high-affinity ligands available that allow in vivo assessment in pathological states ^{11}C-3-methyl-spiperone and ^{18}Fsetoperone were the initial radio ligands used to investigate quantitative levels of 2A receptor expression in schizophrenia [36], autism spectrum disorders as well as Parkinson's and Alzheimer's diseases. Inayama [36] first identified a positive association between the 5-HT2A gene and schizophrenia. Subsequently single nucleotide polymorphisms T102C, his452tyr, and 1438G/A have been implicated in different aspects of the disease. Unfortunately, though further studies failed to replicate these findings with meta-analyses of association studies concluding either no effect or minor effects with odds ratios no greater than 1.18 [34,37]. More promising have been studies in Parkinson's disease. Pimavanserin is a highly selective 5-HT2A receptor inverse agonist devoid of any affinity for dopamine D2 receptors. In randomized placebo-controlled clinical trials pimavanserin was without effect on psychosis in schizophrenic patients [37] but significant improvement in psychosis measures was seen in Parkinson's patients with improvements also in sleep parameters in healthy volunteers [38].

5-HT2A receptors are also notable for their expression within the apical dendrites of cortical pyramidal cells. This postsynaptic localization is consistent with5-HT having direct depolarizing actions sensitive to antagonism by selective 5-HT2AA receptor antagonists. Interestingly psychotomimetic N-methyl-D-aspartic acid antagonists increase glutamate release in the cerebral cortex. Layer V pyramidal cells are excited by 5-HT, blocked by the AMPA/kainite receptor antagonist LY293558 and also by the 5-HT2A receptor antagonist M100907. Group II/III metabotropic glutamate receptor agonists are able to suppress 5-HT2A induced EPSCs and may, therefore, have therapeutic potential as antipsychotics [39]. In two

clinical trials, LY2140023 and its methionine prodrug LY4044039, LY2140023 demonstrated significant improvement in positive and negative symptom scales compared with placebo. However, the effect size was not deemed sufficient to continue development. Genetic factors that might have modulated the results were accordingly sought. A relatively small number of candidate genes were investigated, including GRM2 and GRM3 COMT, dopamine D2, D3, and 5-HT2A and the latter genes showed a significant association between PANNS total score and presence of the single nucleotide polymorphism rs3125 and rs7330461 of the 5-HT2A receptor. Indeed 16 of the 23 SNPs associated with treatment response were in the 5-HT2A locus and a 30 point reduction in PANSS was seen in in the most responsive group for snpr7330461, three times greater than the reduction in the least responsive group [40]. All 5-HT2A receptor agonists induce a head shake response in the mouse. The ability of mGlu2/3 receptor agonists to inhibit the head shake response induced by LSD [41]. It could be reasonably questioned whether the head twitch response bears any meaningful relationship to the hallucinogenic effects of LSD. More convincing would have been a demonstration of the ability to block the interoceptive properties of both LSD and phencyclidine in drug discrimination paradigms, but I could find no evidence that this has been tried, although there is no doubt mGLu2/3 agonist are able to block the haemodynamic response to ketamine [42]. It is unfortunate that Eli Lilly the Pharma company having invested most in glutamate therapeutics have chosen to withdraw from psychiatric drug discovery. The clinical results may not have indicated the identification of the next clozapine. But there is surely enough positive evidence to continue exploring the mechanism, especially since clinically useable mGlu2/3 antagonists are available: might they not also be reasonably capable of exacerbating psychotic symptoms adding weight to the target validation? In schizophrenics the functional consequences of the 5-HT2A polymorphisms need to be urgently addressed lest we lose knowledge already gained and a potential new and beneficial therapeutic intervention will be discarded and never reach patients.

In a study by Holck et al. [43], plasma serotonin levels were examined in depressed subjects who were then treated with the SSRI sertraline for 8-weeks after which plasma serotonin was again assessed. Subjects were defined as responders or nonresponders if they achieved a 50% improvement in the Hamilton Depression rating scale. Responders had significantly lower pretreatment serotonin levels compared to healthy controls

and responders. There was a significant decrease in serotonin levels over treatment in all depressed subjects which was significantly more prominent in responders compared to nonresponders. The authors hypothesize that SSRI response is facilitated by adequate baseline serotonin content and it seems that successful treatment is associated with a decrease in plasma serotonin content. Tryptophan content was also monitored but no significant changes were noted. Dogma has largely dismissed the possibility that peripheral serotonin metabolism reflects neuronal function, perhaps in the light of these findings.

The potential biomarker utility of plasma serotonin determinations should not be so readily dismissed. In a similar vein, not to forget that platelet serotonin transporter function can predict default mode network activity [44]. Indeed if carriers if the transporter short allele have a relative deficiency in transporter expression might this be corrected by infusion of the subjects own platelets and reduce sensitivity to early life trauma.

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CHAPTER SEVEN

Serotonin and sexual behavior

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The last decades' fast developments in the serotonergic field made evident that the serotonin (5-hydroxytryptamine (5-HT)) system in the central nervous system (CNS) is extremely important in all areas where subtle modulation is needed in regulatory processes including mood, anxiety, appetite, aggression, cognition, and sexual behavior [1].

In mammals, 14 5-HT receptors can be divided into seven families, 5-HT_{1-7} [2]. Except for 5-HT_3 receptors, which are ligand-gated ion channels, all serotonergic receptors are 7-transmembrane receptors acting via G-proteins. Moreover, serotonergic neurons possess 5-HT transporters (5-HTT) that actively transport 5-HT back into the neuron after release. The serotonergic system in the CNS consists of various nuclei, in which 5-HT neurons send, via neuronal pathways, information to practically all areas of the CNS and spinal cord. Two raphe nuclei, the dorsal (DRN) and median nucleus (MRN), are prominent in supplying projections to the forebrain, whereas hindbrain and spinal cord are mainly innervated by the caudal raphe nuclei, including magnus, obscurus, and pallidus nuclei [3,4]. Projections from the raphe nuclei play an important role in the modulation of sexual behavior, in addition to other serotonergic areas such as periaqueductal gray and nucleus paragigantocellularis (nPGI) [5,6].

The structure and anatomical localization of the 5-HT system suggest a global, modulatory function. The question can be posed whether 5-HT

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The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00007-4

exerts specific roles, or whether 5-HT operates by having effects on virtually all CNS processes, thus having no specific behavioral and physiological function [7]. Jacobs and Fornall hypothesize that the primary role of the 5-HT system provides coordination of motor, autonomic, and sensory processes, activated with central motor commands and all other behavioral and physiological functions associated with CNS-5-HT are derivatives of this functional relationship [7]. The 14 different 5-HT receptors are widely distributed throughout the CNS and mainly mediate the slow modulatory activity of 5-HT upon fast excitatory (glutamate/aspartate) or fast inhibitory (GABA/glycine) effects, thus influencing all executive functions, aggression, affective behaviors, sexual behavior, etc.

In the sections on serotonin on male and female sexual behavior, some general findings on the role of 5-HT on male and female sexual behavior will be outlined. Most preclinical studies have been performed in rodents (rat and mouse). If possible human data will be discussed.

Sexual behavior system(s) seem to operate under constant inhibitory control to ascertain that sexual behavior is performed only under appropriate conditions. Serotonergic neurotransmission is involved in inhibitory and disinhibitory processes regulating proper sexual behavior. Importantly, 5-HT release is regulated via negative feedback mechanisms, through different presynaptic (5-HT_{1A}, 5-$HT_{1B/1D}$) autoreceptors. Moreover, also postsynaptic serotonergic heteroreceptors are involved in negative feedback on serotonergic cell firing [8]. An important mechanism in maintaining 5-HT levels is the 5-HTT, playing a critical role in homeostatic modulation of the magnitude, duration, and spatial distribution of signals reaching 5-HT receptors [1,9]. Although 5-HT is not considered a central mediator of sexual behavior, but rather modulatory or facilitating, 5-HT activity plays an important role during sexual behaviors, via its machinery of pre- and postsynaptic interactions, thereby critically interfering with GABA-ergic and glutamatergic neurons in many brain areas (prefrontal cortex, hypothalamus, lateral habenula, and DRN). Serotonergic fibers are abundant in many areas of the spinal cord that have been implicated in ejaculatory processes [10]. Postsynaptic serotonergic receptors are, for example, located at lumbar spinothalamic cells [5], suggesting a role of 5-HT in ejaculation at the level of the spinal cord, descending from supraspinal areas like the nPGI. These descending 5-HTergic neurons from supraspinal areas innervate spinal cord mechanisms that control bulbospongiosus muscles, which have inhibitory effects on ejaculation [5]. At hypothalamic level, the medial preoptic area (mPOA) is involved in lowering an ejaculatory threshold via inhibition of an inhibitory serotonergic

tone exerted by the nPGI [11,12], removing a brake on ejaculatory processes. The lateral hypothalamic area (LHA) is also involved in ejaculation; lesions affect ejaculation, but not the preceding mounts and intromissions [13]. Because 5-HT is released in the LHA at the occurrence of ejaculation and infusion of selective serotonin reuptake inhibitors (SSRIs) into this area influences sexual behavior [14], a role of 5-HT is clearly implicated.

The main sources of the serotonergic innervation of the forebrain come from the DRN and MRN. Ascending serotonergic fibers may be divided into a mesolimbic pathway originating in the MRN and a mesostriatal pathway derived from the DRN [10,15,16]. Although the DRN and MRN have also overlapping projections, these do not overlap in the projected structure but go to different subareas [16]. MRN and DRN have reciprocal connections and both structures express high densities of 5-HT_{1A} receptors; however, the main, but unanswered, question is whether and how these extremely complex interactions (and not only between serotonergic structures) interact during the performance of sexual behavior [17,18]. Most research into these areas has been performed in males (mostly rodents) whereas in females data are largely lacking. In how far (serotonergic) systems in the brain involved in sexual processes are (dis) similar in males and females is unknown, but clearly needs experimental studies.

Serotonin and female sexual behavior

Several review articles have nicely and extensively reviewed the role of 5-HT in female sexual behavior [17,18]. Although inconsistencies are present, most evidence supports the hypothesis that increased serotonergic activity is inhibitory whereas reduced 5-HT activity is associated with enhanced female sexual activity. Early studies used rather crude methods to influence serotonergic systems, like administration of reserpine, tetrabenazine, or para-chlorophenylalanine, which reduce monoamine levels, including 5-HT. Such treatments increased lordosis in female rats while treatments that increased serotonergic activity reduced the lordosis reflex [19]. It should be realized that estrogen/progesterone activity plays an important role in female sexual behavior (at least in rodents) and a large interaction occurs between serotonergic action and hormonal conditions

[17]. Apart from this, the emerging picture of a differential serotonergic profile of multiple (14) 5-HT receptors, a 5-HTT, and a complicated serotonergic modulation with pre- and postsynaptic functions makes the notion of a general brake of 5-HT on sexual behavior and more specifically on lordosis, disputable. The abundance of studies on 5-HT and sexual behavior has mainly focused on lordosis, a single reflexive posture [20,21], but female sexual behavior consists of a repertoire of various behaviors [22] often described as appetitive (proceptive behaviors such as hopping, darting, and earwigging), and consummatory behaviors (receptivity and lordosis). Although large differences occur between species in the specific details of female sexual behavior, there is a considerable body of evidence that (serotonergic) mechanisms in the rodent brain have high similarity to the human brain [23,24]. Unfortunately, there is a serious lack of research in female rodents on translational models of human female sexual behavior (dis)functions, for example, hypoactive sexual desire disorder (HSDD) or those induced by psychoactive drugs, like antidepressants [25]. Because antidepressants, in particular SSRIs, enhance acutely and chronically 5-HT levels in the brain, a reduction in female sexual behavior can be expected. Uphouse [17] gave an extensive overview of various manipulations that affect serotonergic neurotransmission in rats, including acute and chronic administration of SSRIs. Measuring lordosis only does not reflect properly the behavioral effects of SSRIs. Snoeren et al. [26] show that acute and chronic paroxetine treatment does not affect sexual behavior in hormonal fully primed female rats, neither in proceptive nor receptive behaviors. There are, however, conflicting results [17], partly depending on the method and SSRI used. It is remarkable that at doses of paroxetine inhibiting sexual behavior in male rats [27], female rats in full estrus do not show any inhibiting effect [26]. The availability of rats lacking the 5-HTT provided a possibility to further study the effects of the 5-HTT on male and female sexual behavior. In fully primed females, homozygous and heterozygous 5-HTT knockout (5-HTT-KO) rats did not differ in any way from wild-type (WT) rats. In contrast, homozygous, but not heterozygous 5-HTT-KO males showed lower sexual behavior than WTs. Of note, 5-HTT-KO rats have enhanced extracellular 5-HT levels, which did not influence female sexual behavior but did so in male rats [28,29]. These results tentatively suggest that enhancing extracellular 5-HT levels in the brain by such a diffuse activation of SSRIs or lack of 5-HTT, leads to a differential effect in females and males. This contrasts with the human experience, where

SSRIs have sexual inhibitory side effects in both sexes. More research is needed on these discrepancies, but hormonal (steroid) differences between males and females probably offer an explanation for the differences.

SSRIs induce a general increase in serotonergic neurotransmission and consequently, each separate serotonergic receptor is stimulated. Prior to the discovery of this diversity (before the 1980s), several serotonergic drugs were used to unravel the role of 5-HT in female sexual behavior (e.g., methiothepin, cyproheptadine, and methysergide) but with mixed results [17]. The emergence of receptor-specific drugs made it possible to study the specific role of individual receptors in female sexual behavior and in particular ligands for 5-$HT_{1A,1B}$ and 5-$HT_{2A/2C}$ receptors have been used most frequently. 5-HT_{1A}-receptor agonists like 8-OH-DPAT and the azapirones (buspirone, ipsapirone, and gepirone) have been tested in female sexual behavior, mostly on lordosis [10,17]. The effects of the azapirones are somewhat puzzling, as both increases and decreases in lordosis have been described [17]. The azapirones are partial 5-HT_{1A}-receptor agonists, while 8-OH-DPAT is a full agonist, and it is therefore possible that the increases in lordosis seen at low doses of the azapirones are due to an antagonistic-like effect. This is supported by data on the 5-HT_{1A}-receptor antagonist WAY100,635 that exerts stimulating effects on lordosis [17]. Snoeren et al. [26] found in a pace-mating situation that 8-OH-DPAT not only reduced lordosis, but also proceptive behavior and time spent in the male compartment which could be antagonized by WAY100,635. Intracranial infusion studies [17] strongly suggest that this inhibitory effect of 5-HT_{1A}-receptor stimulation is mediated via postsynaptic 5-HT_{1A} receptors. Although there is evidence that 8-OH-DPAT might exert its inhibitory effects, next to activation of postsynaptic 5-HT_{1A} receptors, also by stimulating 5-HT_7 receptors, there is quite some evidence that postsynaptic 5-HT_{1A} receptors are mediating this strong inhibitory effect. Snoeren et al. [29] showed in 5-HTT-KO rats a clear desensitization of 5-HT_{1A} receptors compared to WT rats in sexual behavior. However, the absence of the expression of 5-HTT does not affect basal sexual activity in female rats in a paced-mating situation. Apparently, under basal conditions, 5-HT_{1A} receptors do not play a role in female sexual behavior, and only upon activated 5-HT_{1A} receptors, hyposexual behavior is emerging.

The final picture of the role of 5-HT_{1A} receptors in female sexual behavior is complex. One could hypothesize that under normal conditions, 5-HT_{1A} receptors do not play a role. Only under certain conditions

(e.g., stress or 5-HT_{1A}-receptor agonists), (post)synaptic 5-HT_{1A} receptors may exert a (inhibiting) role. Human studies with 5-HT_{1A}-receptor agonists are scarce. Fabre et al. [30] treated women with gepirone and reported a significant reversal of HSDD. Under conditions tested the question is whether gepirone exerted a more antagonistic than agonistic activity, making the interpretation rather complex. A nonselective 5-HT_{1A}-receptor agonist, flibanserin has been developed for female sexual dysfunction, particularly HSDD. In female rats, flibanserin increased sexual motivation [31]. This is a somewhat unexpected finding from a 5-HT_{1A}-receptor agonist and because flibanserin is a nonselective drug, interactions with other neurotransmitter systems might be involved. Stimulation of (probably postsynaptic) 5-HT_{1A} receptors is effective in certain female sexual dysfunctions as shown by the finding [32,33] that combination of a low dose of testosterone with buspirone improves HSDD/FSIAD (female sexual interest/arousal disorder). These findings support a role for 5-HT_{1A} receptors in female sexual dysfunction and may hopefully lead to effective medication [34].

5-HT_2 receptors, and particularly 5-$HT_{2A/2C}$ receptors, have been implicated in female sexual behavior [17], specifically in modulating lordosis. Most evidence comes from studies with rather nonspecific 5-HT_2-receptor antagonists, which decrease lordosis behavior. The systemically administered, relatively selective 5-$HT_{2A/2C}$-receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) did not affect lordosis but stimulated proceptive behavior [17,35], whereas DOI stimulated lordosis when infused into hypothalamic areas. Whether 5-HT_{2A} or 5-HT_{2C} receptors are more or less specifically involved is not clear based on the pharmacology performed thus far [16].

A clear role of 5-$HT_{1B/1D}$ receptors in female sexual behavior is not evident yet. Generally, lack of effects of 5-$HT_{1B/1D}$ ligands in hormonally fully primed rats and a possible stress-involvement [17] make interpretation unclear. Li et al. [36] investigated the effects of CP-94253, a selective 5-HT_{1B}-receptor agonist, on female sexual behavior in a paced-mating situation after acute, subchronic, and chronic administration (5 mg/kg/day/SC). CP-94253 had a slight overall inhibiting profile at all time points, at a dose corresponding to approximately 80% 5-HT_{1B}-receptor occupancy. Those results do not support an important role for 5-$HT_{1B/1D}$-receptors in female sexual behavior.

The available evidence on the role of 5-HT_3 receptors in female sexual behavior is limited [17]. Systemically administered 5-HT_3 ligands have

no effect [37,38], whereas centrally infused 5-HT_3 ligands induce mixed results. Ondansetron, a 5-HT_3-receptor antagonist, was given for 14 days (1 mg/kg, twice daily, SC) and female sexual behavior was recorded after acute, subchronic (7 days), and chronic (14 days) treatment in a paced-mating situation [36]. Ondansetron, at a dose corresponding to approximately 60% brain 5-HT_3-receptor occupancy, did not affect female sexual behavior [36]. Therefore a role of 5-HT_3 receptors in female sexual behavior is unlikely.

Conclusion: 5-hydroxytryptamine and sexual behavior in females

The existing literature generally supports a role for 5-HT in female sexual behavior. Overall activation of the serotonergic system inhibits sexual behavior (particularly lordosis), whereas inhibition facilitates it. There is some evidence for a role of 5-HT_{1A} receptors (inhibitory) and 5-HT_2 receptors (facilitating) in female sexual behavior. Studies are complicated by the hormonal status interacting with the 5-HT system and the frequent lack of drug effects after systemic administration, complicating the results often observed after intracerebral injection. Almost all studies dealing with the role of 5-HT (sub)systems in sexual behavior are performed acutely, whereas, certainly for clinically relevant therapeutic applications, chronic treatment is mandatory. Therefore the study of serotonergic pharmacology and female sexual behavior is indeed in its early infancy [17]. The emergence of potential drugs to treat female sexual disorders (flibanserin, lybrido(s)) will hopefully lead to a research boost into the role of 5-HT (and other systems) in female sexual behavior and its dysfunctions.

Serotonin and male sexual behavior

Several reviews have recently been published on the role of 5-HT in male sexual behavior; the vast majority has been based on male rat sexual behavior. A large difference between male and female rodent sexual behavior is the dependence on gonadal hormones; in females, full sexual activity only occurs if estrogen/progesterone levels are optimal, whereas

in males testosterone is needed but less stringent than in females [39,40]. Veening and Coolen [40] proposed a "funnel-model" of the sequential organization of the sexual behavior of male rats, consisting of three phases. Phase 1 includes a "scanning or initiation" phase, in which the male gets information about the safeness and appropriateness of the environment. If the environmental information signals safety, the male enter phase 2, the "appetitive or precopulatory" phase where the male rat actively explores the environment and the female by approaching, following, and sniffing the female, specifically the anogenital area to obtain olfactory information for initiation of the next "copulatory" phase if the female responds with the appropriate behaviors, darting, hopping, and earwigging [41] leading to the first mount attempt. The latter behavior indicates the start of phase 3, the consummatory or copulatory phase. In this phase a series of successive mounts and intromissions, each typically followed by short bouts of genital grooming, eventually lead to ejaculation, followed by a relatively long (minutes) period of inactivity and male's own genital grooming. In this refractory period (postejaculatory interval (PEI)), the male refrains from sexual activity, but is very aware of his environment, and for that reason, Veening and Coolen [40] include PEI in phase 1. After this period the male starts actively approaching and pursuing the female again, followed by the next copulatory phase, including ejaculation. Male rats may display up to eight ejaculatory series [42,43] to reach sexual satiety after which sexual activity is inhibited for many days [44]. The sequence from phases 1 to 3 is easy to interrupt in phase 1, but increasingly difficult to interrupt in phases 2–3, where the behavior becomes goal-directed, that is, ejaculation [45]. Hypothalamic mechanisms seem to be involved in the early phase, but do not directly influence brain stem mechanisms and spinal reflexes involved in the ejaculatory processes. Ejaculation is a complex process regulated by the spinal ejaculation generator (SEG) [46] in the lumbosacral spinal cord, which receives afferent sensory information and coordinates viscera- and somatomotor systems to produce ejaculation [47]. The SEG is under supraspinal control, descending from brain stem, midbrain, and hypothalamus. Veening and Coolen [40] give an extensive overview of the SEG, its ascending and descending projections and the involvement of sexual reward mechanisms. The serotonergic systems, both the rostral and causal projecting systems, interact with all these mechanisms involved in various aspects of sexual behavior. It is largely unclear where, when, and what these influences are and future research is needed to unravel these complex issues.

Most research on the role of 5-HT on male sexual behavior has been performed on mounting, intromission, and ejaculation. Activation of the 5-HT system (by 5-HT, 5-HT agonists, and SSRIs) generally inhibits sexual behavior, whereas reduction facilitates it [10,17,39,48]. However, this general picture is far more complex when regarding the potential role of (14) different serotonergic receptors and the 5-HTT. In particular, the presence of psychotropics (agonists and antagonists) for these different targets refines the role of the differential contribution of 5-HT mediated by the various receptor contributions.

First, the availability of SSRIs for treatment of depression, anxiety disorders, and obsessive compulsive disorder has boosted the research into the role of 5-HT in sexual (dis)function. SSRIs are by far the most frequent drugs used, in rats and humans as antidepressants, anxiolytics, and antiobsessional drugs. SSRIs induce sexual side effects in humans (anorgasmia, libido loss, and erection problems) but such effects occur mainly after chronic treatment [48]. Apparently, the mechanisms underlying the inhibitory effects of SSRIs reflect changes in 5-HT levels but only becoming manifest after sustained treatment. The underlying hypothesis, increased 5-HT-mediated tonic inhibition, indicates that chronic SSRIs influence underlying circuitry mediating sexual behavior by enhancing 5-HT activity in selected projection areas [49] and presumably activating certain 5-HT receptors there. Although it is not clear how this mechanism specifically acts at different brain levels, it is evident that a very complex network in the brain and spinal cord mediates this 5-HTergic induced action, but also including important roles for norepinephrine, dopamine, and glutamatergic systems [50]. Similar findings are present in rats [48] showing that chronic, but not acute SSRI treatment reduces sexual behavior [27]. Additional mechanisms to 5-HTT inhibition such as norepinephrine or dopamine reuptake blockade may counteract sexual side effects. Recently, antidepressants with an SSRI component but with additional 5-HT_{1A}-receptor agonistic activity (vilazodone) or several other serotonergic mechanisms (vortioxetine) have next to their antidepressant activity, less or no sexual side effects [36,51]. This clearly illustrates the complex regulation of mechanisms involved in sexual processes in which 5-HT plays a role, certainly not a decisive but merely a modulatory one.

Sexual side effects of SSRIs have been therapeutically used to treat premature ejaculation (PE) in men [52,53]. Studies using the intravaginal ejaculation latency time (IELT) demonstrated not only clear IELT-increasing effects after various SSRIs, but also clear differences between

SSRIs in the degree of inhibition [52,53]. This might be indicative not only of associate mechanisms in individual SSRIs but also of differential influence of various SSRIs on serotonergic mechanisms linked to different 5-HT receptors located at different places in the brain and spinal cord. Specifically, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors have been implicated in male sexual behavior. Stimulation of 5-HT_{1A} receptors by various 5-HT_{1A}-receptor agonists has prosexual effects in rats [10], although this is less clear in humans, where buspirone is the only available (partial) 5-HT_{1A}-receptor agonist. 5-HT_{1A}-receptor agonists such as 8-OH-DPAT, flesinoxan, buspirone, ipsapirone, and others [54] in rats decrease the latency to the first ejaculation and decrease the number of mounts and intromissions to reach ejaculation. In a 30-minute test, 8-OH-DPAT may induce up to five ejaculations [42]. The prosexual activity of 5-HT_{1A}-receptor agonists can be blocked by 5-HT_{1A}-receptor antagonists, for example, WAY100,635, which on itself have no intrinsic activity [55]. This indicates that under basal conditions, 5-HT_{1A} receptors do not play a crucial role in sexual behavior. Apparently, 5-HT_{1A} receptors become important when either activated by 5-HT_{1A}-receptor agonists or under conditions of high extracellular 5-HT levels, for example, induced by SSRIs [56]. Adding a 5-HT_{1A}-receptor antagonist to an SSRI (either acutely or chronically administered paroxetine or citalopram) exacerbated the sexual inhibitory effects of the SSRIs [55,57]. This effect can be mediated by inhibition of 5-HT_{1A} autoreceptors that normally limit the increase in 5-HT levels, and/or by blockade of postsynaptic 5-HT_{1A} receptors that lower the ejaculation threshold. A comparable effect was observed in 5-HTT-KO rats where WAY100,635 decreased the ejaculation frequency which did not occur in WT and heterozygous 5-HTT-KO males [58]. 5-HT_{1A} receptors are desensitized in 5-HTT-KO rats. Chronic SSRI treatment also reduced the prosexual effect of 8-OH-DPAT [57] again adding to the conclusion that under normal circumstances 5-HT levels are not high enough (low endogenous tone) to induce a 5-HT_{1A}-receptor-mediated effect on male sexual behavior. Under a high endogenous tone (e.g., after SSRIs), the role of 5-HT_{1A} receptors becomes important. Both 5-HT autoreceptors and postsynaptic 5-HT_{1A} receptors are involved in various aspects of sexual behavior [10] and postsynaptic 5-HT_{1A} receptors are present in many areas of the brain and spinal cord, in line with the involvement of different brain areas in different aspects of sexual behavior [10,18,59]. Although acute administration of 5-HT_{1A}-receptor agonists facilitates male sexual behavior [10,28],

chronic administration (e.g., of buspirone [28] and flesinoxan [36]) leads to diminished effects, although some slight prosexual activity remains present. This appears in line with human findings with buspirone and vilazodone that report lower or no sexual disturbances after chronic administration [27].

5-HT_{1B} receptors are located presynaptically as autoreceptors and postsynaptically as heteroreceptors. Presynaptical autoreceptors inhibit 5-HT release upon activation whereas 5-HT heteroreceptors are inhibitory on various nonserotonergic neurons [60,61]. 5-HT_{1B}-receptor agonists inhibit sexual behavior, although most agonists used (e.g., RU24969, mCPP, TFMPP, and anpirtoline) have also high activity at other serotonergic receptors [62]. Antagonism of the inhibitory effects of anpirtoline by a 5-HT_{1B}-receptor antagonist but not a 5-HT_{1A}-receptor antagonist support the inhibitory role of 5-HT_{1B} receptors, although it is unclear which brain areas are involved [8]. All studies thus far only used acute treatment but a 14-day chronic treatment study with CP-94253 at a dose occupying approximately 80% of 5-HT_{1B} receptors showed both an acute and a chronic inhibitory effect [36]. Such an effect may underlie the inhibitory effects of SSRIs after chronic treatment.

A role for 5-$HT_{2A/2B}$ receptors has been suggested for a long time [18,47]. DOI, a selective 5-$HT_{2A/2C}$-receptor agonist, reduced male rat sexual behavior, which could be antagonized by a 5-HT_2-receptor antagonist [47,63]. mCPP, a 5-$HT_{2C\ and\ 1B}$-receptor agonist, also reduces sexual behavior. mCPP induces penile erections and ejaculations when tested without an estrus female [64] but reduces sexual behavior when tested in a social interaction with an estrus female [65]. Because other 5-HT_{1B}-receptor agonists without 5-HT_2-receptor agonistic activity inhibit sexual behavior without the stimulated penile erections and ejaculation, it seems quite evident that the ejaculatory response induced by mCPP and DOI is 5-HT_{2C}-receptor-mediated [18].

A role for 5-HT_3 receptors in male sexual behavior is unclear. Li et al. [36] found no effects (after acute and chronic studies) of a dose of ondansetron corresponding to approximately 60% brain 5-HT_3-receptor occupancy, in line with earlier studies [37,38].

Very limited data are known about the influence of other 5-HT receptors on male sexual behavior. It is interesting to mention the absence of any sexual side effect of vortioxetine in male or female rats [36]. Vortioxetine is a multimodal antidepressant [66]; in addition to 5-HTT inhibition, vortioxetine is a 5-$HT_{1A/1B}$-receptor agonist and a

5-$HT_{1D/3/7}$-receptor antagonist [66]. As outlined before, several of these 5-HT-receptor subtypes are involved in regulating male and female sexual behavior. Neither acute nor chronic vortioxetine treatment at low (around 40/50%) or high (90%) 5-HTT occupancy did affect male sexual behavior. Human data seem to be in line with these findings [66]. This illustrates the complex regulation of the orchestration of sexual behavior in the brain and spinal cord. Another antidepressant, vilazodone, is an SSRI combined with partial 5-HT_{1A}-receptor agonistic activity with low sexual side effects in humans [67]. In acute and chronic studies, vilazodone, in contrast to paroxetine, had no inhibitory effects on male rat sexual behavior. Moreover, adding buspirone to paroxetine repaired the decreased sexual behavior, indicating that 5-HT_{1A}-receptor activation counteracts the SSRI-induced inhibition of male sexual behavior [51,68]. This once more indicates the complex contribution of serotonergic neurotransmission to sexual behavior.

Conclusion

Most data (primarily from rats) indicate that under normal, healthy conditions, the serotonergic tone in the brain does not seem to play a large role in the initiation, execution, and termination of sexual behavior in males and females. Under diseased conditions, for example, in major depression, the 5-HT system seems to play an important role and SSRIs further exacerbate existing sexual dysfunctions in depressed patients. It is as yet unclear whether genetic background influences the activity of the serotonergic system under rest conditions, although a length polymorphism in the promoter of the 5-HTT (5-HTTLPR) seems to be involved in the vulnerability of humans to, for example, stress [69]. Whether such (and other) polymorphisms exert any influence on the normal activity of the 5-HT system is not clear and certainly not regarding sexual behavior. However, in PE, the presence of an S-allele in 5-HTTLPR genotypes seems to further shorten the already extreme short IELT, whereas the normal distribution of the different genotypes (LL, LS, SS) in the PE population does not differ from the normal population [70]. Comparable data for polymorphisms in the 5-HT_{1A} and 5-HT_{2C} receptor genes [71,72] indicate that polymorphisms in genes that (co)regulate serotonergic

activity may influence sexual behavior. Such data further add to the complexity of the serotonergic modulation of sexual activity and indicate the necessity for translational research into the contribution of normal and abnormal serotonergic activity in sexual behavior.

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CHAPTER EIGHT

Serotonin and cognitive flexibility

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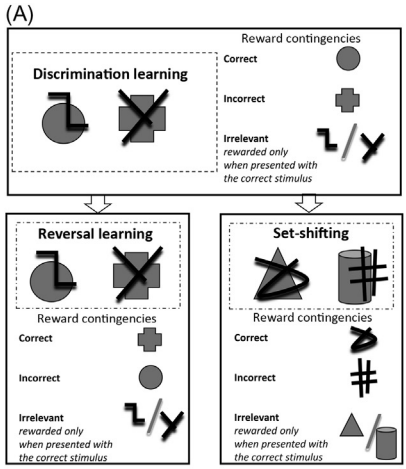
Cognitive flexibility and its relevance

Cognitive inflexibility is a common feature of neuropsychiatric disorders of dissimilar symptom profiles such as attention-deficit hyperactivity disorder, obsessive-compulsive disorder, schizophrenia, substance use disorder, Parkinson's disease, major depressive disorder, and autism [1–6] and can be considered a shared cognitive marker produced by variable etiologies [7]. On this view, the popularity of characterizing cognitive flexibility in patients with psychiatric disorders as well as in experimental animals has rapidly grown [8]. Studies that demonstrate robust cross-species validity of cognitive-flexibility tests as measures of prefrontal-dorsal striatal health and its dependency on 5-hydroxytryptamine (5-HT) has also fueled an interest in this set of cognitive functions [9].

The tests used to assess cognitive flexibility are discussed elsewhere [10,11]. In brief, the tests challenge many overlapping psychological phenomena that have been discussed in contexts such as response control/response inhibition [12,13], excitatory and inhibitory/aversive conditioning [14,15], attentional allocation [16], outcome evaluation [17], and others. Cognitive flexibility is most-often assessed by simultaneous multichoice reversal learning or attentional set-shifting tasks, but can also be assessed by changing stimulus-outcomes established in successive stimulus-presentation tasks such as go/no-go procedures, latent inhibition, extinction learning, and Pavlovian tasks (Fig. 8.1). In the prototypical test of

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00008-6



(B)

Common performance measures in tasks of cognitive flexibility and their definitions

Measure	Definition
Trials to criterion	Trials before achieving the learning criterion
Errors to criterion	Incorrect responses made before achieving the learning criterion
Percent accuracy	The ratio of responses to the correct stimulus
Perseverative error	Incorrect response to the previously correct stimulus, made when responding overall is biased to the previously correct stimulus (typically immediately or soon after a contingency shift)
Regressive error/ learning error	Incorrect response to the previously correct stimulus, made when responding no longer is biased to the previously correct stimulus (typically when the animal is close to reaching learning criterion)
Win-stay/ win-win-stay	Responding to the same stimulus as the previous trial(s), when the previous trial(s) was rewarded
Lose-shift/ lose-lose-shift	Responding to a different stimulus than the previous trial(s), when the previous trial(s) was nonrewarded
Never-reinforced errors	Errors made to a stimulus that is nonrewarded in all test stages

Figure 8.1 Depiction of the principles of reversal learning and attentional set-shifting. (A) Reversal learning often involves a single perceptual dimension and two stimuli. One stimulus is rewarded and the other stimulus is nonrewarded. This rule is reversed after learning an initial discrimination. Attentional set-shifting, however, involves a rule change between two superimposed perceptual dimensions (*figures* vs *lines* in (A)). In the attentional set-shifting test, stimuli in the previously relevant dimension (*figures*) becomes irrelevant and the stimuli in the previously irrelevant dimension (*lines*) become the correct and incorrect stimuli. (B) Definitions of the most common performance measures in these tasks of cognitive flexibility.

cognitive flexibility, the Wisconsin Card Sorting Task (WCST) [18], participants are required to sort cards according to the rule of either color, shape, or number. Once the rule is learned, an unsignaled rule change is introduced and subjects are required to adapt their responses to new outcomes. Human cognitive tasks are nowadays performed by standardized neuropsychological test batteries with visual stimuli on computer monitors and automated data collection systems are designed such a way that dependent variables are consistent across studies (Fig. 8.1) [19]. Tests in experimental animals, however, employ a range of different apparatus, variable sensory domains (e.g., visual, spatial, olfactory, or somatosensory stimuli), and variable outcome measures. Stimulus-outcomes are often appetitive and reward contingencies can be deterministic (where stimuli are either rewarded, or nonrewarded, on all trials) or probabilistic (e.g., the "correct" stimulus is rewarded on 80% of the trials and nonrewarded on 20% of the trials, and the "incorrect" stimulus is rewarded on 20% of the trials and nonrewarded on 80% of the trials). Probabilistic stimulus-outcomes are more difficult for subjects, and may therefore allow for

more sensitive detection of performance improvements after experimental interventions. Probabilistic stimulus-outcomes may also offer a better assessment of reversal-learning mechanisms through measures of response adaptation following spurious negative and positive feedback [20]. Deterministic tasks, on the other hand, are typically more time-efficient.

Primary performance measures vary across studies but often include trials and errors to attain an arbitrary learning criterion (e.g., 85% accuracy within a session for rodents [21], or eight correct responses in a row for human participants [22]; see Fig. 8.1B). These trials and errors are typically subdivided into phases based on the influence of the previously correct and incorrect reinforcement contingencies on current responding. For example, errors can be scored as "perseverative errors" when subjects still have a bias toward the previously correct, now incorrect response, such as when animals perform below 50% accuracy in a session [23] or when human participants make two or more consecutive errors [24]. The phases may correspond with the involvement of different circuitries within and between prefrontal cortical (PFC) and subcortical regions. Circuitries encompassing the orbital prefrontal (OFC) and dorsomedial striatal regions appear to be involved in the early phases of learning directly following a rule change [25,26] while medial prefrontal and dorsolateral striatal regions can support the later phases of learning [21,27]. Multiple lines of data suggest that these networks depend on serotonin for cognitive flexibility. This includes data from central and localized 5-HT depletions studies, which indicate that 5-HT depletions negatively affect cognitive flexibility in rodents as well as in humans and nonhuman primates.

5-Hydroxytryptamine depletion studies and cognitive flexibility

Methods that reduce 5-HT availability can cause cognitive inflexibility in humans and experimental animals alike. Yet the effects on performance in the literature have been mixed with many studies to-date finding no effect of 5-HT depletions on errors and trials to criterion (Table 8.1).

Lowered 5-HT availability in human participants is achieved by acute tryptophan depletion (ATD) through the administration of a tryptophan-free amino-acid solution [49]. Initial studies employing an intradimensional/extradimensional (ID/ED) set-shifting task detected ATD-induced

Table 8.1 Effects of pharmacological procedures that reduce 5-hydroxytryptamine availability on cognitive flexibility in experimental animals and humans.

	Subjects	Assay	Manipulation	Effect, learning	Measure(s) affected
Animals					
Brigman et al. [23]	Healthy mouse	Reversal	PCPA	No effect	–
	Healthy mouse	Reversal	Pet KO	No effect	–
Bari et al. [28]	Healthy rat	Probabilistic reversal	Forebrain 5,7-DHT	Impaired	Win-stay/lose-shift
Clarke et al. [29]	Healthy marmoset	Reversal	PFC 5,7-DHT	Impaired	Errors/perseverative errors
Clarke et al. [30]	Healthy marmoset	Reversal	PFC 5,7-DHT	Impaired	Errors/perseverative errors
Clarke et al. [31]	Healthy marmoset	Reversal	OFC 5,7-DHT	Impaired	Perseverative errors, CS + probe
Clarke et al. [32]	Healthy marmoset	Reversal	Caudate 5,7-DHT	No effect	–
Izquierdo et al. [33]	Healthy rat	Reversal	PCPA	No effect	–
Lapiz-Bluhm et al. [34]	Healthy rat	Reversal	PCPA	Impaired	Trials to criterion
Masaki et al. [35]	Healthy rat	Reversal	PCA	Impaired	Sessions to criterion
van der Plasse et al. [36]	Healthy rat	Reversal	ATD	No effect	–
Wallace et al. [37]	Healthy rat	Reversal	PCPA	Impaired	Trials to criterion
Humans					
Cools et al. [38]	Healthy human	"Pavlovian" reversal	ATD	Improved	Punishment prediction
Evers et al. [39]	Healthy human	Probabilistic reversal	ATD	No effect	–
Finger et al. [40]	Healthy human	Probabilistic reversal	ATD	Impaired	Errors/lose-stay (5-HTTPLR LL homozygotes)

Gallagher et al. [41]	Healthy human	WCST set-shifting	ATD	No effect	–
Golightly et al. [42]	Schizophrenia patients	WCST set-shifting	ATD	Impaired	Categories completed (order dependent)
Hughes et al. [43]	Euthymic bipolar patients	ID/ED	ATD	No effect	–
Hughes et al. [44]	Healthy human	ID/ED	ATD	No effect	–
Murphy et al. [45]	Healthy human	Probabilistic reversal	ATD	No effect	–
Park et al. [46]	Healthy human	ID/ED	ATD	Impaired	Errors (CDR, IDR, EDS, EDR)
Rogers et al. [47]	Healthy human	ID/ED	ATD	Impaired	Proportion reaching criterion, errors (CDr)
Talbot et al. [48]	Healthy human	ID/ED	ATD	No effect	–

impairments in reversal learning and/or attentional set-shifting [46,47]. The ATD-effects on cognitive flexibility were, however, not reproduced by later studies using similar tasks [43,44,48] or when using probabilistic reversal-learning designs [39,45] or the WCST [41]. A rodent study using ATD also reported no effects on reversal learning [36]. The inconsistent results have been explained by a number of cross-study discrepancies. Different ATD administration procedures may have resulted in different levels of 5-HT depletions with less severe depletions failing to affect cognitive flexibility [44]. The effects of ATD may also be subtler than gross changes in learning rates as measured by trials and errors. For example, ATD can affect response latencies in probabilistic tasks [39,45] and improve punishment predictions in a "Pavlovian" reversal task [38]. The mixed effects can also be explained by population differences in baseline 5-HT activity across studies; ATD-induced 5-HT depletion impairs attentional set-shifting in schizophrenia patients [42] where 5-HT transmission is already disrupted [50]. ATD also selectively impairs probabilistic reversal learning in healthy subjects that are homozygotes for the long allele of the common 5-HTTLPR polymorphism of the 5-HT transporter gene (*SLC6A4*) [40], a genotype associated with increased 5-HT transporter expression [51]. Thus reduced 5-HT levels through ATD may interact with genotype, psychopathology, and baseline 5-HT levels to produce cognitively inflexible profiles.

Animal studies have targeted 5-HT availability either by systemic inhibition of 5-HT synthesis through para-chlorophenylalanine (PCPA) and para-chloroamphetamine (PCA), or by local lesions of 5-HT neurons through 5,7-dihydroxytryptamine (5,7-DHT). Similar to the effects of ATD protocols in humans, the effect of PCPA/PCA-induced depletions on cognitive flexibility in experimental animals have been mixed (Table 8.1). PCA impaired operant go/no-go learning in rats [35], and the reversal-learning impairment in this task correlated with the severity of 5-HT depletion in various brain areas, including the OFC. PCPA impaired bowl-digging reversal in rats [34,37] but did not affect visual touch-screen reversal learning in rats [33] or mice [23]. More apparent impairments in cognitive flexibility have instead been achieved through 5,7-DHT-produced lesions throughout the PFC [29,30] or in the OFC selectively [31] in marmosets, and 5,7-DHT lesions of the rat forebrain [28].

It is notable that the effect of 5,7-DHT lesions can depend on testing experience. The impairment produced by intracerebroventricular 5,7-DHT in rats disappears with repeated testing [28]. Conversely, marmoset monkeys with PFC-specific 5,7-DHT-induced 5-HT depletions are not

impaired on the initial reversal test but are robustly impaired when tested on additional, serial, reversals [29]. The ATD-effects in humans are also absent if the participants have experienced the task before the ATD procedure [42,46]. These interactions between 5-HT depletion and the behavioral procedures suggest a complex relationship between 5-HT, initial learning, and cognitive flexibility, and maybe more thoroughly charted by techniques such as optogenetics or fiber photometry in experimental animals [52]. Such techniques might also overcome potential caveats that need to be recognized when interpreting 5-HT depletion data, including the issues of off-target or compensatory effects on other transmitter systems [53,54].

Pharmacological studies of 5-hydroxytryptamine and cognitive flexibility

The efficacy of the most successful psychotropic drugs, including antidepressants and atypical neuroleptics, are reliant on diverse actions on the 5-HT system. The effects of such drugs and more selective serotonergic compounds have been thoroughly investigated in preclinical tests of cognitive flexibility. These studies show remarkably wide-spread cognitive enhancing effects (Table 8.2). We identified more than 45 reports, using more than 20 different drugs that target serotonergic neurotransmission, that conclude cognitive flexibility-enhancing properties in experimental animals. Many of these studies have used healthy animals, or animals pretreated with the NMDA receptor antagonists phencyclidine (PCP) or ketamine, which model aspects of schizophrenia [86], where cognitive inflexibility is a robust feature [1]. Yet concerns regarding the validity of the positive indications remain and are discussed later.

Antidepressants improve cognitive flexibility in experimental animals. Improved reversal learning or attentional set-shifting has been reported from variable doses and administration procedures of citalopram [28,34,55,58], escitalopram [56,57,60], fluoxetine [23,60], milnacipran [61], and vortioxetine [37]. As PFC 5-HT content can correlate with reversal performance [35,53,55], the effects have been discussed as related to the drugs' capacity to increase extracellular 5-HT. However, the acute effects of antidepressants on 5-HT transmission are strongly dependent on dose [87,88] and it is unclear whether the different drugs and dosing

Table 8.2 The effect of pharmacological manipulations on cognitive flexibility in experimental animals.

	Model	Assay	Drug	Effect, learning	Dosing	Measure(s) affected
Antidepressants						
Bari et al. [28]	Healthy rat	Probabilistic reversal	Citalopram	Impaired	1 mg/kg, acute	Reversals completed, lose-shift
			Citalopram	Improved	10 mg/kg, acute	Reversals completed, lose-shift
			Citalopram	Improved	5 mg/kg, 7 days	Win-stay
			Citalopram	Improved	10 mg/kg, 5 days	Reversals completed
Barlow et al. [55]	Healthy rat	Reversal	Citalopram	Improved	1 and 10 mg/kg, acute	Trials/errors
Bondi et al. [56]	Stress model, rat	Set-shifting	Escitalopram	Improved	10 mg/kg, 26 days	Trials
	Healthy rat	Set-shifting	Escitalopram	Impaired	10 mg/kg, 26 days	Trials
Brigman et al. [23]	Healthy mouse	Reversal	Fluoxetine	Improved	>2 weeks in water	Error/perseverative errors
Brown et al. [57]	Healthy rat	Probabilistic reversal learning	Escitalopram	Improved	0.3−1.0 mg/kg, acute	Trials, regressive errors, win-stay
Furr et al. [58]	Stress model, rat	Reversal	Citalopram	Improved	3 weeks osmotic delivery	Trials
Lapiz-Bluhm et al. [34]	Stress model, rat	Reversal	Citalopram	Improved	5 mg/kg, acute	Trials
Nikiforuk [59]	Healthy rat	Reversal/set-shifting	Escitalopram	No effect	0.3 mg/kg, acute	–
	Healthy rat	Set-shifting	Escitalopram + SB2669970	Improved	0.3 mg/kg, acute	Trials
Nikiforuk et al. [60]	Healthy/Stress model, rat	Set-shifting	Escitalopram	Improved	1 and 3 mg/kg, acute	Trials
			Fluoxetine	Improved	1 and 3 mg/kg, acute	Trials
Wallace et al. [37]	5-HT depleted, rat	Reversal	Vortioxetine	Improved	10 mg/kg, 3 days or acute	Trials
	Stress model, rat	Reversal	Vortioxetine	Improved	>3 weeks in diet	Trials
Naegeli et al. [61]	Stress model, rat	Set-shifting	Milnacipran	Improved	>3 weeks, 30 mg/kg	Trials

5-HT$_{1A}$R						
McLean et al. [62]	PCP model, rat	Reversal	Buspirone	Improved	0.31 and 0.62 mg/kg, acute	Percent accuracy
			WAY100635	No effect	0.3 and 1.0 mg/kg, acute	–
Rajagopal et al. [63]	PCP model, mouse	Reversal	Tandospirone	Improved	5 mg/kg, acute	Percent accuracy
5-HT$_{2A}$R						
Amodeo et al. [64]	BTBR model, mouse	Probabilistic reversal	M100907	Improved	0.01 and 0.1 mg/kg, acute	Trials, regressive errors
Amodeo et al. [65]	BTBR model, mouse	Probabilistic reversal	M100907	Improved	0.2 and 0.6 μg, intra-DMS	Trials, regressive errors
			M100907	Impaired	0.2–0.6 μg, intra-OFC	Perseverative errors
Baker et al. [66]	Healthy rat	Set-shifting	Ketanserin	Improved	0.5 mg/kg, acute	Trials, regressive errors
Boulougouris et al. [67]	Healthy rat	Reversal	M100907	Impaired	0.03 and 0.1 mg/kg, acute	Trials, errors, perseverative errors
Boulougouris et al. [68]	Healthy rat	Reversal	M100907	No effect	0.1–1 μg, intra-OFC/ PFC/NAc	–
Furr et al. [58]	Healthy/Stress model, rat	Reversal	M100907	Impaired	0.1–2.0 nmol, intra-OFC	Trials
Idris et al. [69]	PCP model, rat	Reversal	M100907	Improved	0.16 and 0.31 mg/kg, acute	Percent accuracy
Rodefer et al. [70]	PCP model, rat	Reversal/Set-shifting	M100907	No effect	0.08–0.32 mg/kg, acute	–
5-HT$_{2C}$R						
Alsiö et al. 2015 [71]	Healthy rat	Reversal	SB242084	↑↓	0.1–1.0 mg/kg, acute	Improved "early," impaired "late" learning
			SB242084	Improved	1 and 3 μg, intra-OFC	Perseverative errors
Baker et al. [66]	Healthy rat	Set-shifting	SB242084	No effect	0.05–2.0 mg/kg, acute	–
Boulougouris et al. [67]	Healthy rat	Reversal	SB242084	Improved	0.1–1.0 mg/kg, acute	Trials, errors, perseverative errors
Boulougouris et al. [68]	Healthy rat	Reversal	SB242084	Improved	0.1–1 μg, intra-OFC	Trials, errors, persev. + learning errors

(*Continued*)

Table 8.2 (Continued)

	Model	Assay	Drug	Effect, learning	Dosing	Measure(s) affected
			SB242084	No effect	0.1−1 μg, intra-NAc/PFC	–
Del'Guidice et al. [72]	Healthy/Tph2 KI, mouse	Reversal	CP809.101	Improved	0.5 mg/kg, acute	Trials
McLean et al. [62]	PCP model, rat	Reversal	SB243213A	Improved	10 mg/kg, acute	Percent accuracy
Nilsson et al. [68]	Healthy mouse	Reversal	SB242084	Improved	0.5 mg/kg, acute	Trials, CS − probe
Nilsson et al. [73]	Healthy mouse	Reversal	SB242084	Improved	0.5 mg/kg, acute	Errors, CS + probe
5-HT$_3$R						
Arnsten et al. [74]	Healthy rhesus	Reversal	Ondensatron	No effect	1 ng/kg, acute	–
			SEC-579	No effect	0.1 μg/ kg, acute	–
Barnes et al. [75]	Healthy marmoset	Reversal	Ondensatron	Improved	1 and 10 ng/kg	Trials
Domeney et al. [76]	Healthy marmoset	Reversal	Ondensatron	Improved	1−10,000 ng/kg	Trials
			Ondensatron	Impaired	100 μg/kg	Trials
5-HT$_6$R						
De Bruin et al. [77]	PCP model, rat	Reversal	GSK742457	Improved	0.63 and 5 mg/kg, acute	Percent correct
Burnham et al. [78]	Healthy rat	Set-shifting	WAY181187	Improved	10 and 30 mg/kg, acute	Trials
			SB399885	No effect	10 mg/kg, acute	–
Hatcher et al. [79]	Healthy rat	Reversal/set-shifting	SB399885	Improved	10 mg/kg, 8 days, bi-daily	Reversal/ED trials, total errors
			SB271046	Improved	10 mg/kg, 8 days, bi-daily	Reversal trials, total errors
Idris et al. [69]	PCP model, rat	Reversal	SB742457	Improved	2.5 and 5 mg/kg, acute	Percent correct
Mohler et al. [80]	Healthy rat	Set-shifting	PRX-07034	Improved	1 and 3 mg/kg, acute	Trials, perseverative + regressive errors
Nikiforuk et al. [81]	Healthy/Ketamine, rat	Set-shifting	EMD386088	Improved	2.5 and 5 mg/kg, acute	Trials
Rodefer et al. [70]	PCP model, rat	Set-shifting	SB271046	Improved	10 mg/kg, acute	Trials

5-HT$_7$R						
McLean et al. [62]	PCP model, rat	Reversal	SB2669970	Improved	3 and 10 mg/kg, acute	Percent correct
Nikiforuk [59]	Healthy/stress model, rat	Set-shifting	SB2669970	Improved	0.3 and 1.0 mg/kg, acute	Trials
Nikiforuk et al. [82]	Ketamine model, rat	Set-shifting	SB2669970	Improved	1.0 mg/kg, acute	Trials
Nikiforuk et al. [83]	Healthy rat	Reversal/set-shifting	AS19	No effect	10 mg/kg, acute	–
Rajagopal et al. [63]	PCP model, mouse	Reversal	SB2669970	Improved	4 mg/kg, acute	Percent correct
			AS19	No effect	10 mg/kg, acute	–
Other targets						
Del'Guidice et al. [72]	Healthy/Tph2 KI, mouse	Reversal	5-HTP (5-HT)	Improved		Trials
Nikiforuk et al. [84]	Healthy/ketamine, rat	Set-shifting	SB699551 (5-HT$_{5A}$R)	Improved	0.3–3 mg/kg, acute	Trials
Nikiforuk et al. [82]	Healthy/ketamine, rat	Set-shifting	Amisulpride (5-HT$_{7/2B}$R)	Improved	3 mg/kg, acute	Trials
Zhukovsky et al. [85]	Healthy rat	Reversal	Moclobemide (MAO-A)	Improved	3 and 16 mg/kg, acute	Trials, perseverative errors

procedures all converge on similar potentiating effects on 5-HT. The correlation between PFC 5-HT and reversal learning also appears dependent on striatal dopamine levels [53], with high extracellular PFC 5-HT content facilitating reversal learning only when striatal dopamine levels are low. Improved cognitive flexibility from serotonin-reuptake inhibition has also been observed when animals are depleted of 5-HT [37] and the drug effects on striatal acetylcholine and noradrenaline transmission [23,28,34,55,57] are likely also relevant for the performance-enhancing effects.

Aside from targeting overall 5-HT levels, modulation of several 5-HT receptor classes improves cognitive flexibility in experimental animals. Improved reversal learning or attentional set-shifting has been seen after 5-HT_7R antagonist treatment in healthy rats and in a rat stress model [59,82] and also in rats treated with NMDA antagonists [62,63,82]. At least six different 5-HT_6R antagonists and one 5-HT_6R agonist have documented positive effects on cognitive flexibility both in NMDA-antagonist–treated [69,70,77,81] and healthy rodents [70,78–81]. 5-HT_{2C}R antagonists can improve reversal learning when given systemically or locally in the orbitofrontal cortex in healthy rodents [67,68,71,73,89] and when given systemically to PCP-treated rats [62]. Improved cognitive flexibility has been reported following treatments by a 5-HT_{1A}R agonists in PCP-treated animals [62], the nonselective 5-HT receptor antagonist amisulpride in ketamine-treated rats [83], and 5-HT_{5A}R antagonists [84] in healthy animals. Reducing 5-HT degradation through monoamine oxidase inhibition [85] and administering the 5-HT precursor 5-HTP [72] also improve reversal learning. There were also early reports of 5-HT_3R antagonists improving reversal learning in nonhuman primates [74]; but see [76]. In contrast to such beneficial actions of serotonergic agents, 5-HT_{2A}R antagonism has shown mixed effects. The 5-HT_{2A}R antagonist M100907 improves reversal learning when given systemically to PCP-treated rats [69] and when given systemically, or locally in the dorsomedial striatum, in the BTBR mouse model of autism [64,65]. The same 5-HT_{2A}R antagonist has also been without effect [70,89] and has impaired cognitive flexibility [67]. The mixed effects are difficult to reconcile by varying dosing procedures as the studies employed overlapping doses. Nevertheless, 5-HT_{2A}R antagonism can have motor depressant effects [90] and a possible explanation is that 5-HT_{2A}R antagonism has ameliorating effects only in models where task parameters enable dysregulated processing speed and/or hyperactivity to contribute to the deficits in cognition (see later).

Challenges to validity and translation

The above summary shows that the potentially procognitive effects of serotonergic compounds are well-documented in experimental animals. However, several alternative behavioral factors could explain the pharmacological effects in animal tests of cognitive flexibility and diminish the relevance or translatability of the findings. Such considerations become pertinent as the possible candidates and mechanisms listed in Table 8.2 have, at this time, no robust documented positive effects on cognitive flexibility in humans. Furthermore, some of the drugs in Table 8.2 can produce improvements over large dose ranges and variable administration protocols, while dominant theories would predict nonlinear relationships between cognition and transmitter function and, more frequently, quadratic dose–response curves [91]. Many SSRIs also have acute effects in rodent assays of cognitive flexibility, which would be in apparent contrast with their delayed antidepressant actions in human. There are a few interdependent confounding factors that may contribute to false positives in preclinical pharmacological studies.

Such confounds may include the use of over-sensitive assays; tests that detect improvements by 5-HT drugs also report improved cognitive flexibility by drugs that lack clinically meaningful effects on cognition, including neuroleptics [65,82,92,93]. Improved performance has also been observed after corticosterone treatment [94] and acute stress [27], which currently is of unclear therapeutic relevance. Procedural oversensitivity may be a product of the complexity of the tasks. The tests engage a range of different psychological and behavioral constructs that involve 5-HT [12–14,16,17], and the manipulations may affect processes that are unaffected in psychiatric disorders and are of minor relevance for cognitive flexibility in healthy individuals.

The majority of studies presented in Table 8.2 have also used healthy animals, or NMDA-antagonist–treated animals; models whose validity for drug development and relevance for human disorders of cognition have been questioned [23,95–97]. Conclusions regarding cognition in behavioral neuroscience have traditionally suffered confounds whereby the experimental manipulation interact with the model in ways that are irrelevant for cognitive enhancement [98]. An important aspect of manipulations to the 5-HT system is that serotonergic drugs, as well as challenges with NMDA antagonists, affect behavior by targeting motor function

and/or motivation. Such disruptions may mediate behavioral changes rather than effects on learning and/or "executive function" per se [90,99–102]. These factors may also explain the equivocal effects of depletion studies across studies, as the behavioral effects of 5-HT manipulations may depend on parameters that are unstable across tasks (i.e., response requirements and reinforcement value) rather than parameters that are shared by the tasks (i.e., shifting reward contingencies). These concerns are exaggerated in the many studies that employ NMDA-antagonist models but do not assess the 5-HT drug in the absence of the NMDA antagonist. The concerns are also relevant to studies that demonstrate potential procognitive effects over very large dose ranges.

The translational significance of the rodent data could be enhanced by using animals with clearer relevance for psychiatric disorders, and by including negative pharmacological controls (e.g., neuroleptics) when possible. Some of the above questions can also be addressed by dedicated attempts to dissociate learning processes from motoric, sensory, and motivational effects. Rather than reducing performance effects to trials and errors before achieving an arbitrary learning criterion, additional measures such as response latencies, activity measures, trial-by-trial analyses, and measures of reward motivation should be standard additions that provide a "global" response profile and informs on the mechanisms that are more likely responsible for, or concomitant with, the altered cognitive flexibility.

An additional method to overcome the somewhat arbitrary analysis of learning and chunking of behavior into phases based on predefined criteria (e.g., perseverative and regressive errors) is to use computational models in which learning rates for reward and punishment, as well as subtler influences of side-bias and factors unrelated to cognitive flexibility, are estimated. This approach has been employed in human studies [24] and animal experiments [103] and is likely to strengthen the translational potential of preclinical work significantly in the near future [8].

Questions regarding the different psychological constructs affected by the manipulation can also be addressed by more innovative behavioral designs that assess the relative contribution of the underlying cognitive processes. One such example, which has given knowledge of 5-HT function and reversal learning, is a simultaneous three-stimulus task [104,105]. In this task, a constantly nonrewarded stimulus is presented during discrimination and reversal learning, next to the rewarded and nonrewarded stimuli. The stimulus provides an important control measure of responding to a stimulus that does not change reward contingencies. The procedure

was used successfully to show that systemic 5-HT_{2C}R antagonism induces impulsive-like impairments [71] that are concurrent with the previously observed improvements in reversal learning [67,68,89] and thus highlighting potential issues in its clinical utility.

A further task innovation that has provided insight into 5-HT function and cognitive flexibility are stimulus-replacement designs [31,106,107]. These tasks assess the relative contribution of approach versus avoidance tendencies to the rewarded and nonrewarded stimuli by replacing the previous correct or incorrect stimulus with a novel stimulus after a contingency shift [11]. The method has been used successfully in patient populations to demonstrate that different psychiatric groups can show deficits in cognitive flexibility due to impairments in dissociable cognitive components [6,108]. It has also been used in the marmoset to show that OFC-specific 5-HT depletions selectively retard the ability to overcome rewarded associations in reversal learning [31], and that systemic 5-HT_{2C}R antagonism improves the ability to overcome nonrewarded associations [68]. These experiments indicate that different tasks of cognitive flexibility, despite their superficial similarity, are solved by different cognitive strategies. The findings also reveal that different serotonergic manipulations can have dissociable effects on such processes.

Relative to deterministic tasks, the implementation of probabilistic reward contingencies in animal studies has also grown and such tasks may provide increased resolution by measures of win-stay/lose-shift measures [20]. Probabilistic tasks may also have improved sensitivity for detecting disruptions with relevance for depression [109] and 5-HT availability [28,39] and be more translationally relevant to the bulk of current human studies.

Conclusion

There are now more than 70 cross-species reports of altered cognitive flexibility following perturbations of the 5-HT system as assessed by reversal learning or attentional set-shifting tasks.

A particularly successful approach has been to show that a wide variety of tasks assaying cognitive flexibility in experimental animals are affected—and most-often enhanced—by antidepressants and drugs targeting 5-HT receptors in experimental animals. Other key studies show that

cognitive flexibility in humans and animals is disrupted by depletions of 5-HT. These complementary methods convincingly show that 5-HT level correlates with cognitive flexibility, and demonstrate that tasks of cognitive flexibility are valuable translational tools for understanding how serotonergic mechanisms can be used for cognitive enhancement.

The challenge remains to determine if the effective pharmacological treatments have common effects that can be translated to improved human cognition. The development of more successful therapeutic leads will depend on an understanding of how the psychological and behavioral processes regulated by 5-HT are challenged by tests of cognitive flexibility. We recommend the presentation of global performance measures to help understand the behavioral processes that contribute to altered cognitive flexibility, and the use of innovative behavioral procedures and data analysis that dissociate the cognitive components required for successful performance. These methods may contribute to increased validity of human and animal studies that aim to determine how cognitive performance can be improved by targeting serotonergic neurotransmission.

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CHAPTER NINE

Serotonin and aggression

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Violence in the blood

The Netherlands, 1978. A woman entered the University Hospital in Nijmegen, with the sole aim of seeking help for the troublesome men in her family, who, by her own description were "frightening and aggressive." Many of them were prone to aggression and violence, ranging from arson, exhibitionism, and rape to attempted murder. The woman reported that the family tree showed that this violent streak could be traced back over four generations—as far back as 1870—but only in men, female family members were spared of such behavioral traits. She suspected that something was terribly wrong with her family.

Confirmation of her fears came 15 years later when a clinical geneticist Han Brunner found a link between a genetic mutation and aggressive behavior in this large Dutch kindred [1,2]. Through genetic linkage analysis [1,2], he identified an X chromosome–linked nonsense point mutation in the monoamine-oxidase-A (MAO-A) gene in all affected males as well as in unaffected female carriers in this multigenerational family. Hence, functionally, the affected males were MAO-A knockouts, that is, they completely lacked the MAO-A enzymatic activity. MAO-A catalyzes the metabolic degradation of biogenic amines—serotonin (5-hydroxytryptamine (5-HT)), norepinephrine, and dopamine—making it a critical regulator of neurotransmitter signaling at monoaminergic synapses in the brain [3–5]. Complete MAO-A deficiency thus raises 5-HT levels [1,2]. Inhibition of MAO-A, either genetically or via MAO-A inhibitors, has also been shown to elevate the brain 5-HT concentration and aggression in male mice and rats

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00009-8

[3,4,6–17]. This association of aggressive phenotype and increase in 5-HT was further strengthened by a report that 5-HT2A receptor antagonist reduces hyperaggression in MAO-A knockout mice [3,4]. However, MAO-A inhibitors, widely used as antidepressants, were not reported to cause aggression in the adults [18]. This is likely due to the developmental timing of MAO-A inhibition, underscoring the importance of neurochemical changes during development [7]. In such a scenario, high prenatal 5-HT levels may dysregulate the development of serotonergic brain circuits resulting in elevated aggression postnatally. Thus MAO-A may represent a genetic pathway accounting for the consistent observation that low serotonergic turnover strongly predicts high rates of impulsive aggression.

A complete knockout mutation of MAO-A, as described earlier, is very rare—and therefore not plausibly predictive of aggressive behavior in general population. However, genetic polymorphisms in the MAO-A gene are quite frequent, giving rise to two different alleles: MAO-A L and MAO-A H [19]. The low-activity L allele results in lower expression of MAO-A and therefore less effective degradation of the 5-HT, while the opposite is the case for the high-activity H allele [19]. As it might be expected, MAO-A L genotype has been associated with increased risk of antisocial behavior and violence in human and Rhesus Macaques monkey males, particularly in those who experienced early-life adversity, such as childhood abuse [20–40]. The role of MAO-A in aggressive behavior was further supported by McDermott et al. [41] who examined the genetic predisposition to engage in costly aggressive behavior in the context of environmental provocation, that is, the challenge of having money taken away. The study participants played out a scenario where they believed that they could physically harm another person for taking 20% or 80% of the money that they have earned—and their weapon of retaliation was a hot (spicy) sauce that the opponent does not like. In this situation, MAO-A L carriers were more likely than MAO-A H participants to administer physical punishment in the form of forceful drinking of unpleasantly spicy sauce, and more of it, to the person responsible for the loss of 80% of their earnings [41]. Furthermore, the authors of the study concluded that MAO-A not only affects a person's behavior but also their reactions to other people's behaviors, and that MAO-A L males displayed greater aggression in high provocation situations (loss of 80% of their earnings), while they did not differ from MAO-A H males in low provocation situations (when 20% of their earnings taken away). Interestingly, this punishment was not of an altruistic nature, that is, they did not punish

others for the good of the group, but MAO-A L carriers were rather acting out of spite as they received no positive returns for doling out the punishment. On the contrary, they were paying money to cause physical pain to someone they believed had taken money from them. That MAO-A genotype predicts interindividual differences in reactive aggressiveness as a function of the degree of provocation was also supported by Kuepper et al. [23] and Chester et al. [42], suggesting that disrupted serotonergic systems predispose individual's greater aggression by increasing impulsive and aggressive reactivity to provocation and stress.

In addition to examining the genetic basis of this association, some studies have also focused on investigating the relationship between the enzymatic activity of MAO-A and aggression. Thus PET studies with MAO-A-selective radioligands on healthy adult males have shown that MAO-A activity in multiple cortical and subcortical brain regions is inversely correlated with self-reported aggression, anger, and hostility [26,43]. Similarly, an inverse relationship between MAO-A activity and aggression was also reported in individuals with antisocial personality disorder [44], thus not just underscoring the importance of MAO-A in antisocial and aggressive behavior but also suggesting its catalytic activity in the brain may serve as a biomarker for abnormal aggression.

Despite the overwhelming evidence for the association between MAO-A and aggression, the neurobiological underpinnings of this link are still rather sparse. The primary approach to identify the underlying neurobiology is mainly based on neuroimaging studies conducted on carriers of MAO-A L and H alleles. As mentioned earlier, MAO-A L genotype predisposes for aggression by facilitating the dysregulation of affective information processing and altering the emotional responses to provocation. Several imaging genetic studies have reported that these behavioral alterations reflect dysregulated corticolimbic circuitry for affective arousal and inhibitory control. Hence, fMRI examination of healthy individuals showed that, compared to MAO-A H genotype, the carriers of the MAO-A L variant had hyperresponsive amygdala, hippocampus, and anterior cingulate cortex during negative emotional stimuli [45–47] and diminished activity of dorsal anterior cingulate cortex during conflict resolution [48] and cognitive inhibition task [46]. Furthermore, higher trait aggression and interpersonal hypersensitivity of MAO-A L individuals, compared to MAO-A H carriers, were associated with greater dorsal anterior cingulate cortex reactivity to social rejection and during anger control [47,49], whereas their impulsivity was associated with greater activation of

the superior parietal cortex and extrastriate cortex during a response inhibition Go-NoGo task [50]. As these cortical regions negatively regulate the activity of amygdala [51–56], a brain region implicated in both initiation and expression of aggression [57,58], Buckholtz and Meyer-Lindenberg [59] investigated whether the MAO-A L genotype impacts on functional connectivity between them. Their study, carried on healthy volunteers performing an emotion task, showed that, compared to MAO-A H carriers, men with MAO-A L genotype had stronger coupling between amygdala and ventromedial prefrontal cortex, which regulates amygdala activity indirectly via perigenual anterior cingulate. These findings, later replicated by independent studies [47,60], further support the hypothesis that aberrant connectivity between cortex and amygdala results in emotion regulation deficits as MAO-A L carriers appear not to be able to disengage cortical control circuitry from amygdala arousal. Importantly, these clinical observations of hyperactive amygdala and hyporeactive regulatory prefrontal regions were also recapitulated in *Mao-a* deficient mouse models [61–63], thus providing a good translational platform for elucidation of the pathophysiology of aggression and identification of potential treatment targets. While the common denominator of the earlier-described studies is dysregulated corticolimbic circuitry, particularly a pattern of hyperactive amygdala and hyporeactive regulatory prefrontal regions, due to a MAO-A genotype, Ziermans et al. [64] reported that alterations in frontoparietal circuitry involved in working memory also mediate the relationship between MAO-A genetic variation and aggressive behavior during development.

Due to these genetic and imaging genetics associated with aggression, MAO-A gene has been, quite misfortunately, nicknamed a "criminal gene," and later on the "warrior gene." As stated by Brunner [65] himself "Even if the possible relationship between MAO-A deficiency and abnormal behaviour is confirmed in other kindreds (the second case of a 'Brunner syndrome' was identified more than 20 years after the first report [66], the data do not support the hypothesis that MAO-A constitutes an aggression gene. In fact, because genes are essentially simple, and behaviour is by definition complex, a direct causal relationship between a single gene and a specific behaviour is highly unlikely .... Thus, the concept of a gene that directly encodes behaviour is unrealistic." Human behavior, including aggression, has a multifactorial architecture and when considering neurobiological correlates of any behavioral output, one must bear in mind that behavior is expressed in a complex and

intertwined interaction of biological, psychological, and environmental/social determinants. In other words, a possible genetic predisposition does not mean determination that inevitably leads to fixed outcomes, but a propensity, which is further shaped by numerous environmental influences. Nevertheless, the early assumption that there might be biological roots to aggression, that is, "aggression gene," carries a lot of emotional load due to a fear of placing blame onto the biology of individuals and thus relieving them of responsibility and having possible far-reaching implications for the criminal law and the legal system in general. Indeed, research evidence linking MAO-A with aggressive behavior has already been used in several court trials, in which the defendants' MAO-A genetic profile was not only admitted as evidence but was also used to support a mitigation of criminal responsibility [67]. On the other hand, such genetic information might also be used to elevate perceived predetermined dangerousness of individuals who despite the mutation may never go down the criminal path. Hence, instead of using genetic risk factors to deterministically predict aggressive phenotype, such variants could be used as tools to uncover neural systems linked to aggressive behavior. Indeed, MAO-A inhibitors have been extensively used in the treatment of depression [18,68], without causing an increase in aggressive behaviors in these patients. Similarly, chronic MAO-A inhibition in adult rodents reduces, rather than increases (as is the case with developmental MAO-A inhibition [7]) aggression [69,70]. These studies suggest that low MAO-A activity may have qualitatively distinct outcomes during different developmental stages, which would be consistent with the high expression of MAO-A activity during early development and the importance of serotonin for brain development [71].

Notwithstanding, the association between MAO-A deficiency and impulsive aggressivity is important as its discord with the low 5-HT paradigm of impulsive aggression prompted new research in this field. Low 5-HT (early on affectionately called a "civilizing neurohumor") as a biological underpinning of aggression was proposed in 1970s and 1980s based on the measurements of 5-hydroxyindoleacetic acid (5-HIAA), the main 5-HT metabolite, in the cerebrospinal fluid of impulsive, suicidal, and aggressive individuals [72–79]; see also [80,81]. This paradigm was then further strengthened by findings that lesions of the ascending serotonergic fibers facilitate aggressive behaviors in rodents and that drugs enhancing serotonergic neurotransmission lower aggressive behavior in humans and

rodents alike [82–88]. However, this initial dogma of low 5-HT being the primary cause of aggression is now replaced with a view that low 5-HT is a biological risk factor, that may, at least in part, predispose toward aggression across various neuropsychiatric disorders.

In view of this low 5-HT hypothesis of aggression, previously described finding that 5-HT levels are higher in human and animal males with MAO-A deficiency seems inconsistent. However, reduced 5-HIAA levels may also be caused by MAO-A deficiency [1,2], for example, due to ineffective 5-HT degradation, suggesting that 5-HIAA may not be a reliable marker for 5-HT concentration and serotonergic function.

Tryptophan hydroxylase 2

In addition to MAO-A, there are several other components of the 5-HT system that were associated with aggression, and that can be broadly classified as being involved in 5-HT metabolism, that is, synthesis, transport, and degradation, or 5-HT receptor signaling. We have already described the involvement of the 5-HT degrading enzyme MAO-A and will now concentrate on tryptophan hydroxylase 2 (TPH2), a rate-limiting enzyme mediating 5-HT synthesis from L-Tryptophan [89,89a], and on the 5-HT-transporter (5-HTT; SERT) which is essential for clearance and reuptake of extracellular 5-HT [90].

Like in the case of MAO-A polymorphisms, *Thp2* gene was also shown to have high- (C1437C) and low-activity variants (C1437G) [91–93]. However, unlike the MAO-A polymorphisms, mice homozygous for the low-activity allele had lower THP2 activity in the brain and were less aggressive compared to mice homozygous for the high-activity allele [93]. This positive correlation between aggressivity and THP2 was further confirmed by Takahashi et al. [93a]. Nevertheless, the relationship between THP2 activity and the level of aggression seems to be rather complex. On one hand, it has been shown that *Thp2* knockout mice exhibit normal 5-HT neuron development, but very low levels of 5-HT have a highly aggressive phenotype [94–97], thus seemingly supporting the low 5-HT paradigm of aggression. On the other hand, however, a positive correlation between the THP2 activity and aggresivity was reported for several mouse strains [91]. Therefore it is plausible that 5-HT concentration needs to be finely titrated as both increases and decreases may result in elevated aggressivity.

In humans, *THP2* polymorphisms are associated with emotion regulation. Gutknecht et al. [98] have reported overrepresentation of T allele carriers among patients with personality disorders and have associated it with personality traits related to emotional instability. This allele was previously shown to bias responsiveness of the amygdala, a brain structure critically involved in the regulation of emotional responses, during the processing of faces expressing anger or fear [99–101]. Moreover, polymorphisms of *TPH2* have also been linked to anger-related personality traits [102,103], especially to the expression of anger, with reward dependence [104], affective instability and suicidality [105]. In order to bridge the gap between the genes and behavior, several studies have used imaging genetics approaches to identify possible intermediate phenotypes. Yoon et al. [103] have identified orbitofrontal cortex as an intermediate phenotype that bridges serotonin synthesis, that is, *THP2* polymorphisms, and anger-related traits in healthy subjects, whereas Inoue et al. [104] have found a correlation between *THP2* polymorphisms, reward dependence and amygdala and hippocampal volumes. Furthermore, links between the *THP2* polymorphisms and amygdala reactivity to emotional stimuli of both negative and positive valence were reported by Brown et al. [99] and Canli et al. [100,101], thus indicating that allelic variations in THP2 function may contribute to individual susceptibility to stress and aversive environments.

Serotonin transporter

While 5-HT regulates the activity of numerous neural circuits and requires coordinated enzymatic activity for its synthesis, only one protein transports it to presynaptic neurons. This 5-HT transporter (5-HTT; SERT), encoded for by the *SLC6A4* gene, is distributed along the axons and synaptic terminals of serotonergic neurons and plays a key role in spatiotemporal fine-tuning of 5-HT signaling as it removes 5-HT from the synaptic cleft back to the synaptic boutons where it gets reused. As the 5-HTT is the main regulator of the bioavailability of 5-HT, it is natural to expect that any change in its expression would have behavioral consequences. Indeed, the 5-HTT has been associated with aggression in both mouse and human studies and as such has served as a target for pharmacological interventions—it is a target for selective serotonin reuptake inhibitors (SSRIs), as well as for some drugs of abuse [106]. SSRIs elevate

5-HT levels in the pre- and postsynaptic regions of 5-HT neurons by binding to the 5-HTT and blocking the removal of 5-HT from the synaptic cleft [107–109]. SSRIs have been shown effective in reducing aggressive behavior in both humans and animals [86,110–119,119a–d]. However, there are also some contrasting reports indicating that SSRIs may actually increase aggressiveness [120–122]. The most infamous example for the latter being the case of Joseph Wesbecker, who shot dead eight people and injured 12 others before killing himself at his place of work in Kentucky in 1989. Prior to that, Wesbecker was taking fluoxetine for 4 weeks and this led to a legal action against its makers, Eli Lilly. These discordant findings reiterate once more the notion that the link between the 5-HT and aggression is not simply a matter of the 5-HT levels. A recent study by Molero et al. [123] have suggested the moderating role of age, that is, children and adolescents react differently than adults to SSRIs, as was previously shown in preclinical studies [124–126].

The *SLC6A4* gene locus also has a number of polymorphisms—two most important ones being termed variable number tandem repeat (VNTR) polymorphisms and are located in noncoding DNA [127]. One of them, termed 5-HTTLPR exists in two variants containing either 14 (short; S) or 16 (long; L) copies of 22 bp repeat [128–130]. The second one contains 9, 10, or 12 copies of 16–17 bp repeat and is accordingly termed STin2.9, STin2.10, and STin2.12 [127]. The S allele is associated with decreased transcription efficiency, resulting in a lower amount of 5-HTT and subsequently reduced reuptake of 5-HT and decreased serotonergic function [106,129,131]. The S allele has been associated with a number of affective disorders, including anxiety, psychosis, depression, suicide, aggression, and antisocial behavior [37,80,106,131–139]; also see [140]. The mechanism is unclear but was suggested to involve amygdala hyperreactivity during mood recovery [100,101,141–146], as well as less effective inhibitory feedback circuits in prefrontal cortex resulting in dysregulated limbic emotion centers [147]. To further investigate the mechanisms by which the 5-HTT affects aggressive behavior, knockout mouse models were developed resulting in decreased levels of aggressivity [142,148–151]; also see [152]. These studies are therefore consistent with the low 5-HT hypothesis of aggression, that is, higher extracellular, including synaptic levels of 5-HT, are associated with the lower intensity of aggressive behavior. Furthermore, it appears that the contribution of the S allele to increased aggression is moderated by environmental factors, for example, stress and childhood adversity [153–155]. However, a recent

meta-analysis by Tilebeek et al. [156] showed that although there is a significant interaction effect between the 5-HTTLPR genotype and environmental adversities on antisocial behavior, it is not clear whether the significant interaction effect is driven by the S or by the L allele. On the other hand, there are some indications that the contribution of risk by the 5-HTT polymorphisms for impulsive and aggressive behavior is strongest for the combination of both the 5-HTTLPR and STin2 polymorphisms [157,158]. Interestingly, whereas 5-HTTLPR alleles were mostly associated with reactive aggression, the STin2 VNTR12 allele was recently reported to be associated with increased levels of appetitive aggression, characterized by the primary intrinsic enjoyment of the aggressive activity, but with decreased levels of reactive aggression [159]. Further along those lines, it has recently been reported that individuals homozygous for the 5-HTTLPR L allele have psychopathic characteristics, and are more likely to engage in planned, cold-blooded aggression [160].

Imaging genetics studies have been used to further explore the link between genetic variation in the 5-HTT gene and brain activity. Hariri et al. [141] have compared activity in the amygdala between the 5-HTTLPR S allele carriers and individuals homozygous for the L allele while they were viewing pictures of angry and afraid faces. During that task, the S allele carriers showed a larger increase in amygdala activity compared to L allele homozygotes, suggesting that this may reflect a hyperresponsiveness to environmental stimuli, which may predispose an individual toward stress-related psychopathology. Importantly, however, the amygdala response in L allele homozygotes was nearly zero, as previously observed in studies of psychopaths [161]. Interestingly, lower amygdala reactivity in L allele homozygotes appears to be specific to negative, but not positive stimuli [162]. Furthermore, functional connectivity between the amygdala and the ventromedial prefrontal cortex, a region important in decision-making that integrates input from the amygdala to guide appropriate behavioral responses, is also reduced in L allele homozygotes [51,162]. Similar findings have previously been reported individuals with psychopathic traits [163].

Serotonin receptors

Following its synthesis and transport to presynaptic neurons, 5-HT exerts its effect(s) by acting on 5-HT receptors—of which there are at least 14 different subtypes. Here, we will present the findings related to

the four most commonly investigated 5-HT receptors in the context of aggression—5-HT1A, 5-HT1B, 5-HT2A, and 5-HT3. These receptors are present in brain areas implicated in aggression control and are sensitive to alterations in 5-HT activity.

As an autoreceptor, 5-HT1A is present on raphe serotonergic neurons and postsynaptically as inhibitory heteroreceptor on nonserotonergic hippocampal, hypothalamic, septal, and cortical neurons [163a], where it mediates inhibition [164]. In preclinical studies, treatment with 5-HT1A agonists was reported to reduce aggressive behavior, particularly in mice after alcohol exposure or in animals with high or escalated aggression [165–168]. Importantly, as shown by de Boer et al. [168] in rats, 5HT1A receptor activation modulates offensive, but not defensive, agonistic behaviors. The 5HT1A receptor stimulation was also reported to be sufficient in inhibiting aggressive behavior in mice with depleted brain 5-HT [117]. However, the 5-HT1A receptor agonist 8-OH-DPAT has been shown to produce different results in females and males suggesting that the expression of 5HT1A receptors may be sexually dimorphic [169]. This finding was further supported by a study of Parsey et al. [170] showing greater 5-HT1A receptor binding in females compared to males, likely due to the sex steroid hormones that are known to modulate 5-HT1A binding [171]. Interestingly, 5-HT1A receptor knockout mice do not show altered aggression, whereas those having 5-HT1A receptor overexpression in raphe serotonergic neurons exhibit increased aggressivity [172]. In clinics, the 5-HT1A receptor partial agonist buspirone has been used for the management of aggression [173–175]

5-HT1B receptors are present on serotonergic raphe neurons where they act as autoreceptors and on nonserotonergic projection neurons from the striatum and hippocampus where they act as receptors [163a] and regulate neurotransmitter release [176]. Similar to 5-HT1A receptor stimulation, 5-HT1B receptor agonism also inhibits various types of aggressive behaviors in rodents [177–187,187a,b]. Thus direct application of 5-HT1B receptor agonists into the dorsal raphe nucleus inhibits aggressivity in male mice and rats [181,188], whereas direct application of 5-HT1B agonists into postsynaptic locations decreases maternal aggression in rats [187]. The importance of 5-HT action in prosocial behaviors was recently supported by a study in a 16p11.2 mouse model of autism spectrum disorder [189]. They have shown that a 5-HT neuron-specific deletion of 16p11.2 results in deficits in social behavior accompanied by a decrease in dorsal raphe 5-HT activity during social interaction. Decreased sociability

of these mice was rescued by optogenetic stimulation of dorsal raphe 5-HT neurons. However, this effect was dependent on 5-HT1B receptors in the nucleus accumbens, as infusion of a 5-HT1B receptor antagonist (NAS-181) into the nucleus accumbens before optogenetic stimulation abrogated its positive effects on sociability [189]. Interestingly, activation of 5-HT1B receptors in the nucleus accumbens with a 5-HT1B receptor agonist (CP93129) was sufficient to rescue social deficits in 16p11.2 deletion mice [189]. Moreover, Dolen et al. [190] have shown that the rewarding properties of social interaction are dependent on the coordinated activity of 5-HT1B receptors and oxytocin in the nucleus accumbens and that they could be blocked by 5-HT1B antagonist (NAS-181). These examples suggest that similar to 5-HT1A receptors, the effects of 5-HT1B receptor stimulation on aggression are species, region, and sex-dependent. However, unlike 5-HT1A receptor knockout mice, those lacking 5-HT1B receptor activity exhibit heightened aggression [191–193]. Moreover, as with the case of 5-HT1A receptors, the jury is still out on whether the aggression decreases because of a dominant autoreceptor effect or due to an inhibitory effect on nonserotonergic neurons, the latter of which supports the 5-HT deficiency hypothesis of aggression. To disambiguate between these two possibilities, researchers have neurotoxically lesioned the dorsal raphe nuclei in mice and treated them with 5-HT1A and mixed 5-HT1A/5-HT1B agonists 8-OH-DPAT and eltoprazine, respectively [194,195]. Interestingly, this depletion of 5-HT1A autoreceptors did not affect the antiaggressive effects of these drugs suggesting that their behavioral actions are mediated by action on postsynaptic sites of these 5-HT receptors. On the other hand, however, de Boer and Newman-Tancredi [196] have shown that the selective 5-HT1A agonist F13714 that preferentially activates 5-HT1A receptors is more potent in inhibiting aggression than F15599, an agonist preferentially targeting postsynaptic 5-HT1A heteroreceptors, thus indicating a critical role of 5-HT1A autoreceptors in aggressive behavior.

The 5-HT1B receptor agonists have also formed a basis for the development of antiaggressive compounds known as serenics. Early serenics, such as eltoprazine, were mixed 5-HT1A/5-HT1b agonists, whereas the new generation of serenics, such as zolmitriptan, is far more selective for 5-HT1B receptor [197]. Zolmitriptan was shown to reduce alcohol-induced aggression in both mice [198] and humans [199], thus highlighting its translational value, which, at the moment, seems to be rather neglected.

Compared to the wealth of studies that have examined the role of 5-HT1 receptors in relation to aggression, fewer studies have investigated the link between the activation of other 5-HT receptors and aggressivity. Preclinical studies have supported the role of 5-HT2A receptors in the promotion of impulsive and aggressive behavior [200–204], whereas clinical studies have revealed association between 5-HT2A receptor polymorphisms and aggression and impulse control disorders [205–212]; also see [213–215]. In patients diagnosed with various neuropsychiatric disorders, antipsychotic risperidone, which shows 5-HT2A antagonist action, has been used in the management of aggressive outbursts [216–218,218a].

The 5-HT3 receptor is distributed both pre- and postsynaptically [163a] and it is the only excitatory 5-HT receptor. This receptor affects the motivation and reward by mediating mesolimbic dopamine release [219] and its upregulation has been correlated with increased cocaine-induced aggressive responding [220]. In preclinical studies, agonists and antagonists of 5-HT3 receptor were shown to increase and decrease aggressiveness, respectively [220–224]. Genetic polymorphisms of this receptor have been associated with impulse control disorders [225]. Thus stimulation of 5-HT1 and 5-HT2 receptors, and blockade of 5-HT3 receptors, inhibit aggression in preclinical studies.

Conclusion

At the end, as we have seen, 5-HT affects numerous neural processes in the brain, starting from its development to ensuring normal everyday functioning. The evidence and studies reviewed here are consistent with the role of 5-HT in mediating aggression in both humans and animals. However, the exact relationship with aggressivity is far from clear as both high and low levels of 5-HT have been associated with aggressive behavior. On the other hand, aggression per se is a complex, fundamental social behavior, and it is not reasonable to expect that one gene, neurotransmitter or a brain region rules it all. There are still many open questions. Does the 5-HT play a role under normal conditions when aggression is needed (i.e., self-defense) or does it only switch on in pathological conditions? To complicate the complex story even more, but also to finish in a positive tone, let's consider a cleverly executed study by Crockett et al. [226]. They have shown that serotonin also affects our moral judgment and social behavior. Crockett et al. [226] have

demonstrated that a blockade of serotonin reuptake with citalopram, that is, increasing brain 5-HT levels, influences moral judgment in emotionally salient personal scenarios, making people less likely to reject unfair offers but also less likely to endorse harming one innocent person to save many others, suggesting that 5-HT promotes prosocial behavior by enhancing the aversiveness of harming others. Seemingly, this finding goes hand in hand with the low serotonin hypothesis of aggression; however, the jury is still deliberating.

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CHAPTER TEN

Serotonin and sleep

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The modulation of sleep states has long been associated with serotonergic activity since it was noted that arousal closely correlates with the firing rate of dorsal raphe neurons. The pharmacological tools for investigating serotonin metabolism became more selective with respect to affinity for the many subtypes of serotonin receptor. The most commonly used serotoninergic drugs, the SSRIs, have been found to strongly suppress rapid eye movement (REM) sleep. The injection of serotonin into the cat lateral ventricle elicited first arousal followed by prolonged synchronous discharges characteristic of slow-wave sleep. Injecting serotonin toxins showed a reduction in sleep in parallel with the reduction in serotonin content. The depletion of serotonin using para chlorophenyl alanine also markedly reduced sleep, which is readily reversed by administration of 5-hydroxytryptophan. In contrast the indoleamine melatonin can ameliorate sleep disturbance. Compounds with high affinity for the 5-HT2A receptor increase slow-wave sleep in both man and animals. The supracuprachiasmatic nucleus in the hypothalamus functions as a master circadian pacemaker. Mutant fruit files have demonstrated that circadian rhythms are heritable, some strains being arrhythmic, while others showing periodicity in excess of 24 hours. These observations gave rise to the concept of the existence of clock genes with the hypothetical master gene termed *Period*. An antibody to period has been obtained, which showed that there was a 24 hours cycling in the amount of period protein expressed. *Period* is a nuclear protein which shuttles between the cell nucleus and cytoplasm in a temporally regulated manner which is abolished in the timeless mutant. It has been suggested that melatonin secreted from the pineal gland serves as a temporal feedback on the oscillator since the suprachiasmatic nucleus regulating circadian phase and maintaining rhythm stability. Compounds with affinity and selectivity for 5-HT6 and 5-HT7 receptors are prime candidates for influencing sleep. 5-Ht7 is strongly expressed in

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00010-4

the suprachiasmatic nucleus and an agonist action at the receptor increases wakefulness and reduces REM sleep. The selective 5-HT6 receptor antagonist Ro4368554 reduces sleep latency and wakefulness. It is surely significant that there is a very strong relationship between sleep disturbance and depression with 50%–60% of young adults with depression having sleep disturbance perhaps linking serotonin to the underlying the antidepressant effects of sleep deprivation. Sleep electrosomnography is a key tool in the clinical profiling of any new serotonergic compound.

The relationship between serotonin and sleep has a long history. It starts with Brodie [1] in 1955, who noted that depletion of brain serotonin with reserpine-induced a sleep-like state and triggered pontogeniculo occipital waves [2] in the cat even during waking the first electrophysiological recordings of the raphe nuclei. It was immediately noted that firing rate closely correlated with behavioral arousal, achieved via activation of the ascending reticular activating system in which the dorsal raphe is intimately entwined. The reticular system consists of several circuits connecting the brain stem to the cortex via synaptic relays in the intralaminar nucleus of the thalamus and thence to the cortex. The reticular formation includes the dorsal and median raphe as well as the locus coeruleus, pedunculopontine nucleus, and the parabrachial nucleus. In vitro, however, dorsal raphe neurons are not spontaneously active indicating that afferent inputs are necessary for tonic firing. Agonists of three arousal related systems impinging on the dorsal raphe, namely the orexin/hypocretin, histamine, and noradrenaline systems cause an inward current and increase firing rate. The dose–response curve for orexin B suggests an effect mediated by OX2 receptors and both OX1and OX2 receptors have been found to be expressed in tryptophan hydroxylase positive neurons [3]. Sleep is typically defined in both man and animals by the recording of electrical field activity. Scalp electrodes record the EEG while electrodes placed in skeletal muscle record the electromyogram. Together the physiological monitoring constitutes the electrosomnograph. Wakefulness is defined by low voltage fast EEG activity with high muscular tone. Nonrapid-eye-movement sleep (NREM) is characterized by high amplitude, low-frequency EEG and decreased muscle tone, whereas REM sleep has low voltage fast EEG coupled with complete loss of muscle tone and characteristic rapid eye movements [4].

The histochemical information that elucidated the existence of the serotonin-containing raphe nuclei suggested some obvious experiments that would either selectively stimulate or depress raphe activity given that

electrical stimulation was so closely correlated with arousal. Bradley [5] first injected serotonin into the lateral ventricle of the cat which elicited an initial arousal followed by prolonged synchrony after giving doses of 5−30 μg. The appearance of the selective neurotoxic agents dihydroxylated tryptamines-5,7 and 5,6-dihydroxytryptamine allowed for the selective lesioning of raphe nuclei. Michel Jouvet [6] did so in the cat and found that a reduction in sleep parallels the reduction in serotonin. Depleting serotonin by inhibition of tryptophan hydroxylase with para-chloro-phenylalanine also markedly reduced sleep. The insomnia was reversed by administration of tryptophan or 5-hydroxytryptophan [7]. The first study of raphe unit recording throughout the sleep-wake cycle was published in 2006 [8]. The firing of raphe neurons was most frequent during waking and reduced during slow-wave sleep, with complete loss of activity during REM sleep. These findings are consistent with the pattern of serotonin release as indicated by brain dialysis studies [9]. Manipulating extracellular serotonin by administration of selective serotonin reuptake inhibitors results most consistently in a reduction of REM sleep and reduced slow-wave sleep [10]. Dissecting the sleep effects of serotonin subtype selective compounds is not straightforward since many subtype selective receptor agonists can be confounded by the induction of competing for motor stimulation. For example, the 5-HT1A selective agonist 8-OHDPAT stimulates locomotion when given systemically, but infusion via reverse dialysis directly into the dorsal raphe decreases serotonin release and increases REM sleep. Separately the indoleamine melatonin can ameliorate sleep disturbance. In a meta-analysis of sleep studies including 284 subjects from 17 independent studies, melatonin significantly reduced sleep onset latency by 4 minutes, increased sleep efficiency by 2%, and increased total sleep duration by almost 13 minutes [11].

While the effects of serotonin on sleep seem to be generally well replicated, the role of serotonin receptor subtypes is more controversial, given the high degree of heterogeneity and the relative lack of selectivity of many of the pharmacological tools available. The situation is not too different from studies of GABA A receptor subtypes. Generally GABA mimetic drugs promote sleep, sedation, and enhance general anesthesia [12]. GABA transmission within the reticular system increases wakefulness and decreases sleep. The first neurochemical theory of sleep posited that acetylcholine plays a primary role in brain activated states of wakefulness and REM sleep [13,14]. In particular the M2 muscarinic receptor plays a key role in the generation of REM sleep via the laterodorsal tegmental

nuclei and the basal forebrain. The laterodorsal and pedunculopontine nuclei can be divided into two populations based on their firing pattern [15]. One population discharges maximally during wakefulness and REM sleep-referred to as wake-on and REM-on and a second population fires only during wakefulness-wake-on REM-off. In investigating the specific roles of serotonin receptor subtypes in sleep control, transgenic mice lacking individual receptors have been extremely useful. Interest first arose in the 5-HT2A receptor because the antipsychotics ritanserin [16] and clozapine [17] clearly increased slow-wave sleep in both man and animals. The more selective 5-HT2A receptor antagonists ritanserin and M100907 induced similar effects. In all mammals, a small group of nerve cells in the hypothalamus, the suprachiasmatic nucleus, functions as a master circadian pacemaker controlling the timing of the sleep-wake cycle and coordinating this with circadian rhythms in other brain areas and tissues. Circadian rhythms are ancient and conserved throughout evolution. Even the leaves of some plants when placed in continuous darkness still open and close rhythmically during the day. Erwin Brunning is credited with finding the first indications that circadian rhythms are heritable giving rise to the concept of clock genes. Seymour Benzer then identified mutant fruit flies with differing circadian phenotypes. One mutant was arrhythmic while another had a longer period of 28 hours. The hypothetical gene was given the name *Period*. With advances in gene cloning and sequencing technology, an antibody to *Period* was obtained, which showed that there was a 24 hours cycle in the amount of period protein expressed in fly brain neurons, peaking during the night [18]. The mRNA encoded by *Period* also showed circadian variation in quantity, with the peak occurring several hours before the period peak. A nonsense mutant of *Period* failed to induce oscillating levels of period mRNA while the addition of wild type period protein reinstated mRNA expression. These observations gave rise to the negative autoregulatory feedback model whereby the accumulation of *Period* protein attenuates the expression of its mRNA. Subsequently the *Period* protein was identified as a nuclear protein which shuttles between the cell nucleus and cytoplasm in a temporally regulated manner. The period protein cycle was found to be abolished in the timeless mutant. This is described as the *Transcription Translation* feedback loop. But how does melatonin engage with this system? Cassone suggested that melatonin secreted from the pineal gland serves as a temporal feedback on the oscillators in the suprachiasmatic nucleus, regulating circadian phase and maintaining rhythm stability. It is thought that melatonin reaches the SCN via

the CSF and the third ventricle where it modulates protein levels [19]. Of the 14 different subtypes of serotonin receptors in the brain, two have been identified for having positive effects on sleep. 5-HT2A antagonists increase human slow-wave sleep [20]. 5-HT6R ligands were suggested to have a role given the expression of these receptors in the thalamus, hypothalamus, and striatum. Interestingly, these receptors also modulate the GABAergic and cholinergic systems. The selective 5-HT6 receptor antagonist Ro4368554 was duly found to reduce sleep latency and wakefulness [21]. 5-HT7 receptors are most expressed in the suprachiasmatic nucleus. Systemic administration of a selective 5-HT7 agonist increased wakefulness and reduced REM sleep [22]. At the interface between serotonin and sleep it is significant that there is a very strong relationship between sleep disturbance and depression, with both difficulty in initiating night-time sleep, coupled with early morning awakening. Sleep symptoms have been found in 50%–60% of young adults with depression aged 21–30. In a population sample of 8,580 patients with depression, the incidence of insomnia increased with age. The incidence of insomnia is so consistently found in depressed patients that those diagnosing depression in the absence of the symptoms of insomnia should exert caution. In addition to a reduction in slow-wave sleep, the REM latency is shortened and the duration of the first REM period is prolonged. In contrast sleep deprivation for one night exerts an immediate but short-lived antidepressant effect. Borbely and Wirz-Justice hypothesized that in depressed patients a sleep-dependent process, *Process S* is deficient. This process may lead to the inhibition of REM in the early part of the night. An antidepressant effect has been reported following 3 weeks of REM-sleep awakenings but is not seen with slow-wave sleep awakenings. In the context of serotonin, it would be reasonable to conceptualize that sleep deprivation acting as a stress; indeed most methods involve either regular handling or the positioning of an animal on an upturned flower pot in a bath of water into which the animal is plunged as soon as muscle tone is lost, pushing more L-tryptophan through to serotonin. However, dialysis studies have failed to record any change in serotonin release following sleep deprivation, at least in the frontal cortex and hippocampus. However, an earlier study [23] did find changes in 5-HIAA and serotonin, as well as dopamine and its metabolite HVA in the basal forebrain. Corticosterone levels were unchanged. Interestingly, local perfusion of corticosterone into the dorsomedial hypothalamus has been found to potentiate the increase in extracellular serotonin induced by D-fenfluramine. The authors hypothesized

[24] that corticosterone inhibits the postsynaptic clearance of 5-HT from the extracellular fluid by blocking the organic cation transporter-3. Overall the results suggest an involvement of serotonin in the antidepressant actions of sleep deprivation. Ketamine, the only other agent to have antidepressant effects in treatment-resistant patients, induces serotonin release in the prefrontal cortex, which is attenuated by electrolytic lesions of the pedunculopontine tegmental nucleus or by intra raphe injection of dihydro-beta-erythroidine, an $\alpha 4\beta 2$ nicotinic receptor antagonist [25]. The antidepressant actions of electroconvulsive shock have long been thought to be mediated by changes in serotonin release. Shen et al. found that repeated electroconvulsive shocks led to an increase in the expression of serotonin transporter mRNA specifically in the dorsal raphe [26]. The reduction in serotonin neurotransmission that follows from ingestion of a tryptophan-free meal is associated with a reduction in the density of REM sleep and the first and second REM periods were shorter than those of subjects given placebo [27].

But what of the relative functions of the different sleep stages? Slow-wave sleep seems to be most correlated with the restorative function. However, REM sleep is associated with intense neuronal activity, ocular saccades, muscular atonia, and dreaming. Imaging the brain during sleep indicates regional cerebral blood flow increased [28] during REM sleep in the pontine tegmentum, left thalamus, both amygdaloid complexes, the anterior cingulate cortex, and right parietal operculum. Decreases in perfusion were observed bilaterally in the dorsolateral prefrontal cortex as well as in the supramarginal gyrus, posterior cingulate cortex, and precuneus [28]. The authors suggest that the amygdaloid activation indicates a role in emotional memory, perhaps this explains its high sensitivity to inhibition by serotonin? [28]. Some authors have suggested REM sleep is an aid to functional maturation of the brains of babies and young children who spend longer in REM sleep than adults or the young of precocial species. The neuronal activity associated with REM sleep maybe crucially important in guiding neuronal maturation and synaptic connectivity [29]. Serotonergic hyperactivity as may occur in autism spectrum disorders [30] may thus play a role in delaying connectivity and impending brain maturation [31]. Although the roles of sleep remain enigmatic, most accepted is its restorative function and our own personal experience would surely concur with that. One of the most exciting hypotheses is that sleep is of great importance to cognitive function in particular learning and memory. The British Psychologist Steven Hartley was the first to suggest that

dreaming might alter associative learning. It is recognized that sleep might alter the initial encoding of the information learned, or the subsequent consolidation of the information which enables the encoding to be stabilized [32] and less susceptible to time-dependent decay. Retroactive and proactive interferences are well-established descriptors of the degradation of memory by prior or subsequent sensory stimulation. Slow-wave sleep at the very least will diminish sensory interference thus reducing pressure on the process of consolidation. It has been reported that on recall of previously consolidated information, the memory returns to an unstable state, once more requiring consolidation or reconsolidation, but we have not yet defined the mechanistic differences between consolidation and reconsolidation [33]. A memory in its not yet final consolidated (active) state is susceptible to interference by amnesic agents, examined the role of beta-adrenergic receptors in the reconsolidation of a positively reinforced radial arm maze task and a footshock reinforced conditioned emotional response task. Following training, the animals were re-exposed to the maze. If given the beta-adrenergic antagonists after exposure, a temporally graded impairment occurred after the reactivation trial. When repeated with the conditioned emotional response task, the impairing effects of propranolol were greater when given after a reactivation trial than when given immediately after training. The results suggest that reactivation of memory triggers a beta-adrenoceptor dependent cascade of intracellular events, recapitulating what occurs following the initial acquisition [34]. That serotonin might possess similar properties is suggested by similar data achieved by administration of 5-HT6 receptor agonists and antagonists. It is also possible that propranolol might exert its effects via blockade of 5-HT1A receptors. Although early theories of sleep assumed that it occurred at the level of the whole organism, governed by central control mechanisms, there is evidence that sleep might be regulated at a more local level and might be a fundamental property of neuronal networks with duration-dependent on prior activity in each network. The homeostatic regulation of sleep, whereby prolonged wakefulness is followed by rebound sleep, as can be seen with some but not all wake-promoting drugs like amphetamine or modafinil. Interestingly wakefulness induced by a metabotropic mGlu5 receptor positive allosteric modulator such as LY2814617 [35] can produce many hours of wakefulness that is not followed by any rebound hyper-somnolence. Sleep is traditionally thought of as a property of the whole organism which is either awake or asleep. However, the homeostatic regulation of sleep might not be at the level of the whole organism

and might occur in any brain region in response to use. Clinical evidence suggests sleep can be a property of less than the whole brain. People with parasomnias (sleepwalkers) are thought to be simultaneously awake and asleep, awake because they can navigate obstacles, and asleep indicated by their lack of awareness of their actions [36]. Cross-species comparisons also indicate the ability of sleep and wake to occur simultaneously. For example, dolphins do not exhibit high amplitude delta oscillations a defining characteristic of nonrapid-eye-movement sleep, simultaneously in both cerebral hemispheres. Seals birds and whales similarly experience uni-hemispheric sleep. In man, NREM delta power increases in frontal cortex earlier than in posterior cortical areas, consistent with the idea that sleep intensity is a regional phenomenon in the brain. Cortical columns are anatomically well-defined examples of neuronal networks or assemblies. Cortical columns oscillate between functional states as defined by changing input−output relationships. If cortical columns are probed with different sensory stimulation and the amplitudes of the induced evoked potentials measured, awake-like and sleep-like states can be distinguished. In the sleep-like state, the evoked potentials are higher than those that occur during waking. During whole animal sleep most of the cortical columns are in this sleep-like state. The cortical column sleep-like state is predictable and homeostatically regulated. The probability of finding a column in a sleep-like state is dependent on the length of time the column has spent in the awake-like state so the longer a column spends in the awake-like state, the greater is the probability it will transition into a sleep-like state. There is evidence that parts of the brain that are disproportionately used during wakefulness require more sleep, local increases in slow-wave activity have been observed in many species. In man, sensory stimulation of one hand increases slow-wave activity in the opposite hemisphere during subsequent sleep [37]. Similarly in rats slow-wave activity is greater in the somatosensory cortex during the dark period, when whisking is more frequent and greater in the visual cortex during the light period [38]. Yasuda et al. hypothesized that whole organism sleep is an emergent property of the synchronization of loosely coupled local processes [39]. It has been known for many years that substances accumulation the cerebrospinal fluid during different sleep phases and that when transferred to the fluid of awake animals elicit sleep [40]: so-called sleep regulatory substances [41]. It is known, for example, that interleukin-19IL1 and tumor necrosis factor 9 (TNF), adenosine, nitric oxide, prostaglandin D2, and growth hormone-releasing hormone are all

involved in regulating NREM duration and intensity. These substances work in the biochemical cascades that form the NREM. Thus, IL-1 and TNF induce each other's production and activate factor κβ which in turn stimulates nitric oxide and adenosine. IL-1 and its receptor are constitutively expressed in normal brain [42]. IL-1 has reliably been shown to increase NREM sleep and inhibit REM-sleep. Furthermore central administration of an IL-1 antagonist or antibodies against IL-1 reduces spontaneous REM sleep and also inhibits NREM sleep rebound following sleep deprivation [43]. IL-1 stimulates serotonin release in the hypothalamus. An intact brain serotonergic system is required for full manifestation of IL-1 effects on sleep [44]. Indeed, the microinjection of IL-1 into the dorsal raphe. In vitro experiments using guinea pig brain slices showed that NREM sleep was significantly increased when IL-1 was injected into the dorsal raphe and concomitantly reduced the amount of REM-sleep [44]. In vitro 80% of serotonergic neurons were inhibited by bath application of IL-1. Overall the data suggest that the dorsal raphe is the site of action of IL-1 in enhancing NREM sleep [45].

On commencing this review, I only ever considered sleep to be a manifestation of the whole animal. The evidence suggesting it to be an emergent property of synchronized neuronal assemblies was impactful and surely has significance for our understanding of consciousness. The findings that individuals in a vegetative state can respond to commands as do normal individuals as indicated by brain activation patterns revealed by fMRI scans [46]. Clearly as with sleep you can be both simultaneously conscious and unconscious just like you can simultaneously be both asleep and awake. What does this tell us about inferring changes in brain function by measuring brain connectivity using slow oscillations in BOLD signals in situations when the individual cannot avoid oscillating in and out of sleep [47].

Sleep disorders

Narcolepsy is a disabling disorder characterized by excessive daytime sleepiness and irresistible sleep episodes plus or minus automatic behaviors such as cataplexy: a sudden loss of muscle tone triggered by strong emotion, abnormal rapid eye movements, sleep paralysis, and hypnogogic hallucinations. The etiology of narcolepsy is associated with genetic

mutations in the orexin gene OX2 and orexin deficiency is the most frequent cause. Human symptoms are fully expressed in animals with experimental OX2 gene deletion [48]. The management of narcolepsy is based primarily on treatment with amphetamines and similar stimulants, now replaced by modafinil and selective serotonin reuptake inhibitors. Modafinil seems to be working primarily by inhibiting dopamine reuptake doses that are wake-promoting and occupy the dopamine transporter [49]. In 2014 the first in class dual orexin 1/2 receptor antagonist suvorexant was approved for the treatment of sleep disorders [50] confirming the importance of the orexin system in the control of sleep disorders.

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CHAPTER ELEVEN

Serotonin and the psychedelics

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We have already considered in Chapter 1, The metabolism of indoleamines, the profound impact that Albert Hoffman's discovery of lysergic acid diethylamide (LSD) had on Erspamers's discovery that the smooth muscle contraction that he isolated from mammalian gut, "enteramine," was in fact 5-hydroxytryptamine, an indoleamine structurally related to LSD whose actions on the gut could indeed be antagonized by LSD. As knowledge of the effects of LSD on perception and consciousness grew, it became apparent that LSD was in fact a member of a family of mind-altering substances, the psychedelics, that have been used in spiritual ceremonies for millennia. Best known are mescaline, ayahuasca, and psilocybin.

There are many examples of psychedelic compounds (Table 11.1). Dimethyltryptamine (DMT) is present in the Amazonian bevrage ayahuasca derived by boiling the bark of the banisteriopsis tree together with leaves of other locally sourced plants. The bevrage is hallucinogenic and widely used for ritual and recreational purposes by aboriginal tribes of the Amazon Basin. The active ingredient is DMT which is not active orally. Interestingly the beverage contains the β-carbolines harmine, harmaline, and tetrahydroharmine compounds known to have inhibitory action on the enzyme monoamine oxidase which is thought to protect DMT from degradation after oral administration [1]. Takini was also used in the Amazon Basin, notably for medicinal use against rheumatisms. This is prepared from the latex of *Brosimum acutifolium* [2]. Initial descriptions seemed to indicate that takini was more toxic than psychedelic. However, on analysis, it was found to contain 5-methoxydimethyltryptamine, otherwise known as bufotenine found in the skin of the toad genus *Bufo* including *Bufo marinus*. There are over 200 known species of *Bufo* toads and all have paratoid glands on their backs that produce a wide variety of biologically active substances, including norepinephrine, serotonin, and bufotenine. The use of *Bufo* toad venom is thought to date back to Neanderthal times, 100,000 years ago. It is not clear, however, that bufotenine is psychedelic;

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00011-6

Table 11.1 Examples of psychedelic compounds that act as serotonin agonists.

Compound	Chemical name of main constituent	Origin
LSD	Lysergic acid diethylamide	Synthetic
Mescaline	3,4,5-Trimethoxyphenethylamine	Peyote cactus
Ayahuasca	Dimethyl tryptamine	Banisteriopsis tree[a]
Takini	5-Methoxydimethyltryptamine	*Brosimum acutifolium* tree
Bufotenine	5-Methoxydimethyltryptamine	*Bufo alvarius* (*Bufo* toad)
Psilocybin	*N*,*N*-dimethyl-4-phosporylphosphoryloxytryptamine	Basidiomycota mushrooms (majority Psilocybe) and synthetic
25-NB (NBOMe)	Phenylethylamines	Synthetic

[a]Consumed with other plants that contain harmala alkaloids that prevent metabolism, making DMT orally available.

given intravenously it certainly increases blood pressure and induces irregularities of heart rate and respiration. One type of toad venom however from *Bufo alvarius* does cause delirium and hallucinations [3].

The classical psychedelics comprise three main chemical classes: (1) plant- or animal-based tryptamines; (2) ergoline derivatives, and (3) phenethylamines such as mescaline. All psychedelics have affinity for 5-HT_2 receptors, but also variously interact with 5-HT_1, HT_4, HT_5, HT_6, and HT_7 receptors. Given the somewhat promiscuous nature of most serotonin receptor antagonists, it is difficult to single out one particular subtype responsible for *all* psychedelic effects, and certainly *all* effects of psychedelic compounds. Based on drug discrimination work, and supported by blocking studies [4], 5-HT_{2A} is the strongest candidate. Preclinically, the head twitch response has proved exceedingly useful. It is clearly absent in transgenic mice in which the 5-HT_2 receptor has been deleted [5] and it is not a feature of selective 5-HT_1 receptor agonists. In human studies the psychedelic effects of LSD and psilocybin, and cognitive effects of psilocybin that overlap with psychosis are blocked by ketanserin, a nonselective 5-HT_2 antagonist with the highest affinity for 5-HT_{2A} receptors [6–9]. Some cognitive changes following psilocybin remain unaffected by ketanserin [6,7] suggesting a mediation through 5-HT_{1A} or other receptors.

The cardinal signs of a psychedelic are monitored reliably using the five-dimensional altered states of consciousness rating scale or 5D-ASC [10] capturing factors of:

1. oceanic boundlessness,
2. insightfulness,
3. religious experience,
4. blissful state, and
5. experience of unity.

Also scored on the 5D-ASC are feelings of impaired cognitive control, anxiety, and disembodiment. To ascribe all these profound changes to agonism of a single subtype of serotonin receptor is perhaps too simplistic, especially given that not all 5-HT_{2A} agonists are psychedelics, such as lisuride and ergotamine [11]. Opposing effects via other 5-HT receptors may explain this in part, but other mechanisms are likely involved [12]. Through careful biochemical investigations, Gonzalez-Maeso et al. [11] have found that hallucinogenic but not nonhallucinogenic compounds induce a characteristic 5-HT_{2A}-dependent regulation of gene expression in mouse somatosensory cortex. They hypothesize that 5-HT_{2A} receptor agonists recruit specific intracellular signaling pathways which are differentially activated by hallucinogenic versus nonhallucinogenic compounds. A candidate receptor that is responsible for the differential recruitment of signaling pathways is the group 2 metabotropic glutamate receptor (mGlu). Rodents given group 2 mGlu antagonist treatment chronically show reductions of 5-HT_{2A} receptor levels and reduced behavioral and gene expression effects of LSD in the frontal cortex [13]. The group 2 mGlu knock out mouse does not show the characteristic behavioral and gene expression effects of the 5-HT_{2A} agonists 2,5-dimethoxy-4-iodoamphetamine (DOI) and LSD and the high affinity binding site for DOI was not detected in the group 2 mGlu knock outs [14]. A selective G-protein-coupled pathway is now thought to be responsible for these group 2 mGlu 2 dependent effects of psychedelics [15]. 5-HT_{2A} receptors are closely related to the 5-HT_{2C} subtype and share many similar pharmacological properties. Both receptors are coupled to hydrolysis of phosphatidyl inositol and it has been hypothesized that activity at 5-HT_{2C} receptors might contribute an important action of hallucinogenic drugs. Indeed, hallucinogenic compounds act as 5-HT_{2C} receptor agonists while LSD is a potent agonist, its nonhallucinogenic analogue 2-bromo-LSD and lisuride are potent antagonists at 5-HT_{2C} receptors. 2-Bromo-LSD is reported to attenuate the effects of LSD in humans [16].

The subjective mind-altering properties of NMDA antagonists are markedly different from serotonergic psychedelics. NMDA antagonists do not induce the head twitch response and LSD does not generalize to the phencyclidine (PCP) discriminative stimulus, although, LSD has been shown to antagonize the PCP discriminative stimulus [17], perhaps by an antagonist action at 5-HT_{2C} receptors?

Serotonin was known to possess the ability to both excite and inhibit neuronal firing. Administration of the serotonin precursors tryptophan and 5-hydroxytryptophan together with monoamine oxidase inhibitors

could markedly enhance serotonin concentrations in the brain. With the advent of radioligand binding techniques, it was found that serotonin receptors in the brain can bind ^{3}H-labeled serotonin and ^{3}H-LSD and ^{3}H-spiroperidol. ^{3}H-serotonin, ^{3}H-spiroperidol, and ^{3}H-5-HT were shown by Peroutka to bind two distinct classes of receptors. Those binding 5-HT with high affinity were designated as serotonin 1 receptors and those binding spiroperidol were designated 5-HT$_2$. The two subtypes were further discriminated by their sensitivity to regulation by guanine nucleotides. Thus serotonin 1 receptors have high affinity for 5-HT and are guanine nucleotide sensitive. This discrimination was highly controversial at the time: the serotonin 1 receptor being dismissed as a methodological artifact. Administration of serotonin precursor plus monoamine oxidase inhibitor induces in the rat a complex behavioral syndrome known as the serotonin syndrome. The syndrome consists of a number of easily recognizable behavioral components, but early attempts to quantify the syndrome using an all or nothing binomial approach with the presence of any one component scoring positive if seen in any one observation period. With this scoring method, the syndrome was antagonized by the dopamine receptor agonist apomorphine, but when scored by frequency or intensity, apomorphine was seen to inhibit hind limb abduction (flat body posture), but enhance reciprocal forepaw treading (piano playing). Also present is the head twitch or wet dog shake response. The total syndrome can be seen after administration of many different drugs. Antagonist studies mostly led to the conclusion that these behavioral effects resulted from activation of 5-HT$_2$/5-HT$_{2A}$ receptors. Further investigation revealed that the 5-HT$_2$ receptor was in fact a composite of at least two subtypes, revealed by the displacement of ^{3}H-5-HT by spiroperidol over a very wide range of concentrations originally named 5-HT$_{2A}$ and 5-HT$_{1C}$, which is now known as 5-HT$_{2c}$. It was the high affinity component that was given the appellation 5-HT$_{2A.}$

Most prominent in inducing the 5-HT behavioral syndrome are our psychedelics, notably LSD, methoxy-*N*,*N*-dimethyltryptamine, mescaline, and quipazine. All these compounds also induce the head twitch response or wet dog shake, characteristic of compounds which are hallucinogenic in humans. The behaviors could indeed be blocked by neuroleptics which had affinity for 5-HT$_2$ receptors [18]. Then came the receptor agonist 8-hydroxy-2-di-*n*-propylaminotetralin (8-OHDPAT), which was able to displace ^{3}H-5-HT from the 5-HT$_{1A}$ component of the radioligand binding curve with an affinity several orders of magnitude higher than for

other components of the curve. This raised the question of which receptor was responsible for which behavioral component of the overall syndrome. The syndrome induced by these compounds is not a reflection of an action at postsynaptic receptors, that is, it is not dependent on the release of serotonin as is the case with a compound such as fenfluramine. However, if the affinities of compounds for the putative receptor subtypes are known then attempts to correlate this information with ability to induce or antagonize the individual components can be made. At the time the available compounds showed little if any selectivity, so a broad pharmacological analysis was required. Many of the behaviors induced by 8-OHDPAT can be blocked by spiroperidol, methiothepin, and α_1-adrenoceptor antagonists such as prazosin. Clearly, it is very difficult to accurately define the role of any one receptor. The breakthrough in this impasse came with the finding that, though independent of 5-HT release, some components of the 5-HT syndrome persisted in animals depleted of monoamines with the indole alkaloid, reserpine. Induction of the behaviors in reserpinized rats allowed its investigation in the absence of interference from the catecholamine systems [19]. Somewhat surprisingly a compound like prazosin, an α-1 adrenergic receptor antagonist, which was a very effective antagonist of the whole syndrome, was completely without effect in reserpinized rats [19]. Indeed remaining components of the syndrome in reserpinized rats, like reciprocal forepaw treading, were no longer subject to blockade by spiroperidol or ketanserin or the aforementioned prazosin. Luckily, radioligand binding showed that the β-adrenoceptor antagonists propranolol and pindolol (also a partial agonist at the 5-HT_{1A} receptor) were able to stereoselectively displace 5-HT from the 5-HT_{1A} recognition site. (−)-Pindolol and (−)-propranolol were able to antagonize reciprocal forepaw treading induced in reserpinized rats by 8-OHDPAT consistent with this behavior being mediated by the putative 5-HT_{1A} receptor. This conclusion has since been confirmed by use of the selective 5-HT_{1A} receptor antagonist WAY100635 [20].

Another putative 5-HT_1 receptor agonist RU24969 was of interest [21] because unlike 8-OHDPAT, the only behavioral component of the serotonin syndrome that is induced by the compound is hyperlocomotion. It was fascinating to find that when given to reserpinized rats, all the components of the syndrome became apparent. Clearly the 5-HT behavioral syndrome is a clear unambiguous behavioral endpoint to investigate agonist and antagonist drug actions, but here we are interested in the desire to try to quantify the more behaviorally subtle response to psychedelics.

The operant drug discrimination paradigm informs on the subjective effects of compounds and exploits the ability of the body to use internal stimuli to guide subsequent behavior, that is, the stimulus induced by a "training" drug such as LSD is used to guide the experimental animal to press a lever to obtain a food reward or avoid a shock. The beauty of the model is that at least two robust measures of drug response can be objectively recorded: (1) the choice of lever, that is, the one pressed most frequently following repeated pairing with the training drug, compared to (2) the response rate on the lever only ever associated with administration of the placebo or drug vehicle. With daily (5 days per week) training sessions, reliable discrimination can be achieved usually with 2–3 weeks of training at which point—or when the "correct" choice is made on 80%–90% of trials—varying the drug dose will yield asymptotic dose–relationship curves. The question can then be asked how similar to the training drug is this compound in its interceptive stimulus properties? Perfect generalization is achieved when the animal only selects to press the lever associated with reward when receiving the training drug. Response rate on the placebo lever serves as a measure of nonspecific effects. Complete generalization is said to occur when lever choice is directed toward the lever associated with food during training in the absence of any change in response rate for the placebo lever.

Drug discrimination studies in humans are rare and there are no published examples with psychedelics although the use of biomarkers such as neuroimaging can theoretically be used to contrast drug effects and test similarities across existing cohorts or in the same cohorts given multiple psychedelics (e.g., see Ref. [22]). To date, modern in vivo neuroimaging of brain function has been an approach that is beginning to yield much about the mode of action of LSD, psilocybin, ayahuasca, DMT, and mescaline.

Given modern in vivo imaging of brain function, observing changes in brain activation patterns following the administration of psychedelic agents is an approach that can yield much about the mode of action of LSD, psilocybin, ayahuasca, mescaline, and other agents. Earlier work [23] using EEG monitoring showed a clear reduction in oscillatory power for hallucinogens, particularly in the lower range frequency bands, but an increase in α-wave activity. Increased cortical synchrony is also a feature of the response to ayahuasca [24], which was proposed to relate to the intense synesthetic experience. Using the more robust magnetoencephalography which provides more accurate source localization, the subjective effects of

psilocybin began within seconds of its infusion accompanied by reductions in spontaneous cortical oscillatory power from 1 to 50 Hz in posterior association cortices and from 8 to 100 Hz in frontal association cortices. Large decreases in oscillatory power were seen in the default mode network an area associated with self-orientated memory and attentional processes, an area that appears central to understanding the effects of psychedelics [25]. Functional connectivity within the default mode network does appear to be altered with all psychedelics studied. With functional MRI, sensitive to the blood oxygen level dependent signal that follows neural activity, psilocybin decreases connectivity between anterior and posterior nodes of the default mode network, reduced connectivity between the default mode network, and other networks associated with generic executive/attention task performance (reviewed in Ref. [26]). Psilocybin also reduces the stability of connections, creating a multitude of transient functional connections of low stability and short cycle persistence, possibly indicating greater communication or integration of information across the brain [27]. Indeed, changes outside the default mode network are plenty [28] with decreased diversity of connections with the parahippocampal structures associated with "ego-dissolution." These analyses were all derived from the same single-blind, placebo-controlled study of healthy volunteers [29]. Using a similar experimental approach, LSD was shown to increase global functional connectivity (connections between the target regions and the rest of the brain) in the thalamus and association cortices, also increasing connectivity between primary visual cortices and several cortical and subcortical regions—the visual cortex connectivity correlated with hallucination ratings [30]. Decreases in connectivity were also observed in the parahippocampal region and the default mode network, which were both associated with "ego-dissolution," with widespread decreases in the connectivity of other brain networks except the visual system and right hemisphere frontoparietal network. Pharmacological MRI effects of LSD were blocked by the 5-HT_{2A} antagonist ketanserin again pointing to this subtype as the critical mediator of many psychological and neurophysiological effects of LSD [9]. Ayahuasca also decreases connectivity within the default mode network [31] and alters activity in the same structures during a verbal fluency task.

During emotional processing tasks, psilocybin reduces amygdala reactivity to both negative and neutral faces [32]and also reduces the impact of threat on modulation of amygdala connectivity [33]. The reactivity

reductions correlate with enhanced positive mood although it is not known if these brain changes are important for the emerging antidepressant properties of psychedelics [34–36].

Computational and mathematical models of the effects of psychedelics on brain function propose that altered form constants from bifurcation and group theory [37] contribute to hallucinations and altered prediction coding [38]. The latter could underpin multiple experiences through enhanced gain function via the 5-HT_{2A} receptors [39], which has been used to model neural changes measured with fMRI.

Limitations of these neuroimaging accounts must be overcome for a clear account of psychedelic effects including the use of larger sample sizes—an issue which has marred some of the earlier studies where many findings did not survive multiple comparisons correction [26]—overcoming the difficult issue of placebos and blinding [40], and dealing with neurophysiological confounds such as nonspecific global effects and neurovascular coupling [41,42].

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CHAPTER TWELVE

Serotonin and nociception: from nociceptive transduction at the periphery to pain modulation from the brain

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Introduction

Pain is widely defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain) [1,2]. By motivating individuals to withdraw from damaging situations, to protect a damaged body part while it heals or to avoid similar harmful experiences in the future, pain constitutes an ancient physiological system of alert and protection, critical for organisms' survival and homeostasis [3,4].

Intrinsically subjective in its nature, pain experience is a complex phenomenon involving the orchestration of different multilayer mechanisms accounting for the transduction, transmission, and modulation of the nociceptive input at different scales [5]. By definition, nociception is a submodality of somatosensation that encodes both quantitative (temporal, spatial, and intensity characteristics) and qualitative (via distinct subsets of nociceptors) information about noxious stimuli [1]. Peripheral tissues are densely innervated by a wide variety of nerve fibers that are specialized for detecting environmental stimuli [5]. Nociceptors, specialized in detecting and transmitting noxious stimuli, form a subset of these sensory fibers [5]. The peripheral processes of a subset of sensory ganglion neurons that are dedicated to nociception extend into and terminate in tissues as unmyelinated "free nerve endings" [5]. Many nociceptive fibers express the transient receptor potential V1 (TRPV1), a nonselective cation channel involved in detection of noxious

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00012-8

chemical and thermal stimuli [6]. The cell bodies of the sensory neurons for peripheral nociceptors are located in dorsal root ganglia (DRG) alongside the spinal cord and in the trigeminal (V) ganglion (for cranial nociceptors) [5]. Nociceptive signaling typically begins with activation of the terminal endings of certain sensory neurons, termed as nociceptors (*Transduction*), which trigger a train of impulses conducted by primary afferents to the neurons in the spinal cord or in the trigeminal medulla (in the case of cranial nociception) (*transmission*) [5]. Signals are integrated and processed in the dorsal horn of spinal cord or trigeminal medulla (especially the caudal medullary dorsal horn) and then projected ascendingly to the higher brain (*ascending pathways*) where they elicit the perception of pain [5]. However, it has long been known that several factors such as emotional states, anxiety, attention and distraction, past experiences, and memories can either enhance or diminish the subjective experience of pain [7,8]. At least a part of these effects is operated by top-down modulatory pathways from the brain (*descending pathways*), which by local release of neuromodulatory substances (including the inhibitory neurotransmitters GABA and glycine but also serotonin and noradrenaline) concur to facilitate or inhibit the transmission of nociceptive information at the spinal cord (Fig. 12.1) [7,8]. Although several areas of the

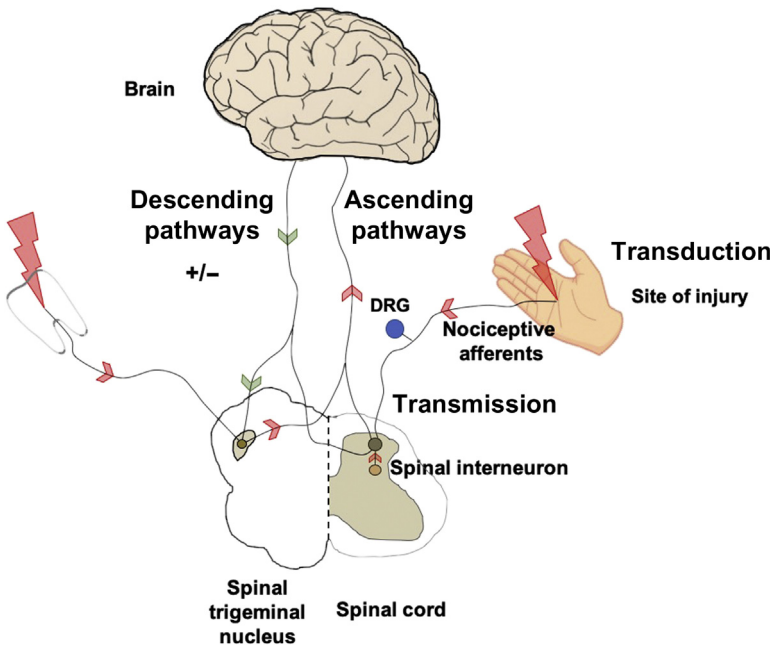

Figure 12.1 ***Pain pathways.*** *DRG*, Dorsal root ganglia.

brain seem to be involved in descending modulation of pain (cortical and subcortical), most of the descending modulatory pathways converge to brain stem relay stations such as the *locus coeruleus*, the rostral ventromedial medulla (RVM), the dorsal reticular nucleus among others, from where descending modulatory projection neurons arise to reach the dorsal horn of the spinal cord or of the medulla [8]. Past research has been critical to elucidate some of the pathways involved in nociceptive transduction, transmission, and modulation as well as its underlying neurochemistry. Despite significant advances over the last half-century, pain remains at the cutting edge of neurobiological research.

By definition, acute pain typically arises from disease or injury, being self-limited in time and not outlasting the time required for healing [2]. Chronic pain, in contrast, represents an ongoing state in the absence of physical stimulation and is an independent clinical phenomenon, associated with the persistence of painful symptoms that outlast the healing process and has no recognizable end point [2]. Chronic pain may arise after disease/injury (reflecting a maladaptive response of the nociceptive system to noxious impulses, involving either processes of peripheral and central sensitization), when previous innocuous stimuli come to be perceived as painful but also from psychological states or other less clearly identified causes (functional chronic painful syndromes) [9]. In the last cases, the physiopathological mechanisms underlying pain remain mostly elusive and are likely to involve disturbances of central modulatory pathways implicated in pain inhibition and amplification [9].

Serotonin (5-hydroxytryptamine (5-HT)) is a pivotal neuromodulator of nociception at several organizational levels from pain transduction to pain modulation from the brain [10–12]. Its actions are complex and depend on a number of factors such as the site of action, the cell type, and the type of receptor targeted, exerting both facilitatory and inhibitory actions [10–13]. Recent studies have demonstrated the implication of plastic changes in serotoninergic signaling (both in the peripheral and central nervous systems) in the pain disturbances observed during chronic pain [14,15]. Serotonin is also one of the biological targets of some of the most used pharmacological agents [serotonin–noradrenaline reuptake inhibitors (SNRIs) and tricyclics antidepressants] for the management of clinical pain during chronic painful syndromes [16].

This chapter facilitates an overview of our current knowledge about the role of serotonin in pain (including nociceptive transduction, transmission, and modulation from the brain) and the contribution of its

dysregulation to pain disturbances during chronic pain states. Implications of this knowledge for pharmacological management of pain and drug development are discussed as well.

Serotonin in central pain processing: spinal processing and descending modulation of pain from the brain

The vast majority of 5-HT in the mammalian body is located in peripheral tissues where 5-HT is actively taken up and released with other chemical mediators by platelets, mast cells, and immune cells [11,17]. Early in the immune response, 5-HT acts as a proinflammatory mediator, inducing platelet aggregation and changes in vascular tone [17]. Some of the sensory neurons responsible for noxious transduction at the lesion sites show a high expression of 5-HT receptors. Indeed, messenger RNAs for the 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, and 5-HT_7 receptor subtypes were detected in the DRG, suggesting the presence of these receptors on peripheral sensory nerves [18–20]. 5-HT_{2A} receptors were directly demonstrated on unmyelinated axons at the dermal–epidermal junction, proposing that 5-HT can act directly on these fibers [20]. This hypothesis was supported by the observation that 5-HT can exert excitatory effects on single peripheral nerve fibers [21], including specific effects on nociceptors [22]. Moreover, 5-HT increases at the periphery in situations associated with pain [23]. Some examples include (1) in skin wounds of guinea pigs, 5-HT was shown to increase for up to 24 h after the injury [24]; (2) after peripheral nerve transection and chronic constriction injury of the sciatic nerve, the 5-HT content in the lesioned nerve increased [25]; and (3) in humans, elevated 5-HT levels in the masseter muscle as retrieved by microdialysis were associated with increased pain and allodynia [26]. Activation of serotonin receptors at the peripheral termination of nociceptive C-fibers may present with pronociceptive and antinociceptive responses depending on the receptor's type activated [11] (Fig. 12.2). Data from studies of pharmacological and genetic manipulation of specific 5-HT receptors have suggested that activation of the 5-HT_{1A} [27,28], 5-HT_{2A} [29,30], 5-HT_3 [31,32], 5-HT_4 [30,33], and 5-HT_7 [34] receptor subtypes present mainly with pronociceptive effects,

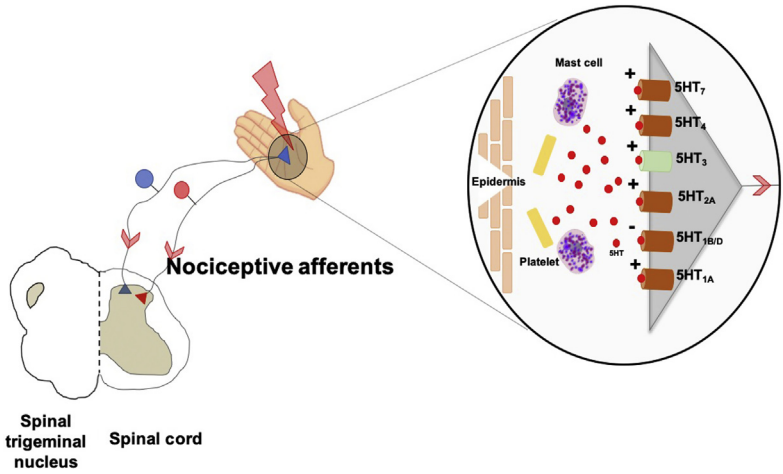

Figure 12.2 Serotonin in pain transduction at the periphery.

while the activation of the 5-$HT_{1B/D}$ [35] receptor seems to be majorly antinociceptive.

How can serotonin's signaling influence pain transduction at the periphery? Direct and indirect mechanisms of action have been proposed. The most compelling evidence of direct action on nociceptors was the discovery that 5-HT, like other inflammatory mediators, modulates tetrodotoxin-resistant sodium currents [36]. 5-HT increased the magnitude of the current and increased its rate of activation and inactivation. The 5-HT_3 receptor, which has been identified as one important receptor for 5-HT actions in the periphery, is a ligand-gated ion channel [37]. Therefore it is a possible scenario that activation of this receptor might directly enhance neuronal activity. In contrast, most studies investigating physiological actions of 5-HT in the periphery found an effect on pain or on neuronal activity only in combination with other inflammatory mediators (i.e., inflammatory soup) [38]. These studies are compatible with indirect mechanisms of sensitization of nerve fibers to algesic agents, by increasing their receptor's sensitivity/number or by convergence and mutually enhancement of downstream signaling subcellular pathways [39,40]. Interestingly, there is compelling evidence that 5-HT may act as a neuromodulator of nociception by altering TRPV1 signaling [13]. Indeed, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{3A}, but not 5-HT_{2C}, receptor mRNAs have been specifically detected in sensory neurons that express TRPV1 [41]. Depleting 5-HT, by *p*-chlorophenylalanine administration, attenuates capsaicin-induced visceral pain and reduces TRPV1

activation [42]. Treating cultured sensory neurons with 5-HT enhances intracellular calcium accumulation in sensory neurons that respond to the TRPV1 agonist capsaicin [43]. 5-HT enhances and prolongs capsaicin-evoked thermal sensitivity [41].

Bearing in mind that 5-HT is mostly pronociceptive in the peripheral nervous system, a putative interesting therapeutically option may include the local-site targeting of the peripheral serotonergic system (an approach that holds the potential to avoid some of the undesired effects associated with the use of serotonin-targeting drugs—which mainly derive from effects on central serotonin signaling). Preliminary clinical studies have reported, for instance, that local injection of the 5-HT_3 receptor antagonist, granisetron, reduces hyperalgesia and allodynia in humans [44]. Similar results were achieved with the topical application of another 5-HT_3 receptor antagonist, ondansetron, which attenuated nociception induced by intradermal capsaicin [45]. Although scarce in number, these studies are promising in their preliminary findings. Further studies evaluating the clinical relevance of peripheral 5-HT receptors-targeting for pain relieve will be more than welcomed.

Serotonin in central pain processing: spinal processing and descending modulation of pain from the brain

Alongside its role in pain transduction in the periphery, accumulating evidence over the last decades have supported the notion that 5-HT represents an important neuromodulator of nociceptive processing in the central nervous system [8]. Through many experimental studies, 5-HT has been identified (alongside noradrenaline) as one of the main neurotransmitters involved in endogenous supraspinal pain-modulating systems. These observations inspired the clinical use of drugs increasing the availability of 5-HT, like antidepressants, in the treatment of chronic pain [46].

Spinal 5-HT is largely derived from serotonergic neurons located in the RVM of the brain stem (and in particular in the nucleus raphe magnus) [47]. Serotonergic projecting tracts descend to the spinal cord through the dorsolateral funiculus and synapse with neurons in the superficial laminae of the dorsal horn of spinal cord or medulla [8]. The serotonergic RVM neurons are activated by noxious stimuli through

opioid mechanisms [8]. RVM electrical stimulation evokes the release of 5-HT in the spinal cord and 5-HT antagonists reduce the analgesia produced by this stimulation [48]. In ex vivo slice experiments containing RVM, some raphe neurons exhibit spontaneous firing, suggesting that 5-HT may be released tonically onto the dorsal horn neurons and may participate in a tonic control of spinal nociceptive balance [49]. Local injections of 5-HT-receptor agonists into the spinal cord have shown both inhibitory and facilitatory effects on pain behavior in animals, depending on the type of receptor targeted, the stimulus quality, and the test used to assess pain behavior [50–53]. Diverging results have been obtained in studies with electrical stimulation of the RVM as well, where both serotonin-mediated pain descending facilitation and inhibition have been obtained as a function of the stimulation parameters [54].

Descending serotoninergic pathways may either facilitate or inhibit nociceptive processing. But what are the mechanisms underlying these differential effects? In the spinal cord, 5-HT receptors are present on terminals of primary afferent neurons, on ascending projection neurons, and on excitatory and inhibitory spinal intrinsic interneurons [10]. It is, thus, not surprising that 5-HT effects on spinal nociceptive processing are complex and likely result from the convergence and interaction of different modulatory actions at different elements of the spinal nociceptive network. 5-HT receptors are located presynaptically at the primary afferent terminals conveying nociceptive information to the superficial layers of the dorsal horn of the spinal cord. Depending on the type of receptor-activated, presynaptic 5-HT modulation of afferents transmission can result in increased or decreased release of the excitatory peptides participating in the stimulation of spinal neurons: while activation of the $5\text{-HT}_{1B/D}$ receptors are thought to decrease the release of excitatory amino acids/peptides (such as glutamate, calcitonin gene-related peptide, substance P, and others) and, thus, the excitation of ascending spinal projecting neuron [55]; activation of 5-HT_{2A}, 5-HT_3, and 5-HT_4 receptors seems to increase their release, thus facilitating pain transmission [56]. 5-HT may also exert direct effects on ascending projection neurons: activation of 5-HT_{1A} receptor decreases neuronal activity, promoting pain inhibition [56]; in contrast, activation of the 5-HT_{3R} increases neuronal excitability and promotes pain facilitation [56]. 5-HT modulation of spinal nociceptive processing also involves in direct regulation of spinal inhibitory interneurons, one of the pivotal mechanisms of pain gating control at

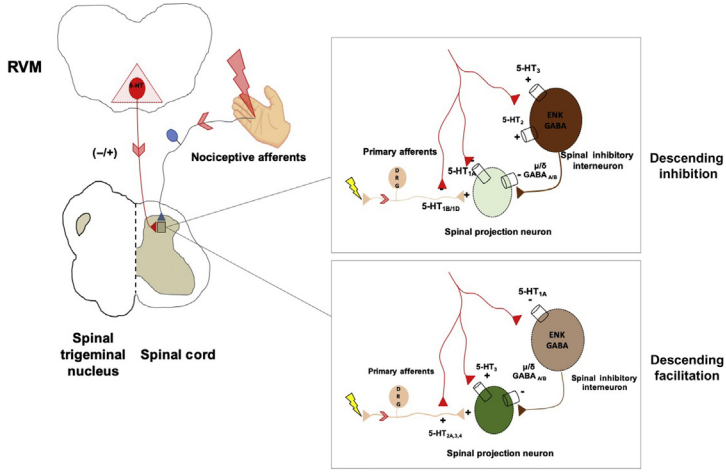

Figure 12.3 ***Mechanisms of serotoninergic descending modulation of pain.*** *DRG*, Dorsal root ganglia; *RVM*, rostroventromedial medulla.

the spinal cord: activation of 5-HT_{3R} (and presumably 5-HT_{2R}) increases intrinsic spinal GABAergic/enkephalinergic interneurons activity and promotes GABA and enkephalin release, which by stimulating μ/δ opioid and $GABA_{A/B}$ receptors at spinal ascending projecting neurons decrease its excitability, thus promoting analgesia [57,58]; activation of 5-$HT1_A$ receptor results in opposite effects, thus promoting pain facilitation [59] (Fig. 12.3).

From the electrophysiological point of view, the RVM encompasses three different functional classes of cells: ON cells, OFF cells, and neutral cells. These cells are characterized by their response to nociceptive input [60]. OFF cells typically show a transitory decrease in firing rate right before a nociceptive reflex [60]. Activation of OFF cells, either by morphine or by any other means, results in antinociception [61]. ON cells show a burst of activity immediately preceding nociceptive input and are theorized to be contributing to the excitatory drive underlying descending pain facilitation [60]. Neutral cells show no response to nociceptive input [60]. For now, there is no consensus regarding the functional class of RVM serotoninergic neurons [62]. Some authors suggest that some serotonergic RVM neurons might be "ON cells" themselves, based on the fact that these cells are labeled with the neural activity marker c-fos after noxious stimulation in a rodent model of acute pain [63]. However, electrophysiological evidence supporting this hypothesis is lacking. In contrast, most authors dispute that RVM's serotonin

neurons are not either ON or OFF cells and regard them as an independent pain-controlling system that may be under opioidergic control [62]. This hypothesis is supported by the observation that 5-HT in the *nucleus of raphe magnus* seems to be necessary for the analgesic action of morphine [64]. Supporting evidence also comes from the observation that $Lmx1b^{f/f/p}$ mice (a transcription factor required for the differentiation of postmitotic 5-HT neurons), which lack central serotonergic neurons [65], have no analgesia after administration of a kappa opioid receptor agonist and significantly reduced analgesic effects of μ- and δ-opioid receptor agonists at both spinal and supraspinal sites [66]. In contrast, morphine tolerance and morphine reward behavior develop normally [66]. Central serotonergic system is, thus, a necessary component of the supraspinal pain modulatory circuitry mediating opioid analgesia. Interestingly, local administration of serotonin at the RVM was shown to nonselectively modulate the functional activity of both ON and OFF cells, with a predominant facilitatory effect on their evoked and ongoing activity. Activation of 5-HT_{1A} by iontophoretic administration of a selective agonist was shown to depress the activity of both cells, suggesting that different receptor subtypes with different functions may coexist in this area and be functionally relevant [67]. This regulatory activity of serotonin on the ON−OFF system may represent an intrinsic RVM's regulatory mechanism through which these two systems may interact to regulate descending modulation.

The descending serotonergic pathways were also hypothesized to be a key underlying component of diffuse noxious inhibitory controls (DNIC), a unique form of descending endogenous analgesia in which the activity of trigeminal and wide dynamic range neurons is strongly inhibited when a nociceptive stimulus is applied to any part of the body, distinct from their excitatory receptive fields [68]. Originally proposed to derive from the subnucleus reticularis dorsalis (SRD), DNIC involves a complex interplay between pathways comprising the dorsolateral funiculus [68]. Human brain imaging studies had recently shown that signal changes associated with the human counterpart of DNIC, the conditioned pain modulation (CPM), including the SRD and parabrachial nucleus, with the former being controlled by cortical influences [69]. The involvement of serotonin in DNIC was initially suggested by the observations that cinanserin and metergoline (serotonin receptor blockers) [70] or *p*-chlorophenylalanine (an inhibitor of the tryptophan hydroxylase and thus of serotonin synthesis) [71] strongly reduce the inhibitory effects of DNIC while having no

significant effect on the nonconditioned responses. These data are supported by the observation of potentiation of DNIC after administration of 5-hydroxytryptophan, a precursor of serotonin synthesis [70]. Altogether, these results account for an important role of descending serotonergic pathways in DNIC; however, anatomical lesions of the RVM do not seem to abolish DNIC responses [68] (an observation that raises interesting questions about the exact anatomical pathways involved in serotonin modulation of DNIC). Converging evidence could be achieved in human studies of CPM where genetic variation in the serotonin transporter involving significant differences in serotonin transporter expression and thus serotonin availability was found to influence CPM responses of healthy volunteers in the direction predicted by preclinical studies evaluating DNIC [72].

The involvement of serotonin signaling in the analgesic effects achieved after administration of some of the most commonly used drugs for pain management during chronic pain has also been intensively demonstrated. As expected, experimental evidence supports the notion that the analgesic effects of antidepressants depend on central serotonin signaling. Indeed, fluoxetine [a selective serotonin reuptake inhibitor (SSRI)] was found to not produce any effect on thermal pain in the $Lmx1b^{f/f/p}$ mice [73]. In the clinical setting, antidepressants are far from the expected efficiency in terms of long-term pain relieve: around 50% of chronic pain patients seem to not respond to antidepressants administration and within the responders, rates of symptomatic pain relieve do not typically go beyond of 50% (sight should not be lost to the fact that SNRIs (i.e., duloxetine) and tricyclic antidepressants (i.e., amitriptyline) are in fact more efficient in pain relieving than SSRIs or selective noradrenaline reuptake inhibitors alone, according to recent clinical trials; this observation emphasizes the importance of dual targeting of serotonin and noradrenaline for higher rates of pain relieve achievement—however, this aspect is out of the scope of the current chapter) [74]. Part of this therapeutic inefficiency is likely to be explained by the heterogeneous effects of 5-HT on pain processing at several of its levels, where facilitatory effects in some elements of the pain circuit can counterbalance the inhibitory effects in others. Interestingly, the spinal excitatory action of 5-HT on 5-HT_3 receptors was also shown to be necessary for the analgesic effect of gabapentin, an anticonvulsant commonly prescribed to manage painful symptoms during neuropathic pain [75], not typically assumed to target serotonin.

Serotonin in trigeminal cerebrovascular nociception and migraine

Migraine is one of the most frequent neurological disorders in the adult population worldwide, affecting up to 12% of the general population [76]. Headache is the primary clinical manifestation and it has been associated with a hereditary or predisposed sensitivity of neurovascular reactions to certain stimuli or to cyclic changes in the central nervous system [76]. The end point of the migraine process, namely the generation of head pain, requires activation of pain-sensitive trigeminovascular afferents that initiate the overall nociceptive process through the release of vasodilator substances (especially the calcitonin gene-related peptide) and increased plasma protein extravasation after local vasodilation of intracranial and extracerebral blood vessels [77]. In animal models, such activation can be induced by cortical spreading depression (CSD), a condition of neuronal depolarization and ionic shifts has been visualized in migraine sufferers by functional brain imaging techniques [78].

Among the many neurotransmitters in the brain, the serotonergic system has been one of the most convincingly implicated in migraine pathophysiology [79]. Changes in serotonin metabolism and in the processing of central serotonin-mediated responses during and in between migraine attacks have led to the suggestion that migraine is a consequence of a central neurochemical imbalance that involves a low serotonergic disposition [80,81]. Although the exact cascade of events that link abnormal serotonergic neurotransmission to the manifestation of head pain and the accompanying symptoms has yet to be fully understood, recent evidence suggests that a low serotonin state facilitates activation of the trigeminovascular nociceptive pathway, as induced by CSD [82]. Whether the activation of trigeminovascular fibers can be achieved by a change in the firing of dorsal raphe neurons remains speculative and the mechanisms involved poorly described.

Direct anatomical connection between the serotoninergic raphe neurons and brain blood vessels had been described [83]. Moreover, brain vessels seem to be able to respond to changes in central serotonin neurotransmission [84]. The vascular consequences of intracerebrally released serotonin point to a major vasoconstrictor role, resulting in cerebral blood flow decreases in several brain regions such as the neocortex. However, vasodilatations, as well as changes in blood–brain barrier permeability,

which are blocked by serotonin receptor antagonists also can be observed (for an overview of the serotonin's involvement in the regulation of brain microcirculation, see Ref. [84]). Interestingly, lesions of the dorsal raphe nucleus seem to induce hypersensitivity to serotonin in isolated cerebral arteries [85]. This observation is compatible with the suggested hypersensitivity of neuronal 5-HT_1 receptors in migraine sufferers [86], which one may speculate can arise due to their chronic low serotonin disposition. Based on this evidence, some authors have suggested an abrupt increase in brain stem serotonin neuron activity and/or platelet discharge of serotonin following a stressful stimulus may activate sensitized neuronal and vascular serotonin receptors and triggers the pain-generating process during migraine attacks [87–90]. Supporting evidence came from the observation that rats in low serotonin state following treatment with the serotonin depleting drug *p*-chlorophenylalanine display enhanced CSD waves and an increased number of activated neurons within the *trigeminal nucleus caudalis* [91]. These results strongly support a link between low 5-HT neurotransmission and migraine headache by showing that a low brain 5-HT disposition facilitates CSD-induced trigeminal nociception, probably through increased cortical excitability and sensitivity of the trigeminovascular pathway.

Trends in serotonin drug development for pain: the paradigmatic example of 5-HT_{1F} agonists in migraine attacks

Migraine acute therapy is nowadays based on specific and nonspecific drugs but up to 40% of episodic migraineurs still present with unmet treatment needs [76]. Triptans constituted one of the first attempts to develop more specific serotonin-targeting drugs for pain management, in this case related to the trigeminal system [92]. Triptans all bind with high affinity to three serotonin receptor subtypes: the 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} [92]. 5-HT_{1B} mRNA is densely localized within the smooth muscle and at a less extent in the endothelium of cerebral blood vessels [93]. This vascular distribution of 5-HT_{1B} receptor has been shown to mediate the vasoconstrictive properties of triptans, responsible for potential cardiac adverse events, which significantly limits

its use in high cardiovascular risk populations [94]. 5-HT_{1B} is also expressed in the nociceptive afferents conducting nociceptive information to the spinal cord/trigeminal ganglia and its activation in this location was found to reduce the release of facilitatory neurotransmitters responsible for the stimulation of central nociceptive circuits [95,96]. Some years past after the introduction of triptans in the clinical practice, the already accumulated evidence suggests that over 35% of chronic migraine pains do not benefit from triptans administration and in another significant percentage of the patients, triptans are contraindicated [97]. In an attempt to override some of its unfavorable side-effects profile, attention has been recently redirected to the 5-HT_{1F} receptor, a serotonin receptor number 1 subtype (previously known as 5-HT_{1Ebeta}) [98]. This receptor is located primarily in the hippocampus, cortex, and dorsal raphe nucleus but it is also present in blood vessels, within the trigeminal ganglion and the trigeminal nucleus caudalis (Sp5C) [98]. Contrary to the 5-HT_{1B} receptor, 5-HT_{1F} receptor lacks vasoconstrictive properties, making it an attractive target for new antimigraine drugs [99]. Promising results were found in the observation that selective activation of 5-HT_{1F} receptor potently inhibited markers associated with electrical stimulation of the trigeminal ganglia in migraine models [99,100]. So far two selective 5-HT_{1F} agonists have been tested in human trials for migraine: LY334370 and lasmiditan [101]. Both molecules were efficient in attenuating migraine attacks with efficacy in the same range as oral sumatriptan 100 mg, the gold standard for triptans [101]. The LY334370 project withdrew because of toxicity in animals, while lasmiditan is still under testing. Preliminary evidence from the first clinical trials of lasmiditan in migraine showed that, although most likely it is effective in the treatment of migraine attacks, a high incidence of central nervous system related could be identified after oral dosing [102,103]. If confirmed in larger phase III studies, this might adversely limit the use of this highly specific nonvascular acute treatment of migraine. Clearly larger studies including the parameters of patients' preferences are necessary to accurately position this new treatment principle in relation to the triptans. Although 5-HT_{1F} agonists may reveal clinically irrelevant in the next future, it is undeniable that they constitute, alongside triptans, a good example of the effort and investment necessary to improve the management of pain by targeting specific nociceptive inhibitory elements of the serotonin signaling cascade.

Serotonin disturbances during chronic pain

The mechanisms involved in the development of chronic pain are varied and complex [104]. Pain processes are plastic and unrelieved pain may lead to changes in the neural structure and function involved in pain generation [105]. The exact mechanisms involved in the pathophysiology of chronic pain are not well understood, but rapid and long-term plastic changes are thought to occur in several parts of the somatosensory system involved in the transmission and modulation of pain following injury [105]. Peripheral and central sensitization of sensory nerve fibers is the primary mechanism underlying hypersensitivity to pain after injury—both for noxious (hyperalgesia) and innocuous (allodynia) stimuli [106]. Prolonged or intense exposure to noxious stimuli, for example, chemical mediators released during inflammation, enhances the responsiveness of nociceptive nerve fibers. This process, termed peripheral sensitization, involves alterations in the threshold for activation of nociceptors, normally accompanied by an upregulation of voltage-gated sodium channels [107]. Peripheral sensitization leads to increased action potential firing and transmitter release in the dorsal horn of the spinal cord, where somatosensory information is processed [107]. Dorsal horn neurons react to this rising input with amplified excitability, a process designated central sensitization. In the case of neuropathic pain (pain caused by damage or disease affecting the somatosensory nervous system), peripheral nerve lesion evokes stimulus-independent (ectopic) activity in nerve fibers [107]. Intrinsic spinal disinhibition further enhances the abnormal input from the lesioned nerve [108]. Worsened by a relative deficit in transmitter uptake, increased glutamatergic transmission causes excitotoxic cell death, reducing the number of inhibitory interneurons [107]. Their loss and a shift in descending modulatory pathways from the brain stem produce a profound imbalance between inhibition and excitation, favoring pain facilitation [109].

Considering its wide role in pain generation, processing, and control, several studies along the last decades have been examining the role of serotonin disturbances in chronic pain. The already accumulated evidence is generally compatible with a contribution of serotonin dysregulation for pain disturbances during chronic pain, although the direction of its effects is not completely clear [14]. Selective ablation of descending serotonergic RVM neurons reduces tissue or nerve injury-induced allodynia and hyperalgesia [110]. Electrophysiological and behavioral studies have confirmed

that nerve injury is indeed associated with an enhancement of the descending 5-HT facilitatory modulation, inducing mechanical hypersensitivity by activation of spinal 5-HT_3 receptors [62,111]. Spinal 5-HT_3 receptors have also been reported to be involved in the pronociceptive effects of descending 5-HT in chronic inflammatory [112], postoperative [113], and diabetic pain models [62]. In contrast, others have suggested that the development of neuropathic pain results from a decrease in the descending inhibitory 5-HT pathways [114]. Supporting this hypothesis, in mice deficient for the serotonin transporter a reduced level of 5-HT in the spinal cord is correlated with bilateral mechanical allodynia in a neuropathic pain rodent model [115]. In fact, decreases in the basal release of 5-HT in the spinal cord has been reported in several models of neuropathic pain [116–118]. However, in chronically inflamed animals, the release of 5-HT in the raphe seems to be increased [119]. Similar results were reported in a diabetic neuropathic pain model, where 4 weeks after diabetes induction, mechanical hyperalgesia and chemical allodynia were accompanied by an increase in the number of serotonin-producing neurons at the RVM and an increase in the spinal serotonin content, when compared to healthy control animals. These findings are strongly supported by a recent optogenetic study demonstrating that selective activation of RVM 5-HT neurons exerts a predominant effect of pain facilitation under control conditions [120], suggesting that enhanced serotoninergic descending facilitation may indeed represent an important mechanism accounting for the transition from acute to chronic pain. These modifications in 5-HT release are often accompanied by other changes in descending serotoninergic pathways that may interact with the modulatory effect of 5-HT. Preclinical studies in rodent models have suggested that chronic pain is associated with a strong upregulation of 5-HT receptors in DRG and dorsal horn in spinal cord [121]. Profound subtype-specific changes in the functional modulatory activities of spinal 5-HT receptors after spinal nerve ligation was described as well [122]. In the clinical setting, abnormal activity of the 5-HT system is also present in some diffuse pain states, such as the fibromyalgia syndrome [123]. Decreased level of serum 5-HT and its metabolite 5-HIAA was reported in the cerebrospinal fluid of patients with fibromyalgia. In these patients, serum 5-HT concentrations correlate with the intensity of painful symptoms [124]. In addition, 5-HT receptors are increased in platelets of patients with fibromyalgia [123]. These findings match well with the pain disturbances found in rodent models of fibromyalgia-like pain based on serotonin (between other amines) depletion after reserpine administration [125].

Conclusion

Over the last years, considerable research effort has been put to unravel the role of 5-HT on acute or chronic pain states as well as to identify the respective 5-HT receptors involved. Peripheral 5-HT seems to contribute to pain transduction and peripheral sensitization through direct or indirect mechanisms. In the central nervous system (and depending on acute or chronic pain states), the descending 5-HT pathways can exert both an inhibitory or facilitatory influence, depending on the site of action and the subtype of receptor targeted. Such complexity is likely to explain why numerous important questions with regard to the role of 5-HT in pain remain unanswered and disparate findings are sometimes achieved in preclinical and clinical research. Determining how serotonin system is recruited to either inhibit or facilitate nociception under different conditions, as well as the subtype of receptors involved is an important challenge in terms of drug development and repurposing for pain in the near future. In fact, while global enhancers of serotonin neurotransmission seem to present with limited efficiency, specific compounds targeting inhibitory elements of the serotonin signaling family may hold the potential to significantly improve the clinical management of a high number of chronic pain patients.

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CHAPTER THIRTEEN

Serotonin and feeding regulation

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In addition to serotonin's functions in the modulation of mood and emotion [1], serotonin also has a well-established role in the regulation of food intake [2,3]. On the whole, the empirical evidence consistently supports the role of serotonin in the tonic inhibition of food consumption [4–6], and particularly in the inhibition of carbohydrate consumption [7].

The effect of serotonin on food consumption is at least partially mediated by central homeostatic centers of appetite control, including those within the hypothalamus [8–12], the parabrachial nucleus [13,14], and the nucleus of the solitary tract (NTS) [15,16]. However, there is some evidence to suggest that serotonin may also impact feeding via its effects on hedonic processes mediated by the nucleus accumbens [17–20] and the ventral tegmentum [21].

The precise effect of serotonergic activity on feeding is moderated by many factors, including the specific serotonin receptors being stimulated, the location of receptor stimulation, the nutritional state of the animal, and the point within the meal when serotonin or its agonists are introduced [2,21]. The remainder of this chapter will aim to explain the effects of serotonin on feeding under each of these alternate moderating conditions, and will be organized primarily by serotonin receptor type.

Type 1 serotonin receptors

The serotonin 1a receptor

Type 1 serotonin receptors couple to G_i proteins on the cell membrane of neurons, producing hyperpolarization of the cell when activated. As will be explained in further detail later in this chapter, however, the

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00013-X

activity of the 5-HT_{1a} receptor differs depending on the nutritional state of the animal, with important effects for feeding behavior [22].

Most studies have demonstrated that administering high systemic doses of 5-HT_{1a} agonists to food-restricted animals results in a hypophagic feeding response [23–29]. There is evidence, however, to suggest that this decreased feeding may be a secondary effect of behavioral disruption associated with induction of the serotonin syndrome. Indeed, at least half of the studies measuring the effect of 5-HT_{1a} receptor activation in food-restricted animals have reported that the low posture and stereotyped behavior characteristic of the serotonin syndrome coincided with suppressed feeding behavior [24,26–28]. These findings thus cast doubt on the behavioral specificity of 5-HT_{1a} activity in food-deprived animals, raising the possibility that suppression in food intake may reflect general behavioral disruption rather than a specific hypophagic effect. In support of this hypothesis, it has been found that low doses of 5-HT_{1a} agonist 8-Hydroxy-n,n-dipropylaminotetralin (8-OH-DPAT) does not elicit symptoms of the serotonin syndrome, and also fails to yield any significant effect on feeding in food-restricted animals [27,30]. Therefore, this evidence suggests that feeding suppression to 5-HT_{1a} agonism does not occur in food-restricted animals, except when co-occurring with general behavioral disruption.

In non-food-restricted animals, however, the systemic administration of 5-HT_{1a} receptor agonists appears to have a very different effect on food consumption. On the whole, the systemic injection of 8-OH-DPAT in non-food-restricted animals tends to increase feeding [24,27,29–34], although no significant effect on feeding was found by Bovetto & Richard [35]. To add an additional point of complexity, the effects of 5-HT_{1a} activity appear to be moderated by weight status and age as well as nutritional status. Specifically, Voigt, Schade, Fink, and Hörtnagl [36] have found that systemic 8-OH-DPAT administration increases feeding in non-food-deprived, lean 3- and 6-month old Zucker rats, while suppressing feeding in obese rats of the same ages. At 10 months of age, 8-OH-DPAT continued to stimulate feeding in lean rats, while having no effect on feeding in obese rats [36].

As serotonin is primarily produced within the raphe nuclei, it stands to reason that serotonergic activity within this region lies upstream of serotonin's specific effects on feeding. In support of this point, it has been found that injection of 8-OH-DPAT into the dorsal and medial raphe increases food intake in nonfood-restricted rats [37–39]. In terms of the

mechanism of action, the evidence suggests that 5-HT_{1a} receptor activity stimulates feeding in non-food-deprived animals via agonism of somatodendritic autoreceptors on serotonin-synthesizing neurons within the dorsal raphe, thus preventing 5-HT release [27,33,40–43]. The hyperphagic effect of 5-HT_{1a} activity within the dorsal and medial raphe does not appear to be mediated by serotonergic activity within the hypothalamus [39]. This finding is supported by evidence demonstrating that the simultaneous injection of serotonin into the paraventricular nucleus of the hypothalamus does not block the hyperphagic effect of 5-HT_{1a} activity within the dorsal and medial raphe nuclei [39].

The hyperphagic effects of 5-HT_{1a} activity within the medial and dorsal raphe nuclei are more likely mediated by dopaminergic mechanisms [44,45]. Fletcher and colleagues have found that injections of dopamine antagonists, administered systemically or directly into the striatum, antagonize the hyperphagic effects of 8-OH-DPAT in the raphe nuclei [44,45]. This mediating dopaminergic activity appears to be primarily located within the prefrontal cortex [46], as well as within the raphe nuclei [47].

While serotonergic activity within the hypothalamus does not appear to directly mediate the hyperphagic effects of 5-HT_{1a} stimulation within the dorsal and medial raphe, there is evidence to suggest that 5-HT_{1a} activity with the hypothalamus can independently regulate feeding [48]. Injections of 8-OH-DPAT within the arcuate nucleus of the hypothalamus, in particular, have been found to suppress feeding in non-food-restricted female rats in both the diestrous and estrous phases, and 8-OH-DPAT within the lateral hypothalamus suppresses feeding at high doses during the estrous phase [48]. There is some additional evidence to suggest that 5-HT_{1a} activity within the paraventricular nucleus of the hypothalamus also suppresses food intake, and particularly carbohydrate intake [9], although an earlier study did not find any significant effects on feeding [23]. It is notable that this suppression of feeding following central injection of a 5-HT_{1a} agonist in non-food-restricted animals runs counter to the hyperphagic effects seen with systemic injection of the same 5-HT_{1a} agonist. Further research is required to consolidate these discordant findings.

The 5-HT_{1a} receptor may also play a specific role in the modulation of hedonic feeding [21]. The nucleus accumbens and ventral tegmentum are heavily involved in reward processing and motivation, including the "liking" and "wanting" of taste reward [49]. It has been found that stimulation of the 5-HT_{1a} receptor within the nucleus accumbens reduces

feeding in nonfood-restricted male rats [19]. Stimulation of the 5-HT_{1a} receptor within the ventral tegmentum reduces feeding in both food-restricted and nonfood-restricted male rats [21]. These findings suggest a role for serotonin 1a receptors in down-regulating feeding motivation underpinned by hedonic neural circuits.

Despite the evidence for the role of 5-HT_{1a} receptor activity in regulating feeding within wild-type animals, genetic evidence suggests that the 5-HT_{1a} receptor may not be critical for the normal regulation of food intake. The majority of studies have not found any significant differences in food intake or body weight in 5-HT_{1a} knockout mice, when compared to wild-type littermates [50–53] (although see Yadav et al. [54] for an exception). On the whole, these findings therefore suggest a diet- and weight-dependent role of 5-HT_{1a} receptor activity on feeding in wild-type mice, which can be compensated for by other physiological mechanisms in the 5-HT_{1a} knockout mouse model.

The serotonin 1b receptor

Overall, the effect of systemic administration of 5-HT_{1b} receptor agonists on feeding is far more consistent than that of 5-HT_{1a} receptor agonists. The majority of the evidence demonstrates that systemic injections of the selective 5-HT_{1b} receptor agonist CP-94,253 inhibit feeding in both food-restricted [8,55,56] and nonfood-restricted [57,58] mice and rats. Further support for the mediating role of the 5-HT_{1b} receptor in the hypophagic response to CP-94,253 comes from evidence that this hypophagic effect is attenuated by pretreatment with the 5-$HT_{1b/d}$ receptor antagonist GR-127,935 or the selective 5-HT_{1b} receptor antagonist SB 224289, but not the 5-HT_{1a} receptor antagonist WAY-100,635 [58]. Additionally, the hypophagic effect of CP-94,253 has been shown to be stronger in wild-type mice, when compared to 5-HT_{1b} receptor knockout mice [8]. This pattern of evidence thus further adds support for the role of the 5-HT_{1b} receptor in down-regulating subsequent food intake.

Similar hypophagic responses result from systemic injection of the 5-HT_1 receptor agonist RU-24969 in both food-restricted [23,24,26,59,60] and nonfood-restricted [35] mice and rats. This hypophagic effect was blocked by the nonselective 5-HT antagonist metergoline, but not the 5-HT_{2C} antagonist mianserin. This pattern of findings suggests that the 5-HT_{1b} receptor is a more likely candidate for mediating the hypophagic effect of RU-24969 than the 5-HT_{2C} receptor [59]. It should be noted

that the hypophagic effects observed following systemic administration of 5-HT_{1b} receptor agonists are likely mediated by central mechanisms, as systemic injections of the 5-HT_{1b} receptor agonist CP-93,129, which has poor ability to penetrate the blood–brain barrier, do not impact feeding in mildly food-restricted male rats [55].

Dexfenfluramine, a compound which promotes the efflux of serotonin within the brain also suppresses subsequent food intake [61]. There is evidence to suggest that the 5-HT_{1b} receptor may mediate the hypophagic effects of systemically administered dexfenfluramine [62,63]. Specifically, it has been found that 5-HT_{1b} receptor knockout mice do not exhibit the hypophagic response to systemic dexfenfluramine seen in wild-type mice [62,63], thus suggesting that the 5-HT_{1b} receptor is required to mediate this effect.

One central candidate region mediating the effects of 5-HT_{1b} receptor agonists on feeding is the parabrachial nucleus of the pons. Injection of the selective 5-HT_{1b} receptor agonist CP-93,129 into the parabrachial nucleus decreases feeding in both food-restricted and non-food-restricted rats [13]. Further supporting the mediating role of the 5-HT1b receptor in this effect, it has been found that this hypophagic effect can be blocked by pretreatment with the 5-HT_{1b} receptor antagonist SB-216641 [14]. Furthermore, systemic injections of CP-94,253 at hypophagic doses increase c-fos immunoreactivity within the parabrachial nucleus, thus further adding support to the hypothesis that the parabrachial nucleus mediates the hypophagic effects of peripherally administered 5-HT_{1b} agonists [8].

There is evidence to suggest that 5-HT_{1b} activity within the paraventricular nucleus of the hypothalamus also inhibits feeding [23,64]. However, the hypophagic effect mediated by 5-HT_{1b} receptor activity within the paraventricular nucleus is much less potent than that mediated by 5-HT_{1b} receptor activity within the parabrachial nucleus [13]. Nonetheless, systemic injections of CP-94,253 at hypophagic doses increase c-fos immunoreactivity within the paraventricular nucleus, highlighting it as another region which may mediate the effects of systemic 5-HT_{1b} receptor agonists [8].

A likely downstream mechanism of action for central 5-HT_{1b} receptor stimulation involves the action of Neuropeptide Y (NPY) and the melanocortin signaling system [57]. NPY is a potent orexigen that initiates feeding via stimulation of neurons within the arcuate nucleus of the hypothalamus containing Agouti-related protein [65]. These neurons

subsequently exert a hyperphagic effect by inhibiting anorexigenic proopiomelanocortin (POMC) neurons via the Melanocortin 3 and 4 receptors [66].

The selective 5-HT_{1b} receptor agonists CP-94,253 and CP-93,129 have been found to hyperpolarize NPY-responsive cells within the arcuate nucleus, thus decreasing the likelihood of NPY-induced feeding [57]. The hypophagic effect of 5-HT_{1b} receptor stimulation is likely to be mediated downstream by the Melanocortin 4 receptor, as Melanocortin 3 receptor knockout mice exhibit similar hypophagic response to systemic injections of CP-94,253 compared to wild-type mice, while Melanocortin 4 receptor knockout mice do not respond to systemic injections of CP-94,253 [57].

There is mixed evidence for the effect of 5-HT_{1b} activity on hedonic eating [19,21]. 5-HT_{1b} receptor stimulation within the nucleus accumbens does not reduce chow consumption in food-restricted rats [19]. While 5-HT_{1b} agonist administration within the ventral tegmentum can reduce consumption of normal chow consumption in food-restricted rats, it does not impact palatable food consumption in non-food-restricted rats [21]. In a complementary set of findings, administering 5-HT_{1b} antagonists to the ventral tegmentum reduces feeding only for palatable food in nonfood-restricted rats, and does not affect normal chow consumption in food-restricted rats [21]. It is possible that 5-HT_{1b} activity within the ventral tegmentum modulates feeding motivation mediated by dopaminergic outputs, and is moderated by the nutritional state of the animal [67]. However, more research is required to identify the mechanism mediating the opposite feeding effects of 5-HT_{1b} receptor activity within the ventral tegmentum in food-restricted versus non-food-restricted conditions.

Other receptors in the 5HT1 family

Research has not supported a major role for the $5HT_{1d}$, $5HT_{1e}$, and $5HT_{1f}$ receptors in influencing feeding. (NB: It should be noted that there is no $5HT_{1c}$ receptor, as it has been re-classified as the 5-HT_{2c} receptor [68,69]). Although some studies have demonstrated that the 5-$HT_{1b/1d}$ antagonist GR-127,935 can attenuate the hypophagic effect of systemic CP-94,253 [8,58], this effect is likely mediated by 5-HT_{1b} receptors, as CP-94,253 is a selective 5-HT_{1b} agonist.

Type 2 serotonin receptors

The serotonin 2a receptor

Serotonin type 2 receptors couple with G_q proteins on neuronal cell membranes and evoke the depolarization of the cell. Systemic administration of 5-HT_{2a} receptor agonists tend to decrease feeding in food-restricted mice, although interpretation of these findings is made difficult by the nonselectivity of many agonists used to study the 5-HT_{2a} receptor [70]. Fox and colleagues, for example, have demonstrated that systemic injection of the 5-HT_{2a} agonist (4-Bromo-3,6- dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide (TCB2) reduces feeding in food-restricted male mice [70]. Previous studies have found TCB2 to be selective for the 5-HT_{2a} receptor [71]; however, some doubt is cast on these findings based on the fact that the selective 5-HT_{2a} antagonist MDL-11,939 did not block the hypophagic effects of TCB2 in Fox et al.'s study [70]. This may potentially be due to differing levels of blood–brain barrier penetration by TCB2 and MDL-11,939, although future research is required to corroborate this hypothesis.

Further evidence for the hypophagic effects of 5-HT_{2a} receptor activity comes from research using the 5-$HT_{2a/2c}$ agonist 2,5-Dimethoxy-4-iodoamphetamine (DOI). Several research studies have found that systemic injection of DOI suppresses food intake in food-restricted male mice and rats [26,60,70]. The hypophagic effect of systemic DOI is likely mediated by the 5-HT_{2a} receptor, as pretreatment with the selective 5-HT_{2a} antagonist MDL-11,939 has been found to block the hypophagic effect of systemic DOI [70]. However, there is considerable doubt as to whether DOI in fact exerts a behaviorally specific hypophagic effect, as both Simansky and Vaidya [26] and Kitchener and Dourish [60] have found that reductions in feeding were accompanied by hyperactivity and disruptions in the continuity of feeding. Therefore, feeding effects observed from studies utilizing DOI should be interpreted with caution.

Despite the inconclusive findings yielded by studies of systemic 5-HT_{2a} agonist administration described above, there is evidence to suggest that the 5-HT_{2a} receptor influences feeding via central mechanisms involving melanocortin signaling within the paraventricular nucleus of the hypothalamus [10,12]. Injecting NPY directly into the paraventricular nucleus of the hypothalamus consistently evokes feeding via melanocortin

signaling [65,66]. However, pretreatment of DOI within the paraventricular nucleus blocks the hyperphagic effects of NPY [10,12]. It is likely that 5-HT_{2a} activity, specifically, antagonizes the hyperphagic effects of NPY, as co-administration of DOI and the selective 5-HT2a antagonist spiperone does not block NPY's hyperphagic effects [12].

Central 5-HT_{2a} receptor activity may also have a tonic hypophagic effect mediated by the prefrontal cortex, as direct injection of the selective 5-HT_{2a} antagonist MDL-11,939 into the ventromedial prefrontal cortex increases feeding in non-food-restricted male rats [72]. There is no evidence that 5-HT_{2a} receptor activity modulates hedonic or hunger-driven eating underpinned by the ventral tegmentum [21].

The serotonin 2b receptor

There is some evidence to suggest that the 5-HT_{2b} receptor may partially mediate the hypophagic effect of systemically injected dexfenfluramine, as food-restricted mice lacking the 5-HT_{2b} receptor do not exhibit the hypophagic response to dexfenfluramine seen in wild-type mice [73]. Furthermore, the hypophagic effect of systemic dexfenfluramine is abolished in wild-type mice pretreated with the 5-HT_{2b} antagonist RS127445 [73]. The 5-HT_{2b} receptor may also mediate novelty-induced hypophagia in mice, as systemic injection of the selective 5-HT_{2b} antagonists SB215505 and SB20471 have both been shown to attenuate novelty-induced hypophagia in food-restricted mice [74].

Contrary to these systemic effects, there is preliminary evidence to suggest that central 5-HT_{2b} receptor activity may increase feeding, as injection of the 5-HT_{2b} agonist BW 723C86 into the right lateral ventricle increases feeding at low doses (1−10 μg) in rats [75]. There is no evidence to suggest that 5-HT_{2b} receptor activity in the ventral tegmentum affects hedonic or hunger-driven food intake [21].

The 5-HT_{2b} receptor is a poor candidate for pharmacological feeding regulation due to the fact that it is also expressed on cardiac tissue and is believed to have partially mediated deleterious cardiac effects of serotonergic appetite suppressant drugs prescribed in the latter half of the 20th century [76]. As a result, there is a relative scarcity of recent research into the feeding effects of the 5-HT_{2b} receptor.

The serotonin 2c receptor

On the whole, activity of the serotonin 2c (5-HT_{2c}) receptor is associated with a reduction in subsequent feeding, and potentially exerts the most

potent and behaviorally specific effect on feeding of any 5-HT receptor subtype [2]. For example, systemic administration of meta-Chlorophenylpiperazine (mCPP), a 5-$HT_{1b/2c}$ agonist, has been found to have hypophagic effects in both food-restricted and non-food-restricted rats [59,60,77] and in humans [78]. The hypophagic effect of mCPP is at least partially mediated by the 5-HT_{2c} receptor [77]. Evidence for the mediating role of the 5-HT_{2c} receptor comes from research showing that the hypophagic effect of mCPP is attenuated by pretreatment with antagonists that have a high affinity for the 5-HT_{2c} receptor (metergoline and mianserin), but not nonselective 5-HT2 receptor antagonists (ketanserin and ritanserin), or compounds antagonizing other 5-HT receptor families [77].

Additionally, systemic injection of the selective 5-HT_{2c} receptor agonist ORG 37864 has been found to decrease food intake in non-food-restricted female rats [79]. The hypophagic effect of ORG 37864 is further potentiated by pretreatment with the selective 5-HT_{1b} agonist CP-94,253; and the hypophagic effect of mCPP, a 5-$HT_{1b/2c}$ agonist, was found to be greater than equivalent doses of ORG 37864 or CP-94,253 alone [79]. This pattern of findings suggests that the 5-HT_{1b} and 5-HT_{2c} receptors act together in an additive manner to reduce subsequent food intake.

Systemic injections of the selective 5-HT_{2c} agonists Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 also decrease the consumption of palatable food in non-food-restricted female rats [80], and systemic injection of Ro 60-0175 decreases the consumption of regular chow in non-food-restricted male rats [81]. Direction injection of Ro 60-0175 into the paraventricular nucleus decreases subsequent food intake, and particularly carbohydrate intake, in non-food-restricted male rats [9]. Martin et al. [80] found that administration of Ro 60-0175 was associated with penile erection in male rats, which is characteristic of the serotonin syndrome. However, both López-Alonso et al. [9] and Clifton et al. [81] reported that the behavioral satiety sequence (indicating the usual progression from eating, to postingestive grooming, to rest [82]) was maintained following administration of Ro 60-0175, suggesting that the effects of this selective 5-HT_{2c} agonist are behaviorally specific to feeding.

Research using the selective 5-HT_{2c} agonist VER23779 provides further evidence for the hypophagic effects of 5-HT_{2c} activity [83]. Systemic injection of VER23779 decreases consumption of palatable wet mash in non-food-restricted male mice. Systemic VER23779 also decreases chow intake in food-restricted male mice, and does not disrupt the behavioral

satiety sequence [83]. Adding further support to the mediating role of the 5-HT_{2c} receptor in this effect, Somerville et al. [83] also found that the hypophagic effect of VER23779 was blocked by pretreatment with the 5-HT_{2c} antagonist SB242084, but not the 5-HT_{1B} receptor antagonist SB224289 or the 5-HT_{2A} receptor antagonist MDL-100907.

Central injection of VER23779 has been found to induce c-fos immunoreactivity in the posterior part of the NTS, within the paraventricular nucleus of the hypothalamus, and in the central, anterior basolateral, and posterior basolateral nuclei of the amygdala [83]. These findings suggest that the hypophagic effect elicited by systemic administration of 5-HT_{2c} agonists may be mediated by central mechanisms within the NTS, paraventricular nucleus, and amygdala. Further studies testing c-fos immunoreactivity following systemic perfusion of selective 5-HT_{2c} agonists are required to corroborate this hypothesis. There is no evidence to suggest that 5-HT_{2c} receptor activation with in the ventral tegmentum affects the consumption of chow in food-restricted rats, or the consumption of palatable food in non-food-restricted rats [21].

The hypophagic effects of 5-HT_{2c} receptor activity are likely mediated by melanocortin signaling. Support for this hypothesis comes from research showing that melanocortin 4 receptor knockout mice do not exhibit the expected hypophagic response to systemic injections of the selective 5-HT_{2c} agonist BVT.X, as is observed in wild-type mice [84]. Additionally, administration of mCPP has been found to depolarize POMC neurons within the arcuate nucleus, although it is unclear whether this is primarily due to the actions of 5-HT_{1b} or 5-HT_{2c} agonists [85].

5-HT_{2c} receptor activity likely exerts a tonic hypophagic effect, as systemic injections of the selective 5-HT_{2c} antagonist RS-102221 increase feeding in non-food-restricted rats [86]. Administration of the 5-HT_{2c} receptor antagonist cyproheptadine has also been found to increase feeding and weight gain in asthmatic children [87,88], people with tuberculosis [89], people with cystic fibrosis [90], and women with anorexia nervosa [91]. However, interpretation of this effect is confounded by cyproheptadine's nonselectivity. As cyproheptadine has anticholinergic and antihistaminergic action in addition to antagonizing 5-HT_{2a} and 5-HT_{2c} receptors, it is therefore difficult to determine what mechanism of action accounts for cyproheptadine's hyperphagic effects.

There is evidence to suggest that the 5-HT_{2c} receptor also mediates the hypophagic action of the drugs sibutramine [92] and d-fenfluramine

[93,94]. However, both drugs have since been removed from the market due to deleterious cardiovascular side effects [95]. One of the most promising antiobesity drugs currently approved by the FDA, however, is the selective 5-HT_{2c} drug lorcaserin (APD356). The effects of lorcaserin are discussed in further detail in a later section of the current chapter (see *Serotonergic approaches to weight loss*).

Type 3 serotonin receptors

Rather than coupling to G-proteins on the neuronal cell membrane, in a similar fashion to serotonin type 1 and 2 receptors, serotonin type 3 (5-HT_3) receptors are ligand-gated cation channels that result in depolarization of the cell [96]. The 5-HT_3 receptor is commonly known for its antinausea and antiemetic action during chemotherapy treatment [97], although there is evidence to suggest that the 5-HT_3 receptor also plays a specific role in the regulation of feeding [98–100].

There is mixed evidence regarding the feeding effects of 5-HT_3 antagonists when injected systemically. Van der Hoek and Cooper [101], for example, found that systemic administration of the selective 5-HT_3 antagonist ondansetron reduced consumption of a palatable wet mash in non-food-restricted male rats. In a contradictory finding, however, Cooper, Greenwood, and Gilbert [102] found that systemic administration of ondansetron increased feeding of palatable wet mash in non-food-restricted male rats, but decreases consumption of a sucrose solution. Three other studies, however, have not found that systemic injection of ondansetron had any effect on sucrose consumption or chow intake when administered alone [98,100,103]. Direct injection of ondansetron into the NTS, however, increases food intake in in food-restricted male rats [15].

The role of the 5-HT_3 receptor in feeding regulation yields more consistent results in relation to its ability to modulate the hypophagic response of other pharmacological compounds. For example, systemic injection of the 5-HT_3 antagonist ondansetron has been found to heighten the hypophagic effect of d-amphetamine in non-food-restricted male rats [102]. 5-HT_3 antagonists have also been found to mediate the satiety response elicited by duodenal lipid infusions, cholecystokinin (CCK), and gastric distension [15,16,98–100]. Direct injection of an Intralipid solution into the duodenum inhibits subsequent consumption of both chow and sucrose

solution in rats [98]. However, this satiating effect is attenuated by pretreatment with ondansetron [98]. Similarly, Burton-Freeman et al. [99] found that pretreatment with the selective 5-HT3 antagonist Tropisetron reduced latency to the next meal following duodenal Intralipid infusion.

As alluded to above, research consistently supports a role for the 5-HT_3 receptor in mediating the satiating effects of CCK [15,16,100] (although see [104] for an exception). Direct injections of ondansetron into the fourth cerebral ventricle [15] and systemic injections of ondansetron [100] attenuate the satiating effect of CCK in food-restricted male rats. The 5-HT_3 receptor likely mediates the typical hypophagic effect to an amino acid-imbalanced diet, as systemic administration of the 5-HT_3 antagonist tropisetron attenuates this hypophagic response [105]. Furthermore, there is evidence to suggest that 5-HT_3 activity interacts with CCK in inducing the hypophagic response to an amino acid-imbalanced diet, as administration of Devazepide, as CCK_a receptor antagonist, attenuates the feeding recovery induced by tropisetron [105].

The dorsal vagus complex is a promising candidate region at which 5-HT_3 receptors may mediate the satiating effects of CCK and lipid infusions [16,98,100]. Duodenal lipid infusion has been found to result in increased Fos-like immunoreactivity within the dorsal hindbrain in rats, and specifically within the NTS, dorsal motor nucleus of the vagus, and the area postreme. However, ondansetron treatment attenuates immunoreactivity in these regions, thus implicating a 5-HT_3 receptor activity in this region [98]. CCK administration has been found to result in a similar increase in Fos-like immunoreactivity within the NTS, which is also attenuated by ondansetron [16,100]. These findings suggest that 5-HT_3 receptors in the dorsal hindbrain may partially mediate the inhibition of feeding that produced by lipid consumption and CCK.

Despite this role for 5-HT_3 receptors in mediating the satiating effect of CCK, it appears that other feeding mechanisms can compensate for a lack of 5-HT_3 receptors, as 5-HT_3 knockout mice do not exhibit differences in feeding compared to wild-type mice [106].

Type 4 serotonin receptors

Type 4 serotonin receptors (5-HT_4) preferentially couple to G_s proteins on the cell membrane and result in depolarization of the cell.

Although there is relatively little research into the feeding effects of 5-HT_4 receptor activity, the evidence so far indicates that 5-HT_4 receptor activity exerts central hypophagic action localized within the nucleus accumbens [17,20]. Direct injection of the 5-HT_4 agonist endo-*N*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-isopropyl-2-oxo-1*H*-benzimidazol-1-carboxamide hydrochloride (BIMU8) into the nucleus accumbens decreases subsequent feeding and increases levels of Cocaine- and amphetamine-related transcript (CART) in both food-restricted and non-food-restricted mice [17]. 5-HT_4 receptor activity within the nucleus accumbens likely exerts a tonic inhibitory effect on feeding. Evidence for this hypothesis comes from research finding that direct injection of the 5-HT_4 antagonist RS39604 into the nucleus accumbens increases food intake in non-food-restricted mice [17].

There is also evidence to suggest that 5-HT_4 receptor activity within the nucleus accumbens mediates the hypophagic effects of 3,4-Methylenedioxymethamphetamine (MDMA). Francis et al. [20] found that injecting the 5-HT_4 antagonist RS39604 into the nucleus accumbens increased food intake in mice treated with MDMA, but not saline. However, it is likely that 5-HT_4 receptors are not critical to the maintenance of regular feeding and can be compensated for by other regulatory mechanisms; as 5-HT_4 receptor, knockout mice do not display significant differences in food intake when compared to wild-type mice [107].

Type 5 serotonin receptors

Type 5 serotonin receptors (5-HT_5) have multiple intracellular effectors while coupling to adenylyl cyclase and calcium channels [108,109]. Thus far, there is no evidence to suggest that 5-HT_5 receptors play a role in the regulation of feeding [3].

Type 6 serotonin receptors

Type 6 serotonin receptors (5-HT_6) preferentially couple to G_s proteins on the cell membrane, and result in depolarization of the neuron.

Although there is some contradictory evidence [110], on the whole, the evidence supports a role for 5-HT_6 receptors in up-regulating feeding [111].

Two studies, for example, have found that direct injection of the partial 5-HT_6 receptor agonist EMD 386088 dose-dependently increases subsequent food consumption [18,112]. This effect applies both to food-restricted and nonfood-restricted male rats [112], and to *ad libitum* as well as operant feeding paradigms [18,112]. This finding was contradicted by Fisas et al. [110], who found that oral administration of the partial 5-HT_6 receptor agonist E-6837 produced a hypophagic effect when administered as a single dose to lean male rats, and chronically to female diet-induced obese rats. It may be the case that these discrepant findings can be explained by differing affinity of EMD 386088 and E-6837 for other receptors, or the different locations and method of administration. Future research is required to consolidate these contradictory findings.

Recent research on the effect of 5-HT_6 receptor antagonists, and knockdown of 5-HT_6 receptor knockdown, however, has yielded more consistent results. Early studies into the effects of 5-HT_6 receptor knockdown with antisense and missense oligonucleotides failed to find any effect on subsequent food consumption in non-food-restricted male rats [113,114]. Recent opinion, though, has concluded that these null effects were probably due to the poor efficacy of receptor ablation in these early studies, in the order of 25%–30% knockdown [111]. Modern research, capable of more complete receptor ablation, has found that the knockdown of 5-HT_6 receptors with antisense oligonucleotides does, indeed, suppress subsequent feeding in male rats [115,116].

The selective 5-HT_6 receptor antagonist BVT 5182 reduces feeding in a dose-dependent manner when given as a single dose to mice genetically predisposed to obesity (ob/ob mice) [117]. The hypophagic effect of BVT 5182 also persists with chronic administration in diet-induced obese mice, and is accompanied by significant weight loss [117–119].

The 5-HT_6 receptor antagonist PRX-07034 similarly suppresses feeding in lean and diet-induced obese female rats [120]. The preservation of the behavioral satiety sequence following administration of PRX-07034 suggests that this effect is behaviorally specific [120]. When administered as chronic systemic injections, the hypophagic effects of PRX-07034 persist over time in diet-induced obese female rats [121]. However, it should be noted that PRX-07034 also has some affinity for the dopamine D3 receptor, and these findings should therefore be interpreted with a degree of caution.

Oral administration of the selective 5-HT_6 antagonists SB-357134 and SB-399885 also suppress feeding in food-restricted and non-food-restricted rats [122,123]. Furthermore, Garfield et al. [123] found that systemic administration of SB-399855 increased subsequent c-Fos immunoreactivity in the paraventricular nucleus of the hypothalamus and the NTS, suggesting that both are possible central regions responsible for mediating the hypophagic effects of systemic 5-HT_6 antagonists.

Further supporting the role of the 5-HT_6 receptor in the up-regulation of feeding, it has been found that mice carrying a nonfunctioning 5-HT_6 receptor are protected against diet-induced obesity when presented with a high-fat diet [117,124]. However, this may be at least partially due to metabolic factors, as Bonasera, Chu, Brennan, and Tecott [125] did not find any differences in chow consumption between 5-HT_6 receptor knockout mice and wild-type mice.

Type 7 serotonin receptors

Type 7 serotonin receptors (5-HT_7) preferentially couple to G_s proteins on the cell membrane, and result in depolarization of the neuron. There is no evidence to suggest that 5-HT_7 receptors regulate feeding [3].

Interactions with other hormones and neuropeptides

In addition to the direct effects of serotonergic activity described above, serotonin also interacts with a number of other hormones and neuropeptides to influence feeding. These other interacting hormones include: ghrelin, corticotrophin-releasing hormone, oxytocin, adrenaline, CCK, glucagon-like peptide-1, insulin, and leptin.

Ghrelin

As mentioned previously in the current chapter, the anorexigenic effects of serotonergic functioning are at least partially mediated by the melanocortin system (see *The serotonin 1b receptor*). The orexigenic hormone ghrelin and serotonin, however, exert counteracting effects on agouti-related

protein neurons. While serotonin causes hyperpolarization of these cells, thus decreasing the likelihood that feeding will subsequently be initiated [57], ghrelin has the opposite effect [126]. Serotonergic activity therefore has the capacity to indirectly antagonize ghrelin's orexigenic effects, whilst ghrelin antagonizes serotonin's inhibitory effect on feeding [126].

Corticotrophin-releasing hormone

Additionally, serotonin's inhibitory effects on feeding may be partially mediated by activity of the hypothalamo-pituitary adrenal (HPA) axis. Serotonin has been found to promote the production of corticotrophin-releasing hormone (Crh) within the hypothalamus [127–129], which subsequently has a stimulatory effect on the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. Via this mechanism, Crh therefore indirectly regulates corticosteroid release from adrenal glands within a normal fight-or-flight response [130]. Crh has been found to independently reduce feeding by stimulating the release of anorexigen glucagon-like peptide 1 [131], with mixed findings regarding its potential role in mediating the anorexigenic effects of the melanocortin system [132,133]. Therefore, serotonin may also partially inhibit feeding indirectly via the promotion of Crh production.

Adrenaline and noradrenaline

Evidence supporting the direct interaction between serotonin and the adrenergic system further supports the implication of HPA interaction in serotonin's effects on feeding. For example, peripheral administration of the serotonin agonist mCPP stimulates noradrenaline-producing neurons with the NTS [134]. Moreover, the extent of activation of noradrenaline-producing neurons within the NTS has been found to predict the degree of subsequent feeding suppression, thus suggesting a potential mediating role of noradrenaline in the hypophagic effects of peripheral serotonin [134]. Conversely, injecting the potent selective $\alpha 1$ receptor agonist cirazoline into the median raphe nucleus has been found to promote serotonin production, while injection of the $\alpha 1$ receptor antagonist prazosin reduced serotonin production in a dose-dependent manner [135]. In summary, the evidence therefore suggests that adrenaline promotes serotonin production, and vice versa, thereby further reinforcing the anorexigenic effects of both hormones.

Oxytocin

Oxytocin has received increasing attention over recent years for its role in the regulation of feeding [136]. Although the effects of oxytocin on feeding are conditional on a number of factors, including nutritional content of the food presented and the extent of animal socialization [137], a recent meta-analysis has found that a single dose of oxytocin has a strong overall inhibitory effect on feeding in animals [136].

There is extensive evidence to suggest that serotonin promotes oxytocin secretion [138,139], specifically within the paraventricular nucleus of the hypothalamus [140,141]. Serotonin may also exert indirect effects on oxytocin release via its effects on the melanocortin system [142]. It is therefore plausible that oxytocinergic functioning may, at least partially, mediate some of serotonin's anorexigenic effects, although this functional link has yet to be tested explicitly.

Cholecystokinin

CCK is a hormone produced by the intestines with strong satiating effects, which may be partially mediated by serotonergic activity. There is mixed evidence supporting the role of the 5-HT_{1a} receptor in mediating the satiating effects of CCK. Poeschla and colleagues have found that administration of the 5-HT_{1a} receptor agonist 8-OH-DPAT, a compound with hyperpolarizing effects on target neurons, attenuated the satiating effect of systemically administered CCK [143]. However, Ebenezer and colleagues did not replicate this finding [144]. In support of a role for the 5-HT_{1a} receptor in interacting with CCK, it was later found, conversely, that systemic administration of CCK can rather antagonize the onset of feeding elicited by 8-OH-DPAT [145].

Despite the mixed findings regarding the implication of 5-HT_{1a} receptor activity in inhibiting the satiating action of CCK, it is likely that serotonergic activity, more generally, plays some role in mediating the satiating effects of CCK. Support for this hypothesis comes from evidence demonstrating that inhibition of central serotonin release through the peripheral administration of p-chlorophenylalanine attenuates the satiating effects of CCK [146]. The role of serotonin in mediating the satiating effects of CCK is further supported by studies finding that administration of metergoline, a nonselective 5-HT antagonist, attenuates the satiating effects of CCK [147,148]. The mediating action of serotonin likely occurs at central sites within the brain. Evidence for this comes from the fact

that, contrary to the action of metergoline, peripheral administration of xylamidine, a 5-HT receptor antagonist with poor ability to penetrate the blood–brain barrier, does not attenuate the satiating effects of CCK [148].

There is evidence to suggest that the 5-HT_{2c} receptor, in particular, may play a role in mediating the satiating effects of CCK. Pretreatment with peripherally administered mianserin, a selective 5-HT_{2c} receptor antagonist, attenuates the satiating effects of CCK [104]. Furthermore, CCK does not induce satiety in 5-HT_{2c} receptor knockout mice [149].

In addition to evidence that serotonin mediates the satiating effects of CCK, there is also evidence supporting the role of CCK in mediating the satiating effects of serotonin. Cooper and colleagues, for example, found that peripheral administration of (+)-fenfluramine, a serotonin promoter and agonist, produced a strong satiating effect in non-food-restricted rats [150]. However, this effect was almost completely antagonized by peripheral administration of the CCK receptor antagonist MK-329 [150], thus suggesting that downstream activity of CCK is required to mediate the satiating effects of serotonin. This hypothesis was later corroborated by Grignaschi and colleagues in a similar study using the CCK antagonist devazepide [147].

It is likely that the mediating effects of CCK in serotonin-induced satiety are supported by central sites of action. As described above, the evidence supporting a mediating role for CCK in serotonin-induced satiety primarily used the serotonin agonist and promoter (+)-fenfluramine, a compound with high ability to penetrate the blood–brain barrier, to test this effect. However, a the mediating effect of CCK could not be corroborated when CCK antagonists were administered peripherally alongside serotonin, which has poor ability to penetrate to central sites [151,152].

The ability for CCK to mediate satiating effects of serotonin also appears to be dependent on nutritional state. Contrary to effects found in non-food-restricted rats, Francis and colleagues found that the CCK antagonist devazepide did not moderate the satiating effects of fenfluramine in 17-hour food-deprived rats [153].

There is mixed evidence regarding the ability for CCK and serotonin to act synergistically by exerting a hypophagic effect in a supra-additive manner. Zippel, Heidel, and Davidowa [154] found that some neurons in the lateral hypothalamus do exhibit an additive response to 5-HT and CCK administration, thus representing a potential site of action where the effects CCK and serotonin may converge to regulating feeding centrally.

Furthermore, Hayes and Covasa [151] have demonstrated that co-administering CCK and serotonin, each at low doses that would be ineffective when delivered independently, combined to produce a significant hypophagic effect. However, co-administering a CCK promoter alongside the serotonin promoter dl-fenfluramine fails to produce a heightened effect, as compared to the eating suppression resulting from the administration of either compound alone at a functionally effective dose [155].

Glucagon-like peptide 1

Glucagon-like peptide 1 (GLP-1) is a peptide hormone most commonly known for its role in stimulating insulin release following meal consumption [156]. Additionally, GLP-1 has a well-established role in suppressing appetite and food intake in both animals and humans [157–160].

It has been found that GLP-1 promotes the release of serotonin from hypothalamic synaptosomes in vitro, thus suggesting that central serotonergic activity may be one mechanism mediating the anorexigenic effects of GLP-1 [161]. The 5-HT_{2c} receptor, specifically may be primarily involved in mediating the hypophagic effects of GLP-1, as GLP-1 does not reduce food intake when administered to 5-HT_{2c} knockout mice [149,162]. However, it is also possible that the hypophagic effects of GLP-1 are mediated via a mechanism downstream of 5-HT signaling, as the effects of GLP-1 were also found to be attenuated in mice with a heterozygous mutation of the melanocortin 4 receptor [162].

Insulin

It addition to its role in promoting glucose uptake by cells, insulin plays an additional role in glucose homeostasis by producing a satiety response [163,164]. Central infusions of insulin have been found to promote the secretion of serotonin within the ventromedial nucleus of the hypothalamus [165], thus suggesting that downstream serotonergic activity may mediate some of insulin's hypophagic effects. Within the periphery, systemic administration of serotonin reduces circulating insulin levels in plasma without affecting blood glucose levels, thus indicating an overall improvement in glucose homeostasis [166].

Leptin

Both serotonin and leptin play strong independent roles in suppressing subsequent food intake [2,167]. However, the degree to which serotonin

and leptin interact to influence feeding regulation is currently unclear given the current array of contradictory findings.

There is a significant amount of research to suggest that serotonin and leptin serve distinct roles in the suppression of food intake. This research includes evidence that leptin and serotonin activate separate populations of POMC neurons, thus representing distinct central sites mediating the anorexigenic effect of either hormone [85]. Furthermore, despite the fact that serotonin-producing neurons in the dorsal raphe nucleus produce leptin receptor mRNA [168,169], recent evidence has found that serotonin-producing neurons within the brain do not, in fact, express leptin receptors [168].

However, there is evidence to suggest that the 5-HT_{2c} receptor may partially mediate leptin's anorexigenic action. For example, the 5-HT_{2c} receptor antagonist SB 242084 has been found to attenuate leptin's anorexigenic effects [170,171]. Furthermore, intracerebroventricular injections of leptin have been found to enhance subsequent serotonin release in the lateral hypothalamus following food ingestion, thus suggesting that serotonin may partially mediate the satiating effects of leptin activity [172]. Repeated intraperitoneal injections of leptin have also been found to increase serotonin concentrations in the hypothalamus and brainstem of leptin-deficient ob/ob mice, but not lean mice [173]. These findings therefore suggest some interaction between serotonergic activity and leptin activity while the mechanism of action for this interaction, including the receptors involved, remains uncertain.

Serotonin effects on locomotor activity and metabolism

On the whole, the evidence also points to an inhibitory effect of serotonin on locomotor activity. Rozenblit-Susan, Chapnik, Genzer, and Froy [174], for example, found that the selective serotonin reuptake inhibitor fluvoxamine decreased anticipatory locomotor activity observed prior to feeding in mice placed on a restrictive feeding schedule. Furthermore, administration of the tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA), which suppresses subsequent serotonin synthesis, had the opposite effect. That is, rather than inhibiting activity, mice treated with PCPA exhibited increased levels of locomotor activity. The

effect of serotonin on locomotor activity, however, appears to be moderated by lighting conditions and nutritional state, as mice kept in 24-hour dark conditions or on an ad libitum diet did not exhibit altered locomotor activity in response to fluvoxamine, when compared to placebo [174].

There is evidence that activity of the 5-HT_{1a} receptor, associated with hyperpolarization of the target neurons, instead increases locomotor activity. Mignon and Wolf [175] and Lucki, Ward, and Frazer [176], for example, have found that peripheral injections of the selective 5-HT_{1a} agonist 8-OH-DPAT increase subsequent locomotor activity in both healthy and monoamine-depleted rats. This effect, however, may be moderated by dose size as Carey, DePalma, Damianopoulos, Müller, and Huston [177] have found that 8-OH-DPAT stimulates locomotor activity at medium doses (0.4 mg/kg), but reduces locomotor activity at low doses (0.05 mg/kg).

The 5-HT_{2c} receptor may be particularly important in mediating the effect of serotonin on locomotor activity. Activity levels are increased in 5-HT_{2c} receptor knockout mice [178,179]. Furthermore, pharmacological evidence has demonstrated that both selective and nonselective 5-HT_{2c} agonists reduce locomotor activity at low to medium doses in wild-type mice, but not 5-HT_{2c} knockout mice [80,180,181]. The 5-HT_{2c} agonist Ro 60-0175 also reduced activity in 5-HT_{2c} knockout mice at a dose of 10 mg/kg, however, suggesting that Ro 60-0175 suppresses locomotor activity via mechanisms mediated by other receptors at higher doses [181]. Conversely, the 5-HT_{2c} antagonist SB242084 increased locomotor activity in wild-type mice, but not 5-HT_{2c} knockout mice, and reverses the suppression of locomotor activity induced by 5-HT_{2c} agonists [80,181]. This pattern of effects thus supports a role for 5-HT_{2c} receptor activity in mediating the suppression of locomotor activity in mice.

There is mixed evidence for the effect of central serotonin on thermogenesis and overall resting metabolic rate, although the majority of empirical evidence supports a role for serotonin in suppressing thermogenesis and overall energy expenditure. Injecting serotonin directly into the third cerebral ventricle increases subsequent oxygen consumption in male rats [182]. By contrast, administering serotonin specifically to the hippocampus and lateral septum reduces thermogenesis in rats [183]. Furthermore, 5-HT_{2c} knockout mice and mice with the 5-HT_{2c} receptor specifically deleted from POMC neurons in the hypothalamus demonstrate increased energy expenditure [184,185], thus indicating a role for 5-HT_{2c} receptors in suppressing overall resting energy expenditure. Additionally, inhibiting the synthesis of serotonin through the administration of PCPA stimulates thermogenesis and overall

energy expenditure [54,186,187]. This latter finding thus also illustrates a tonic role for serotonin in suppressing thermogenesis.

The 5-HT_{1a} receptor also plays a role in in the serotonergic control of basal metabolic rate. For example, central injections of the selective 5-HT_{1a} receptor agonist 8-OH-DPAT to the raphe pallidus attenuate leptin's ability to increase thermogenesis within brown adipose tissue [188]. Furthermore, administration of the 5-HT_{1a} antagonist WAY-100635 reversed the suppression of energy expenditure induced by 8-OH-DPAT [188]. These findings thus point for a role of central 5-HT_{1a} receptor activity in reducing thermogenesis and overall energy expenditure.

By contrast, peripheral 5-HT_{1a} activity increases basal energy expenditure. This hypothesis is supported by research demonstrating that peripheral injections of 8-OH-DPAT transiently increase daytime energy expenditure and increase overall nocturnal energy expenditure [35]. Peripheral injection of the 5-$HT_{1a/b}$ agonist RU-24969 also increases daytime energy expenditure, whilst attenuating the naturally occurring increase in nocturnal energy expenditure [35]. However, given that peripheral activity of the selective 5-HT_{1a} agonist 8-OH-DPAT increases nocturnal energy expenditure, it is plausible that the attenuation in energy expenditure observed following administration of RU-24969 more strongly reflects activity of the 5-HT_{1b} receptor.

In humans, empirical evidence suggests that selective 5-HT_{2c} agonists do not affect resting energy expenditure, while serotonin reuptake inhibitors increase resting energy expenditure [189–191]. This overall pattern of effects is supported by evidence that oral administration of the selective 5-HT_{2c} agonist lorcaserin does not affect energy expenditure in humans after controlling for weight loss caused by reductions in food intake [189]. However, the selective serotonin reuptake inhibitor fluoxetine increases thermogenesis and energy intake in humans [191], and the nonselective serotonin reuptake inhibitor sibutramine attenuates the natural declines in energy expenditure normally observed with weight loss [190]. These findings therefore suggest that serotonergic agents for suppressing appetite also have the potential to enhance weight loss by promoting thermogenesis in humans.

Serotonin disturbances in eating disorders

Given that serotonergic activity tends to suppress subsequent feeding behavior, it stands to reason that up-regulated serotonergic functioning

might be one underpinning factor in anorexia nervosa, with down-regulated serotonergic functioning present in binge-type eating disorder. Indeed, Kaye and Weltzin [192] have proposed that heightened serotonergic activity acts as a risk factor for anorexia nervosa, both by directly suppressing feeding and contributing to the obsessionality and impulsiveness that maintain restrictive eating behavior.

In the acute stage of anorexia nervosa, however, the evidence rather supports a down-regulation of serotonin. For example, blood tryptophan concentration, a precursor to serotonin, has been found to be significantly reduced in women with anorexia nervosa compared to women without history of an eating disorder [193]. Additionally, cerebrospinal fluid (CSF) levels of serotonin metabolites are reduced in women with acute anorexia nervosa, compared to women without history of an eating disorder [194]. The functional significance of these alterations is supported by evidence that downstream functions of serotonin, including stimulating the release of prolactin, are suppressed in women with acute anorexia nervosa [195]. This down-regulation of serotonergic functioning may largely due to dietary deficiency of tryptophan, resulting from malnutrition during the acute phase of anorexia nervosa.

Evidence of a trait for up-regulation of serotonergic activity rather comes from research with participants recovered from anorexia nervosa. Women recovered from anorexia nervosa exhibit heightened CSF levels of 5-hydroxyindoleacetic acid, a major 5-HT metabolite [196]. Additionally, the prolactin response to the serotonin promoter d-fenfluramine does not differ from controls in women recovered from anorexia nervosa, illustrating a correction of dysregulated serotonin functioning during the acute phase of anorexia nervosa [197].

A more complex picture of serotonergic functioning in anorexia nervosa has emerged from more recent neuroimaging studies. In a positron emission tomography study administering radioactive-labeled WAY-100635, a 5-HT_{1a} receptor antagonist, it was found that that women recovered from the binge-purge subtype of anorexia nervosa exhibit increased 5-HT_{1a} receptor binding in the cingulate cortex, lateral and medial orbital frontal cortex, the parietal cortex, the lateral and mesial temporal cortex, and in the dorsal raphe, compared with women without history of an eating disorder [198]. By contrast, no differences in 5-HT_{1a} receptor binding were found in women recovered from the restrictive subtype of anorexia nervosa, when compared to women without history of an eating disorder [198]. However, these findings are confounded by

more recent evidence that WAY-100635 also binds at the D4 dopamine receptor [199]. In a clearer set of findings, women recovered from the restrictive subtype of anorexia nervosa exhibit reduced binding at the 5-HT_{2a} receptor within the amygdala, hippocampus, the cingulate cortex, and occipital and parietal cortex, when compared to women without history of an eating disorder [200]. These findings thus suggest possible alterations in 5-HT_{1a} receptor binding in women with a history of anorexia nervosa, binge-purge subtype, and alterations in 5-HT_{2a} receptor binding in women with a history of anorexia nervosa, restrictive subtype.

There are mixed findings regarding a genetic underpinning for serotonergic dysregulation in anorexia nervosa. Several early studies provided support for a higher frequency of the -1438G/A genetic polymorphism within the promoter regions of the 5-HT_{2a} gene among women with anorexia nervosa, compared to case controls without an eating disorder [201–203]. Later studies, however, failed to replicate this finding [204,205]. The fact that differences in polymorphism frequency were not replicated when testing for family transmission disequilibrium in a large study with 316 participants suggests that the results of previous case-control studies may have been impacted by stratification bias [205]. Likewise, other case-control studies have also found evidence of a polymorphism on the 5HTR1D gene that is associated to anorexia nervosa, thus implicating the 5-HT_{1d} receptor in the etiology of the disorder [206–208]. However, this finding was not replicated in a larger, more recent study [209].

With regards to bulimia nervosa, there is also evidence of dysregulated serotonergic functioning during the acute phase of the disorder [210–214]. Indeed, the extent to which the prolactin response to d-fenfluramine and dl-fenfluramine is suppressed in bulimia nervosa is correlated to the frequency of binge eating episodes [210] and a history of self-injurious behavior [212]. These findings thus add weight to the hypothesis that serotonergic dysregulation is functionally implicated in the maintenance of bulimia nervosa.

Women with bulimia nervosa also exhibit reduced central availability of the serotonin transporter when compared to women without history of an eating disorder [215]. There is evidence to suggest that degree of serotonin transporter availability may also be implicated in the maintenance of bulimia nervosa, as the degree of serotonin transporter availability is inversely correlated to duration of illness in bulimia nervosa [215]. Some dysregulation appears to correct during recovery, as women recovered

from bulimia nervosa exhibit a normal prolactin response to the serotonin agonist mCPP [216]. However, heightened CSF levels of the serotonin metabolite 5-hydroxyindoleacetic acid persist into recovery [216]. Furthermore, Kaye et al. [217] have found evidence of reduced 5-HT_{2a} receptor binding within the medial orbital frontal cortex in women recovered from bulimia nervosa, when compared to women without history of an eating disorder.

In summary, the empirical literature supports a complex pattern of differences in serotonin functioning in anorexia nervosa and bulimia nervosa. Given the diversity of functions affected and underpinned by serotonin, it is likely that the disordered eating seen alongside differences in serotonin function may be underpinned by both direct effects on appetite, as well as the indirect effect of serotonin on mood [218].

Involvement of serotonin in antipsychotic-induced weight gain

It is well-established that many antipsychotic drug treatments result in marked weight gain over time, with more recent atypical antipsychotic drugs, such as olanzapine and clozapine, having a particularly strong effect [219]. There are likely multiple pharmacological mechanisms contributing to antipsychotic-induced weight gain, with serotonergic activity being one such neurotransmitter system involved [220].

The atypical antipsychotics olanzapine, clozapine, and risperidone each have antagonistic properties at both 5-HT_2 receptors and dopamine D_2 receptors [221–225]. As described above, agonism of 5-HT_2 receptors, and particularly 5-HT_{2c} receptors, tends to reduce subsequent eating behavior, while antagonism has the opposite effect [2]. Therefore, it is plausible to hypothesize that olanzapine, clozapine, and risperidone may partially contribute to weight gain via central antagonism of 5-HT_2 receptors.

Genetic evidence regarding the role of 5-HT_{2c} receptor polymorphisms in contributing to and protecting from antipsychotic-induced weight gain is somewhat mixed. For example, carrying a variant for the 23 Serine amino acid or the 102T allelic variant on the 5-HT_{2c} gene is a risk factor for olanzapine-induced weight gain [226]. By contrast, most studies have found that the -759T allele on the 5-HT_{2c} gene protects against

antipsychotic-induced weight gain [227–232], although Theisen et al. [233] and Hong, Lin, Yu, Yang, and Tsai [234] did not replicate this finding. In a meta-analysis examining the effect of the -759 polymorphism on antipsychotic-induced weight gain, the -759T allele on the 5-HT_{2c} receptor gene was found to be associated with an overall attenuation of weight gain across all studies [235]. This finding may be explained by the fact that the -759C (rather than T) allele is associated with under-expression of the 5-HT_{2c} receptor gene [236], thus resulting in less hypophagic 5-HT_{2c} receptor activity to counteract antipsychotic-induced weight gain.

However, while the genetic evidence stemming from 5-HT_{2c} receptor polymorphisms does support a functional role for serotonin in antipsychotic-induced weight gain, it is unlikely that serotonergic activity is the dominant pharmacological mechanism explaining this weight gain [237]. For example, antipsychotic treatment is also associated with alterations in glucose homeostasis [238,239] and lipid homeostasis [240,241]. Furthermore, H1 histamine receptor activity is a strong predictor for short-term weight gain during antipsychotic treatment [242], and a meta-analysis of 23 studies found that receptor occupancy at the H1 histamine receptor and muscarinic acetylcholine receptor were associated with antipsychotic-induced weight gain, while serotonin receptor occupancy was not [237]. This pattern of effects therefore suggests that while serotonergic activity, and specifically the expression of the 5-HT_{2c} receptor, modulates antipsychotic-induced weight gain, serotonin is unlikely to play a dominant role in mediating this effect.

Serotonergic approaches to weight loss

Given the role of serotonin in reducing food intake and weight in animal studies, perhaps the most obvious implication of these findings is the possibility of modifying serotonergic activity to support weight loss in humans. Especially given the rapid increase in the prevalence of obesity and secondary health conditions seen in Western countries over the 20th and 21st centuries, this proposition seems especially pertinent [243]. However, the use of serotonergic agents for appetite suppression has a particularly checkered past.

Fenfluramine, a serotonin promoter and indirect serotonin receptor agonist was commonly prescribed during the 1980s to suppress appetite

and promote weight loss [244]. However, fenfluramine was later found to increase the risk of pulmonary arterial hypertension, an incurable and often fatal condition [245,246]. Fenfluramine is known to inhibit the reuptake of serotonin by serotonin transporters, thus increasing circulating levels of serotonin [247]. The deleterious cardiovascular side effects are therefore likely to involve vasoconstriction within pulmonary arteries stimulated by chronic high levels of serotonin [248]. Due to these deleterious cardiovascular effects, fenfluramine was later withdrawn from the market in 1997.

In addition to fenfluramine, aminorex fumarate was also a popular prescription drug for weight loss during the 1960s. However, aminorex also inhibits serotonin transporter uptake of serotonin, thus increasing systemic levels of serotonin and producing similar harmful side effects [249]. Aminorex has also since been removed from the market [250].

Due to the deleterious cardiovascular side effects of inhibiting serotonin transporter activity, any pharmacological agent seeking to suppress appetite via this mechanism is no longer a viable avenue in the pursuit of a safe and effective serotonergic weight loss drug. Whilst agonism of the 5-HT_{1b} receptor has a consistent anorexigenic effect in animals [8,55,56], the 5-HT_{1b} receptor is also expressed in vascular tissue [251]. Therefore, pharmacologic agents acting via the 5-HT_{1b} receptor also pose the risk of causing harmful side effects.

The most promising serotonergic anorexigenic agents at present, in terms of both safety and efficacy, instead act via the 5-HT_{2c} receptor [3]. The 5-HT_{2c} receptor agonist lorcaserin received approval from the United States Food & Drug Administration for use in the long-term treatment of obesity in 2012 [252,253]. In rats, systemic injection of lorcaserin has been found to reduce feeding in both food-restricted and non-food-restricted conditions, and did not disrupt the behavioral satiety sequence at low doses [254]. Furthermore, chronic systemic injections of lorcaserin reduced feeding and decreased weight over time in diet-induced obese rats, when compared to vehicle or sibutramine treatment [255].

In humans, daily doses of lorcaserin (10 mg–20 mg/day) promote significantly greater weight loss without greater incidence of cardiac side effects [189,256,257]. At the end of an 8 week trial testing 10 mg daily lorcaserin against placebo, it was found that the treatment group consumed significantly fewer calories in lunch and dinner meals, thus suggesting that this weight loss is at least partially driven by appetite suppression [189].

m-Chlorophenylpiperazine (mCPP), a 5-$HT_{2c/1b}$ agonist has previously been found to reduce feeding in mice [258], and has also been tested for its effect on feeding in humans [78,259–261]. Single-session between-subjects and crossover design studies have so far demonstrated a consistent reduction in subjective appetite in response to mCPP administration, compared to placebo [78,260,261]. However, there is mixed evidence for the effect of mCPP on subsequent food consumption in a test meal presented in the lab, suggesting that effects on subjective hunger may not carry over into actual eating behavior [78,260,261].

A 2-week randomized controlled trial prescribing twice-daily doses of mCPP has provided more ecologically valid evidence for the effect of mCPP on eating behavior in humans [259]. mCPP, as compared to placebo, was found to be associated with greater reduction in body weight and subjective hunger in both men and women. This trial, however, incrementally increased the dose of mCPP over time, therefore leaving room for doubt as to whether administering a repeated constant dose over time would result in tolerance.

Finally, it should also be noted that mCPP is not without side effects. mCPP has been found to increase subjective anxiety in women [261]. Additionally, two single-session studies found that mCPP administration was associated with greater nausea and light-headedness compared to placebo [260,261], thus indicating that the anorexigenic effect of mCPP may not be behaviorally specific. Therefore, even if the anorexigenic effects of mCPP are maintained for periods exceeding 2 weeks, the side effects may not be found acceptable to endure in the long-term.

The drug combination of phentermine and topiramate was approved for use in supporting weight loss by the United States Food & Drug Administration under the commercial name QSymia in 2012. There is some evidence to suggest that phentermine increase levels of serotonin in the hypothalamus in rats [262,263]. However, phentermine has a stronger effect at stimulating the release of noradrenaline than serotonin [264], and it is therefore unclear to what extent phentermine/topiramate promotes weight loss via serotonergic functioning.

In summary, although a number of serotonergic compounds have shown promise at suppressing food intake in animals, translating these findings for clinical use in humans has proved difficult. However, the selective 5-HT_{2c} agonist lorcaserin has so far yielded promising results in supporting weight loss. Future research will be helpful in further testing the extent of lorcaserin's therapeutic effects, such as its potential to reduce binge eating behavior in bulimia nervosa and binge eating disorder.

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CHAPTER FOURTEEN

The outlook for the development of serotonergic drugs as therapeutic medications for psychiatric disorders

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The reader of this book surely, cannot fail to be impressed by the vast range of impact the serotonin system has on psychological function. As the complexity of its physiology and pharmacology became apparent great excitement was rapidly tempered by the reality of attempting to discover compounds with high selectivity and affinity for the myriad potential biochemical control mechanisms controlling 5-hydroxytryptamine (5-HT) neuronal activity. A healthy place to start would have been to isolate the mechanisms underlying the disorders of interest before developing a strategy to correct the metabolic electrophysiological or genetic errors detected or hypothesized to be causative. Sadly, in the Anthropocene age, time to take a leisurely and solidly scientific approach was lost as financial resources built on the foundation of the first generation psychotherapeutics evaporated to be replaced by the quick win, quick kill mentality, where only immediate profitability and share price matter. Yes, it is difficult. We are dealing with the most complex object in the universe. But as scientists our primary concern must be those whose lives have been tragically cut short by crippling depression, psychosis and the many other mental disorders that blight from childhood until old age. Because the first generation of psychotherapeutic agents was discovered by serendipity, it has been hard to overcome the feeling that novel therapeutics are there hanging low on the trees so why waste time and money looking for errors in a haystack that's too big to comprehend? The discovery of the psychedelics thousands of years ago shows what can be done with microgram amounts of substances that just happen to sit on the skin of a toad in the Amazon. Reading about the amazing spiritual effects of psilocybin

The Serotonin System.
DOI: https://doi.org/10.1016/B978-0-12-813323-1.00003-7

reinforces the view that all of human nature can be accessed and modulated by chemical means. Sadly, the attraction of such amazing tools for illicit recreational use and abuse has removed them from our armory just at the time when the tools for investigating their neuronal properties have reached the dizzy level of sophistication that has come with noninvasive brain imaging, electrophysiological monitoring, optogenetic manipulation of brain circuitry, and the collection and analysis of megabytes of genetic data. The unprecedented opportunity to record directly from the living human brain has taught us much about how we should be thinking about neurotransmitter control of physiological functions. A blast of serotonin release of only a few milliseconds duration is enough to alter behavior while we are fixated on restoring tonic activation when pulsatile release patterns might be crucially more important. The last few years have also seen great improvement in behavioral neuroscience with much greater emphasis on experimental design and data reproducibility but particularly in making MRI scanner-friendly paradigms that can readily translate from man to animals especially when they can be coupled with in vivo oxygen amperometry that can record signals that match the BOLD signals of fMRI in rodents [1] performing operant or other conditioned behavioral paradigms. The lack of correspondence between preclinical and clinical proof-of-concept findings has been held as the greatest impediment to drug discovery for CNS diseases. However, not only are compounds tested without evidence that the chosen dose occupies the target receptor, but there is also often a failure to verify that occupation of the target is associated with a pharmacodynamic effect. The discovery of 5-HT3 receptor antagonists. The first of the new wave of subtype selective compounds that were hoped to be both efficacious and side effect free by virtue their high affinity and selectivity was greeted with great excitement following reports that the compounds had anxiolytic, antipsychotic and procognitive behavioral effects at very low doses, but many people failed to reproduce these behavioral effects. Although the 5-HT3 compounds were able to inhibit vomiting induced in the ferret by cytotoxic anticancer drugs, they did that at doses much greater than those claimed to be anxiolytic in rat or mouse. The 5-HT3 receptor antagonists have undoubtedly improved the quality of life for patients undergoing cancer chemotherapy but have had no impact at all on psychiatric disorders. Does this mean we have exhausted the therapeutic possibilities of serotonergic manipulation? The therapeutic benefit observed in Parkinsonian patients against associated psychotic symptoms and insomnia with selective 5-HT2A receptor antagonists suggests not. With 14 different receptor

subtypes only 5-HT1A, 5-HT2, and 5-HT3 receptor antagonists have been commercialized. However, 5-HT4 receptor is also expressed in the brain and a PET ligand [2] has been identified. Moreover, a significant association between 5-HT4 receptor polymorphisms and bipolar disorder has been noted in samples drawn from both the United States and Japan. There are also data showing that activation of 5-HT4 receptors expressed in brain stem respiratory centers can overcome opioid-induced respiratory depression without impact on nociception [3]. Metoclopramide is used to stimulate upper gut motility and to prevent nausea and vomiting respectively. These clinical benefits are attributed to 5-HT4 receptor activation and to antagonism at 5-HT3 receptors. The compound also has some affinity for dopamine receptors which has been reduced in cisapride and metoclopramide. Cisapride and metoclopramide are marketed for gastrointestinal motility disorders [4]. Preclinical data support a role for 5-HT6 receptor ligands in the treatment of cognitive disorders. The pharmacology is complex because, like the 5-HT2 receptor, 5-HT6 receptors are linked to multiple signaling pathways. The Chapter 8 by Nilsson in this volume has discussed some of the preclinical evidence suggesting a role for 5-HT6 and/or5-HT7 receptors in improving cognitive processes. Compounds are in development but as yet no clinical data have been published. Idaloperdine and RVT-101 were reported to be in phase III trials for Alzheimer's disease but failed to meet the primary endpoint when combined with a cholinesterase inhibitor [5]. A second compound with 5-HT6 activity, Dimebon is also acholinesterase inhibitor but similarly failed in clinical trials [5,6]. The 5-HT7 receptor antagonist SB2699970 is notable for its ability to reverse the deficit in working memory induced by the NMDA receptor antagonist MK801 in an operant delayed-nonmatching-to-position translational assay. The compound also reversed the MK-801-induced increase in glutamate release in prefrontal cortex without effect on changes in dopamine. These results [7] are quite impressive and it would be wonderful to see them independently replicated. Having completed a model preclinical evaluation, there is no current indication that the compound has progressed into clinical trials. Although, other preclinical data suggest a role in modulating circadian rhythms, the selective 5-HT1A receptor agonist 8-OHDPAT, which can shift pacemaker neurons in the suprachiasmatic nucleus, achieves this by interacting with 5-HT7 receptors. Preclinical evidence suggests similarities of effects on responses to stress are similar to those of antidepressants. Indeed sulpiride which has antidepressant effects [8] has an appreciable affinity for 5-HT7 receptors. Alternative splicing gives rise to four 5-HT7

receptor isoforms [9] but there is no significant association with either bipolar disorder or schizophrenia. 5-HT7 receptor ligands may also be considered as a possible treatment in autism spectrum disorders since activation of 5-HT7 with LP211 corrected excessive glutamate-mediated long term depression in FMR1 knockout mice (an animal model of Fragile X syndrome) [10]. For the 5-HT7 receptor the future looks promising with potential beneficial effects from mainly antagonists in the treatment of affective disorders and cognitive deficits associated with schizophrenia. Compounds with appreciable affinity for 5-HT7 include the antipsychotics amisulpiride and lurasidone, clozapine and aripiprazole. Compounds with proven antidepressant effects with 5-HT7affinity include vortioxetine and JNJ180386683 [11]. Although these compounds are not selective agents, the preclinical data accumulated in the short time since the receptor was cloned and expressed in 1993 are of high quality and considerably more impressive than that obtained with highly selective 5-HT3 receptor antagonists, and should certainly encourage Pharma companies not to lose hope that drug discovery for psychiatric disorders is still worthy of investment. Not to forget the 5-HT1B receptor agonist zolmitriptan which is an effective antiaggressive agent in mice and human subjects primed to be aggressive by alcohol [12,13], as it is in man [14]. The translational significance of these findings should not be ignored, although it seems they currently have been. Eltoprazine has been trialed in adult ADHD and found to reduce significant hostility. The primary endpoint was met in a double blind placebo controlled trial of 47 subjects with adult ADHD. The secondary endpoint "hostility" was achieved with a significant reduction $P < 0.001$ (Amarantus Bioscience holdings PRNewswire, Feb 4, 2014).

An alternative approach to assessing the role of 5-HT autoreceptors compared to postsynaptic heteroceptors is to use biased ligands. F15599 preferentially targets postsynaptic heteroceptors whereas F13714 preferentially targets raphe presynaptic autoreceptors. The biased agonists were administered to aggressive resident male rats confronting an intruder. Both compounds had antiaggressive effects [15]; so even with these tools there is no clear separation of function between the receptor types. A further complication is the potential involvement of oxytocin which was hypothesized [5] to explain the effects of citalopram on harm aversion [16]. At least to me it seems there are plenty of opportunities for the discovery of novel and useful psychiatric drugs and it is hoped that Pharma companies become aware of these opportunities and resource the field adequately before the expertise is lost.

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THE SEROTONIN SYSTEM

HISTORY, NEUROPHARMACOLOGY, AND PATHOLOGY

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Serotonin plays a key role in a number of behavioral and neuroendocrine mechanisms, including sexual behavior, aggression, impulsivity, mood regulation, sleep, food intake, and endocrine control. The neurotransmitter also has links with numerous neurodevelopmental and neuropsychiatric disorders, including autism spectrum disorders, schizophrenia, and intellectual impairment. In the decades since the discovery of serotonin, the genomic revolution has helped researchers identify 15 different serotonin receptor subtypes in the brain, and new agonists and antagonists have been tried and tested for their beneficial effects in neuropsychiatric disorders. But which of these have real therapeutic efficacy, and against which symptoms?

The Serotonin System provides an up-to-date account of the physiology and pathophysiology of serotonin and the role it plays in behavioral functions and explores the potential roles of 5-HT1 in neurodevelopmental disorders. It summarizes the history of the discovery and development of serotonergic drugs for the treatment of neuropsychiatric disorders and the light they have shed on the circuitry underlying affective disorders. This concise yet thorough volume is the perfect introduction to this critical neurotransmitter for students and researchers new to the study of behavior, neuropsychiatry, or neuropharmacology, and will also assist established investigators with gaining a greater perspective of serotonin beyond their own specialization.

Key Features

- Examines the role of serotonin in physiological functions and in neuropsychiatric disorders, as well as the role of serotonin receptors as drug targets
- Provides in-depth knowledge of all aspects of the serotonin system
- Explores serotonergic receptors as targets for current and new therapeutic compounds

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