Brain serotonergic circuitries

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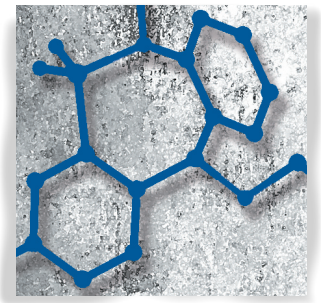

Brain serotonergic circuitries interact with other neurotransmitter systems on a multitude of different molecular levels. In humans, as in other mammalian species, serotonin (5-HT) plays a modulatory role in almost every physiological function. Furthermore, serotonergic dysfunction is thought to be implicated in several psychiatric and neurodegenerative disorders. We describe the neuroanatomy and neurochemistry of brain serotonergic circuitries. The contribution of emergent in vivo imaging methods to the regional localization of binding site receptors and certain aspects of their functional connectivity in correlation to behavior is also discussed. 5-HT cell bodies, mainly localized in the raphe nuclei, send axons to almost every brain region. It is argued that the specificity of the local chemocommunication between 5-HT and other neuronal elements mainly depends on mechanisms regulating the extracellular concentration of 5-HT, the diversity of high-affinity membrane receptors, and their specific transduction modalities.

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Serotonin or 5-hydroxytryptamine (5-HT) is a small indolamine (MW 176.2) widely distributed throughout the animal (from ascidies to human)[1-4] and plant[5,6] kingdoms. In mammals, a gut-stimulating factor called enteramine, distinct from subtance P, was reported in 1940.[7-9] Eight years later, a vasoconstrictor factor named serotonin was isolated from the serum.[10] It was subsequently demonstrated that enteramine and serotonin were the same chemical entity, ie, 5-HT. The biological activity of 5-HT in peripheral nerves and brain was described a few years later.[11-15] Additionally, developmental studies reveal that 5-HT occurs early during fetal life and plays a role in morphogenesis as well as in neural trafficking.[16,17]

Among the large variety of chemical messengers acting in nerve cell signaling, 5-HT is the focus of much interest due to its implication in almost every physiological function (eating, reward, thermoregulation, cardiovascular regulation, locomotion, pain, reproduction, sleep-wake cycle, memory, cognition, aggressiveness, responses to stressors, emotion, and mood) and in several human pathologies. Thus, dysfunction of the serotonergic systems is thought to be associated with irritable bowel syn-

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Selected abbreviations and acronyms

5-HIAA	*5-hydroxyindolacetic acid*
5-HT	*5-hydroxytryptamine*
SERT	*serotonin membrane transporter*
TPOH	*tryptophan hydroxylase*
VGLUT	*vesicular glutamate transporter*
VMAT	*vesicular monoamine transporter*

See also the Appendix for an explanation of some of the terms used in the text

drome,[18] restless legs syndrome,[19] sudden infant death syndrome,[20,21] autism,[22] headache,[23] insomnia,[24] anxiety,[25] depression,[26] anorexia,[27,28] schizophrenia,[29] Parkinson's disease,[30] and Alzheimer's disease.[31,32] At the present time, most of the anxiolytic/antidepressant compounds such as tricyclic and tetracyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs),[33,34] azapirones,[35] setron antiemetics,[36] and triptans used to relieve migraine,[37] all target the serotonergic systems. Besides a well-known dopaminergic component, atypical neuroleptics (eg, olanzapine, clozapine, quetiapine, aripiprazole) interact with serotonergic receptors (ie, 5-HT1A, 5-HT2A-2C, 5-HT6 and 5-HT7).[38-40] Finally, psychotropic drugs including LSD, mescaline, cocaine, and amphetamines powerfully alter 5-HT functions via 5-HT1A, 5-HT2A receptors[41,42] and monoaminergic transporters.[43-45]

5-HT is massively synthesized in the gastrointestinal tract (mainly in enterochromafin cells), whereas only a small percentage is produced within the nervous system.[46,47] There is some evidence that 5-HT synthesis, release by calcium-dependent exocytosis, selective reuptake by an energy-dependent membrane transporter, metabolism and reuptake in vesicles operate in all the neuronal elements of the 5-HT neurons (ie, soma, dendrites, axons, and terminals), together participating in 5-HT homeostasis.[48,49] The widespread distribution of 5-HT axons and terminals throughout the neuraxis *(Figure 1)*, the frequent nonsynaptic neurotransmission (called *diffuse or volume neurotransmission*[48,50-52]), as well as the abundance of 5-HT receptors *(Table I)* contribute to explaining the complex relationships between 5-HT and other neurotransmitter and neurohormonal systems.

The main goal of this review is to discuss the most salient features concerning the neuroanatomy of the serotonergic neurotransmission, ie, the serotonergic circuitries in the human brain. In the first instance, proteins such as enzymes, transporters, and receptors more specifically devoted to the serotonergic functions will be described. Methodological limits of the classical postmortem approaches in the human and new 5-HT in vivo imaging modalities will also be considered. At the present time, more than 100 000 scientific publications concern 5-HT (PubMed). Wherever possible, we have tried to include up-to-date references dealing with the human brain.

The main molecular protagonists in 5-HT neurotransmission

From tryptophan to serotonin

In the brain, neuron subpopulations have a set of enzymes permitting the two-step synthesis of 5-HT from its precursor tryptophan, an essential aminoacid provided by nutrients and actively cotransported with other neutral large amino acids from the blood to the brain.[53] The consequences of tryptophan depletion or loading on physiological functions, including memory, cognition, mood, facial expression of emotion, and sleep, have been reported in detail elsewhere.[53-56] Contrasting with the peripheral glandular serotonergic systems (eg, the enterochromafin cells or the pineal gland) that uses a first tryptophan hydroxylase form (TPOH1), 5-HT synthesizing neurons in the brain express another tryptophan hydroxylase (TPOH2) recently evidenced from knockout studies in mice.[57] The respective sequences of these isoenzymes revealed 30% heterology, offering the perspective of a selective modulation by appropriate drugs in central or peripheral pathologies.[57] Some 5-HT-related neuropsychiatric disorders are possibly correlated with genetic variants of TPOH2.[57-61] Additionally, recent analyses indicate that TPOH1 polymorphisms could increase susceptibility to schizophrenia[62] and suicidal behavior.[63]

5-hydroxytryptophan formed during the first rate-limiting step by TPOH1 or TPOH2 is then transformed into 5-HT via an aromatic L-amino acid decarboxylase (AADC) also present in catecholaminergic neurons. Rare AADC point mutations reported in humans result in deficiency of catecholamines and serotonin with severe neuropsychiatric symptoms.[64]

In the nervous system, 5-HT is mainly metabolized by the monoamine oxidase A (MAOA) and a 5-HT half-life of only a few minutes is reported.[65] Thus, reciprocal 5-HT exchanges between the central nervous system (CNS) and other tissues appear to be limited, although a brain 5-HT

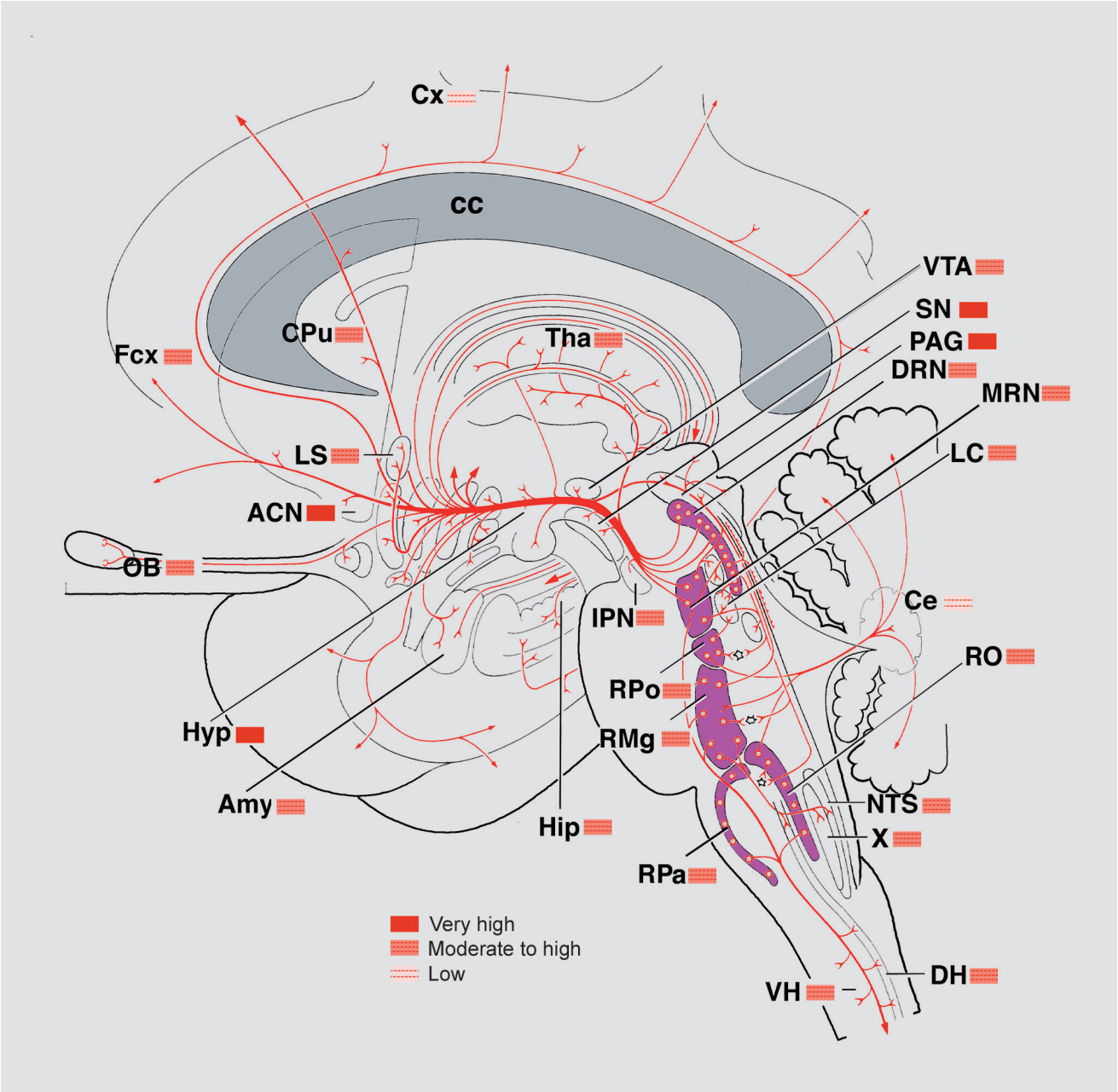

Figure 1. Schematic sagittal view of the human brain showing the distribution of the serotonergic systems. The raphe nuclei containing the majority of the serotonergic cell bodies appear in purple. It is readily seen that these nuclei are exclusively located in the brain stem. The axons issued from them are drawn in red. The trajectories and extensive branching of the axons until the main terminal areas are illustrated. The densities of the serotonergic axonal networks in these terminal areas are given by the colored boxes. X, dorsal motor n of the vagus nerve; ACN, accumbens n; Amy, amygdala; cc, corpus callosum; Ce, cerebellum; CPu, caudate-putamen; Cx, cortex; DH, dorsal horn spinal cord; DRN, dorsal raphe n; Fcx, frontal cortex; Hip, hippocampus; Hyp, hypothalamus; IPN, interpeduncular n; LC, locus coeruleus; LS, lateral septum; MRN, median raphe n; n, nucleus; NTS, n of the solitary tract; OB, olfactory bulb; PAG, periaqueductal gray; RMg, raphe magnus n; RO, raphe obscurus n; Rpa, raphe pallidus; RPo, raphe pontis n; SN, subtantia nigra; Tha, thalamus; VH, ventral horn; VTA, ventral tegmental area
Adapted from ref 129: Nieuwenhuis R. *Monoamines: Chemoarchitecture of the Brain.* Berlin, Germany: Springer Verlag; 1985:33-41.

efflux through the blood-brain barrier was observed in rat species.[66] Abnormality in 5-HT metabolites, especially low 5-hydroxyindolacetic acid (5-HIAA) levels in the cerebrospinal fluid (CSF) was correlated with suicidality and severity of aggressive behaviour.[67,68] Furthermore, an association between CSF 5-HIAA and cholesterolemia was described in certain suicidal patients.[69,70] Although largely conjectural, the neurobiological basis of these observations might be found in the evolution history, a propensity to aggressive behavior in man being related to an ancestral adaptative response to a low-cholesterol diet occurring during starvation and famine.[71]

Serotonin transporter

The main physiological role of a 5-HT transporter is the clearance of released 5-HT from the extracellular space, and thus the control of the duration and magnitude of neurotransmission via 5-HT receptors. Although an active concentrating mechanism of 5-HT by human platelets was already mentioned by Hardisty and Stacey in 1955,[72] selective 5-HT uptake into nerves was only reported at the end of the 1960s. Later, it was observed that certain neuronal subpopulations in brain selectively concentrate exogenous tritiated monoamines by uptake.[73-75] The binding of anti-

5-HT receptor	Locus	Aminoacid length	Human brain regions	Putative functions	Related clinical interests	Ref
5-HT1A	5q11.2-q13	422	Raphe n hyp, hip, amy, CPu, Cx, Fcx	5-HT activity, thermoregulation, feeding, stress, pain, mood, emotion, cognition, learning, memory…	Anxiety/depression, neurodegenerative disorders, schizophrenia	25,147,171
5-HT1B (5-HT1Dß)	6q13	390	SN /VTA, ACN, CPu, ventral pallidum, Cx	5-HT activity,mood, feeding	Anxiety/depression, migraine	131,138,172
5-HT1D	1p36.3-34.3	343	CPu, , ventral pallidum, Fcx	5-HT activity, mood, feeding	Anxiety/depression, migraine	173
5-HT1E	6q14-q15	365	CPu, Hyp, Cx	(?)	(?)	See 174
5-HT1F	3p13-p14.1	366	Ce, Hip, Cx	Mood, emotion	Migraine	175
5-HT2A	13q14-q21	471	Dorsal vagal complex, hypoglossal n, inferior olvary complex, Thal, CPu, Cx, FCx	Mood, respiratory control, feeding, nociception	Schizophrenia, anxiety/ depression, Tourette's syndrome, Alzheimer's didease, anorexia/ bulimia, drug abuse, pain	110,160,176
5-HT2B	2q36.3-q37.1	481	Ce (?), LS (?), Hyp (?) Cx (?)	Brain development (?), feeding (?)	Drug abuse, anxiety (?)	177
5-HT2C	Xq24	458°	Choroid plexus, Ce, DRN, SN, Hyp, Amy, Hip, CPu, ACN, Cx	Mood, impulsivity, feeding, locomotor activity	Anxiety/depression, schizophrenia, drug abuse, obesity	178
5-HT3A-E subunits	11q23.1-27.1	510* (5-HT3A)	Dorsal vagal complex, Hip, Amy, CPu	Vomiting reflex, mood,	Nausea, anxiety/depression	103,104
5-HT4	5q34-q36	402*	Hyp, Hip, ACN, CPu	Feeding, reward, cognition	Anorexia, drug abuse, Alzheimer's disease	139,171, 179,180
5-HT5A	7q34-q36	357	Ce, Hyp, Thal, Hip, Cx	Circadian rhythm, sleep, mood, cognition	Schizophrenia (?) anxiety/depression (?)	181
5-HT6	1p36-p35	440	Hip, CPu, Cx, olfactory tubercle	Cognition, learning, memory, feeding	Alzheimer's disease, dementia, obesity	171,182
5-HT7	10q21-q24	479*	Raphe n., Hyp, Tha, Hip, Amy, Cx	Mood, sleep, cognition	Anxiety/depression, schizophrenia.	183

Table I. Serotonin (5-HT) receptors in the human brain: distribution, putative functions, and related pathologies. Pre-RNA *splicing and ° editing variants. For review see also refs 98 to100. X, dorsal motor n of the vagus nerve; ACN, accumbens n; Amy, amygdala; cc, corpus callosum; Ce, cerebellum; CPu, caudate-putamen; Cx, cortex; DRN, dorsal raphe n; Fcx, frontal cortex; Hip, hippocampus; Hyp, hypothalamus; LS, lateral septum; MRN, n, nucleus; SN, subtantia nigra; Tha, thalamus; VTA, ventral tegmental area

depressants to neurons, platelets, gastrointestinal, pulmonary, and placental brush-border membranes bearing a serotonin transporter (SERT or 5-HTT) was then demonstrated.[76,77] More than 30 years later, a large family of *neurotransmitter sodium symporters* was identified by molecular cloning.[44] Contrary to metabotropic receptors displaying seven transmembrane domains, the predictive topology of monoamine transporters indicated 12 transmembrane domains, a large extracellular loop, and intracellular N and C terminal sequences. The identification of the human SERT sequence as an antidepressant and a cocaine-sensitive transporter[78] in 1993 was just preceded by the description of γ-aminobutyric acid (GABA) and noradrenaline transporter sequences. Interestingly, in 1991, Hoffman and coworkers had already reported a SERT sequence from a rodent leukemia cell line.[79] SERT homologous sequences were also described in invertebrates such as *Drosophila*, suggesting that this gene is phylogenetically ancient.[80] In humans as well as in other mammalian species, SERT mRNA expression in the brain is restricted to 5-HT cell bodies.[81,82] The unique SERT gene includes 14 exons encoding both a short and a long variant in humans and is localized in the long arm of chromosome 17.[78] Several polymorphisms, especially in the promoter region of SERT, are presumed to be associated with psychiatric illness including depression, anxiety, cognitive impairment, eating disorders, alcohol dependence, and primary insomnia.[83-87]

A transcription factor, Pet-1, influences TPOH2 and SERT expression levels in the rodent brain. It was demonstrated that Pet-1-null mice have severe deficiency in 5-HT signaling associated with anxiety-like and aggressive behaviors.[88] However, the role of the human ortholog gene FEV (Fifth Edwin Variant) is less well established.[89] Furthermore, it was recently reported that the level of SERT expression is under influence of a *microRNA* (MiR-16) upregulated by antidepressants such as fluoxetine.[90]

As described for other monoamine transporters, reuptake of 5-HT by SERT is ATP-dependent. It was suggested that SERT-associated proteins (a variety of phosphatase and phosphokinase proteins, nNOS and several others) could regulate the transporter velocity, its downregulation by intracellular sequestration, and its surface membrane targeting.[77,91]

Following its reuptake into the neuronal elements by SERT, 5-HT can be degraded by MAO associated with the mitochondrial membranes. Alternatively, 5-HT is packaged into vesicles by a (H+)-dependent carrier called vesicular monoamine transporter 2 (VMAT2) also present in other monoaminergic neurons. The factors leading to the packaging rather than degradation of 5-HT within 5-HT neurons remain to be elucidated. Very intriguing is the recent report of *vesicular-filling synergy* in serotonergic neurons, a mechanism previously found in certain cholinergic neurons.[81] Thus, it was observed that half of the neocortical and hippocampal subsets of 5-HT neuronal elements lacking SERT coexpress VMAT2 and the vesicular glutamate transporter VGLUT3 on the same vesicles. It was further demonstrated that vesicular glutamate uptake via VGLUT3 allows 5-HT vesicular filling by VMAT2, fostering 5-HT release from tonically active terminals involved in volume transmission. Serotonergic fibers and terminals coexpressing VGLUT3 and VMAT2 but lacking reuptake by SERT could represent sites of powerful regulatory mechanisms in 5-HT neurotransmission (for further details see ref 81). VMAT2 is targeted by several psychoactive drugs such amphetamines, tetrabenazine, and reserpine, which finally facilitate 5-HT depletion within neurons by its release in the extracellular space.[49] Specific haplotypes in the VMAT2 gene are possibly associated with depression symptoms.[92] They are also presumed to be protective in Parkinson's disease[93] and alcoholism.[94]

Serotonin receptors

The first evidence for 5-HT/tryptamine receptors and their desensitization were reported in the guinea-pig ileum during the 1950s. According to their sensitivity to morphine or dibenzyline, 5-HT/tryptamine receptors were called M and D, respectively. It was further suggested that M receptors also act in the nervous system.[95] The presence of 5-HT receptors in the brain was deduced from electrophysiological and pharmacological investigations in the cat lateral geniculate nucleus. Thus, it was demonstrated that lysergic acid diethylamide (LSD) directly influences central 5-HT receptors. Based on binding experiments of [3H]5-HT and [3H]spiroperidol, two distinct 5-HT receptor populations (5-HT1 and 5-HT2) were described in rodent and bovine brain membranes.[96] On pharmacological criteria, four brain 5-HT 1 receptor subtypes (5-HT1A, 5-HT1B, 5-HT1C, 5-HT1D) and a peripheral 5-HT3 serotonin receptor were then described in rodents.[97] From 1987 to the present time,

more than 15 5-HT receptors grouped into seven families were identified by various cloning strategies and characterized as distinct entities encoded by distinct genes *(Table I)*. Additional pre-RNA splicing and editing variants were further demonstrated for 5-HT2C, 5-HT3A, 5-HT4, and 5-HT7 receptors.[98] The same 5-HT receptor diversity was also observed in humans *(Table I)* and other mammalian species, although interspecies differences in their neuroanatomical distribution or their pharmacological profiles were noted.

With a few exceptions, the 5-HT receptor subtypes are expressed in the nervous system[98-100] as well as in the gastrointestinal tract.[46,47,101,102] 5-HT3 receptors[103,104] are ionotropic receptors formed by a pentamer of subunits (mainly 5-HT3A and B), whereas the other 5-HT receptors are metabotropic (G-protein coupled receptors) activating a large variety of signaling pathways.[105,106] As expected, the growing number of 5-HT receptor subtypes stimulates the development of selective interactive compounds of potential interest as therapeutic agents and, more recently, radiopharmaceutical tracers for in vivo imaging. It can be noted that the in silico design (ie, computer simulation) of these compounds gains more and more importance (for example see ref 107).

5-HT receptor subtypes more often coexist in the brain areas enriched in 5-HT-neuronal elements *(Table I, Figure 1)*. In the human brain, like in other species, the substantia nigra, the hippocampal formation, the hypothalamus, the amygdala, the striatum, and the frontal cortex display a large set of 5-HT receptors. Their relative densities show great variation among the brain areas, some of them being highly expressed in a restricted number of regions (eg, 5-HT3, 5-HT4, 5-HT6). Our knowledge of the anatomical distribution of 5-HT receptors in the human brain is not exhaustive, since selective ligands or specific antibodies for certain 5-HT receptor subtypes are not yet available (eg, 5-HT1E, 5-HT2B, 5-HT5A receptors). Consequently, their distribution is only based on their respective mRNA expression obtained by in situ hybridization histochemistry, and thus remains less well characterized.

From pharmacological characterization in human and basic studies in animal models there is evidence that 5-HT receptor density at the surface of the neuronal elements and their activity vary. A sustained stimulation of 5-HT receptors by agonist or endogenous 5-HT results in attenuated receptor responsiveness (or desensitization), intracellular sequestration (or internalization) and receptor recycling back to the membrane (eg, see refs 108, 109). Such mechanisms involve the activation of protein kinase C, phospholipase D and binding to arrestin proteins, uncoupling the transduction by G-protein subunits.[105,106] When stimulated by released 5-HT or 5-HT agonists, somatodendritic 5-HT1A *autoreceptors* in the raphe nuclei and 5-HT1B/1D autoreceptors in 5-HT terminal areas represent a powerful feedback mechanism, decreasing both the firing of the 5-HT neurons and the release of the neurotransmitter. Besides other neuroplastic changes, long-term desensitization and sequestration of these 5-HT receptor subtypes could be implicated in the delayed response of anxiolytic/antidepressants (SSRIs, buspirone, etc). Perhaps of special interest in psychosis, *heterologous* desensitization of 5-HT1A receptors by 5-HT2A receptor activation and close relationships between 5-HT, SERT, and 5-HT2A receptor densities were recently demonstrated in the living human brain.[110] Desensitization is not restricted to metabotropic receptors. Indeed desensitization of 5-HT3 receptor channels following sustained stimulation may play a critical physiological role in the regulation of neuronal excitability via this receptor.[111]

Intriguingly, *homodimerization* between 5-HT receptors (eg, 5-HT2A, 5-HT2C, 5-HT4 receptors) or even *heterodimerization*, an aggregate of two unrelated receptors, such as a 5-HT2A/ metabotropic glutamate receptor 2 dimerized complexes integrating both 5-HT and glutamate signaling, were reported in the human cortex.[112] Furthermore, this complex could increase the affinity of 5-HT2A receptors for hallucinogenic compounds such as LSD.[113] It was also recently reported that the internalization of CRF1 receptors by a CRF agonist enhances 5-HT2A signaling and anxiety-related behavior by recycling this receptor to the plasma membrane from an intracellular pool.[114,115] Finally, a variety of proteins including β-arrestins, serine/threonine protein kinases, protein phosphatase and tensin homolog, calpactin, and PDZ proteins interact with 5-HT receptor subtypes, modifying their functional activity.[105,116] They represent putative new targets for treatment of mood disorders and addiction.

Thus, the status and function of 5-HT receptors in the brain depend on a multiplicity of factors including crosstalk with other homologous and heterologous receptors.[106]

As illustrated in *Figure 2,* 5-HT availability in the extracellular space and target receptor functions are regulated at multiple levels, some of them being closely linked (eg, 5-HT1A, 5-HT1B/1D feedback mechanisms).

Anatomical organization of 5-HT circuitries in the brain

Morphological approaches in the brain

The respective scales of morphological approaches in the brain are called in *Figure 3*. Thus, imaging of the human living brain provides nowadays an incredible amount of information on functionally linked regions and, according to the availability of selective radiotracers, on millimetric clusters of binding sites. Morphological approaches including immunohistochemistry, in situ hybridization histochemistry and autoradiography allow to visualize a nucleus like the dorsal raphe, as well as a single labeled neuronal element of approximately one micrometer in diameter (eg, an axon varicosity) in brain tissue sections *(Figure 3)*. Electron microscopy studies in the human brain and, more often, in other mammalian

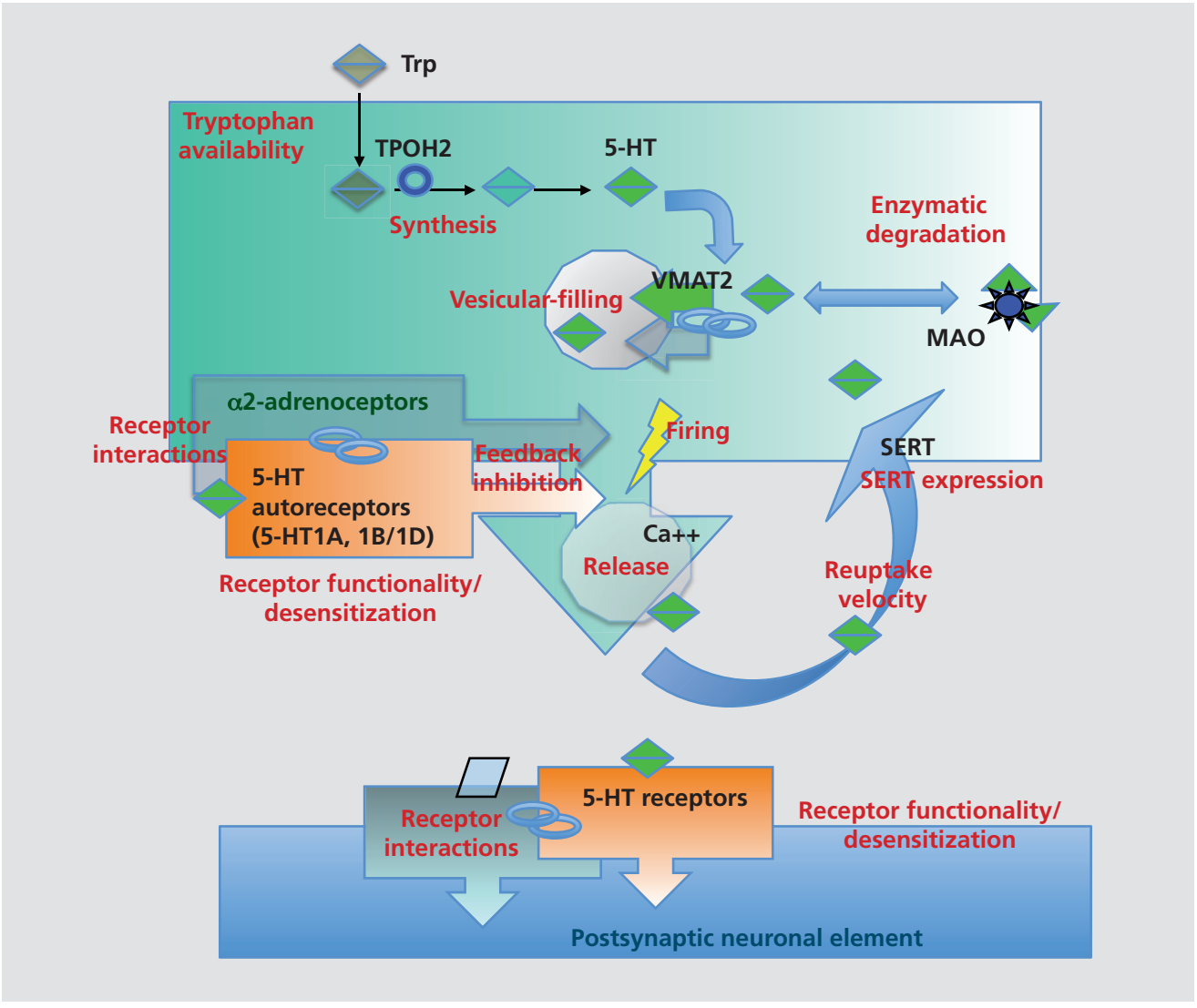

Figure 2. The serotonergic neurotransmission depends on serotonin (5-HT) levels present in the extracellular space and on membrane receptors triggering functional changes in neighbouring neuronal elements. 5-HT synthesis, release and reuptake are regulated by several mechanisms including feedback inhibition by 5-HT1A, 5-HT1B/1D autoreceptors and α-2 adrenoceptors. Other mechanisms of regulation are receptor dimerization and desensitization affecting their trafficking and functionality. See text for further details.

species give ultrastructural details (eg, junctions between neuronal elements or 5-HT1A receptor internalization).[117]

Cellular mapping of 5-HT-producing neurons in the CNS

Due to the postmortem instability of 5-HT[118] and other possible methodological bias,[119] quantitative biochemical estimation of 5-HT in the human brain subdivisions should be interpreted with caution, as illustrated by the numerous discrepant data reported since the 1950s. For the same reason, morphological approaches by formaldehyde-induced fluorescence or immunohistochemistry using antibodies against 5-HT are limited to biopsies and fetal brain tissues. Most of the anatomical studies in human are based on regional autoradiography of SERT binding sites to selective radioligands and immunohistochemical studies using antibodies against TPOH, which represent more stable postmortem markers. Therefore, from these studies and those performed in much detail in other species including rodents,[120] cat,[121] and nonhuman primates,[122] it appears that the anatomy of the serotonergic system has remained somewhat similar between different species of mammals.

The 5-HT systems belong to the neuronal systems composed of a restricted number of neurons emitting extensively branched, non- or poorly myelinated axons that innervate almost all brain nuclei. As first described in human fetuses[123,124] and later in adults by several authors,[125-129] the distribution of the 5-HT cell bodies (approximately 350 000 cells) in the human brain is restricted to the brain stem. As illustrated in *Figure 1*, a large majority of them is concentrated along the midline in the raphe nuclei, extending from the caudalmost level of the medulla oblongata to mid-level of mesencephalon, but a substantial number is located in the reticular formation lateral to these nuclei. The 5-HT neurons form a continuum of cells with loosely defined boundaries along the raphe nuclei. On the basis of studies of cell body localization and their respective projections, the 5-HT neurons can be separated into two groups: a rostral group located in the mesencephalic and rostral pons, sending axons to the forebrain, and a caudal group lying in the rostral pons and medulla oblongata, sending axons in the brain stem and spinal cord (refs in ref 128) In humans, the rostral group contains approximately 85% of the 5-HT neurons. It is composed of neurons located in four nuclei and one area, namely the interpeduncular, the caudal linear, the dorsal raphe (DRN with 165 000 neurons) and the median raphe (MRN with 64 000 neurons) nuclei. The additional area corresponds to the cau-

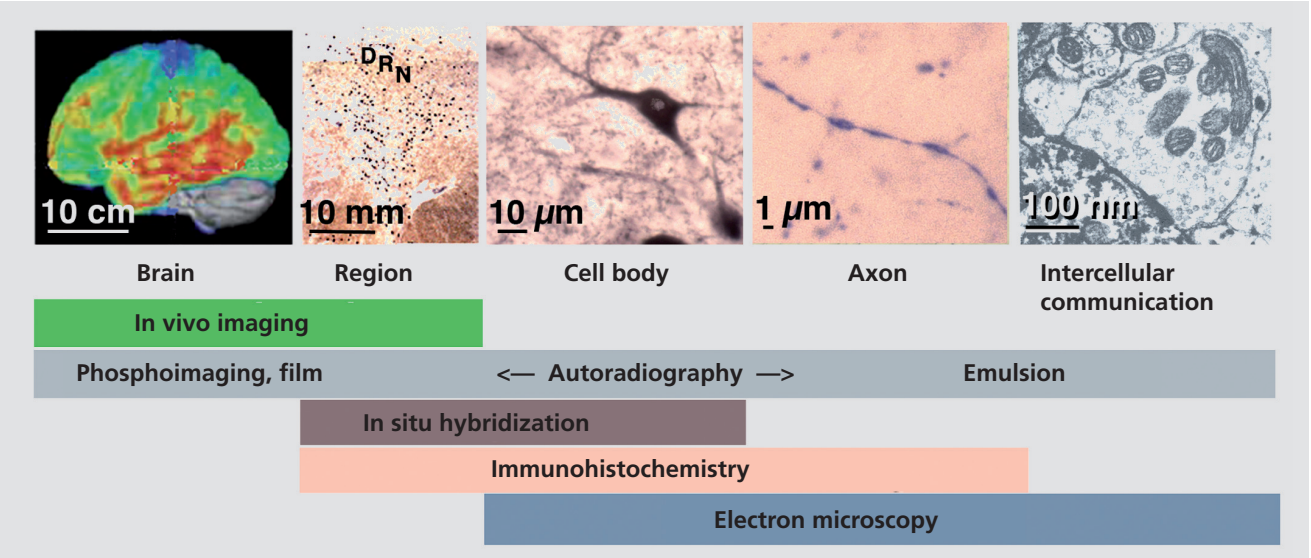

Figure 3. Photographs illustrating the different scales provided by the different anatomical methods used to investigate the brain. In vivo imaging allows regional analyses (from the whole brain to groups of neurons), whereas electron microscopy provides images of neuronal cell bodies and is particularly useful to visualize axonal varicosities and their contacts with neighboring elements. In between are autoradiography, in situ hybridization, and immunohistochemistry. DRN, dorsal raphe nucleus.

dal mesencephalic and rostral pontine reticular formation. 5-HT neurons spread in this area were already observed in the rat and cat species and their large number estimated in human (60 000 neurons).

The caudal group accounts for 15% of all the 5-HT neurons. It is composed of 5-HT neurons located in three raphe nuclei, namely the raphe magnus (30 000 neurons), the raphe obscurus, and the raphe pallidus (1000 neurons), and in the ventral medullary reticular formation lying lateral to the raphe magnus and the pyramids. As noted earlier, the rostral and caudal groups have separate afferent projections, with, however, some overlapping in the brain stem and as far down as the spinal cord. The trajectories of the efferent pathways have been studied in laboratory animals, often combining retrograde tracing with immunohistochemistry. Thus, a rostral and a ventral pathway emerge from the rostral group, rapidly join ventrally and split again into a lateral projection running in the internal capsule to innervate the lateral cortex and a longitudinal rostral projection running in the medial forebrain bundle to innervate the hypothalamus, basal forebrain, septum, basal ganglia, and amygdala. This rostral projection extends into the cingulum and innervates the medial cortex and the hippocampus.

The density of innervation in terminal areas reported in certain human brain areas has been extensively studied in cat and rodents. This density greatly varies from one region to the other and also within a region *(Figure 1)*. In the cerebral cortex, the superficial layer receives more axons than the other layers. A dense innervation is observed in the ventromedial part of the caudate-putamen and in the globus pallidus. Ventral to them, the substantia innominata is also richly supplied in 5-HT terminals. In the amygdala, the basal nucleus stands out for its very high number of 5-HT axons. In humans, like in animals, the 5-HT axons innervating the cortex and the hippocampus display two different morphologies.[130] One category of axons bears spaced small and elongated varicosities while the other category displays closely spaced, large, and round varicosities. It can be noted that the two populations of axons show several interesting properties. First, they are respectively issued from two different raphe nuclei, the DRN and the MRN. Second, the small varicose axons correspond to the numerous 5-HT axons not engaged in true synaptic contacts. For example, it is remarkable that only 5% of the varicosities display synapses in the rat frontoparietal cortex.[48] Thirdly, and of special clinical interest, the small varicose axons are more susceptible to degeneration caused by amphetamine derivatives, like ecstasy.[131] The caudal group of 5-HT neurons sends axons both laterally in the reticular formation and downwards in the spinal cord. In the reticular formation, the 5-HT axons are particularly abundant in the cranial motor nuclei (trigeminal, facial and hypoglossal). In the spinal cord, the 5-HT axons terminate in all subdivisions and along the whole length of the cord. In the dorsal horn, the superficial layers are densely innervated. In the intermediate gray, the preganglionic sympathetic neurons of the intermediolateral column are densely surrounded by 5-HT axons. In the ventral horn, the 5-HT axons are in close apposition to the motor neurons, especially in primates.[132]

In vivo imaging of the brain serotonergic systems

Structural and functional tomography through the living brain is currently possible. Powerful tools, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and pharmacological MRI (phMRI),[133-135] add new information on the functional anatomy of the serotonergic systems in the human brain. PET and SPECT neuroimaging respectively use positron–emitting nuclides (18F, 11C) and gamma-emitters (123I, 125I) coupled to a small heterocyclic compound selective for one 5-HT receptor subpopulation, SERT or MAO A.[87,136,137] Since the radiotracer is injected at trace level, 5-HT receptors or SERT can be localized in vivo and their relative concentration/affinity estimated from binding potential (BP). A submillimeter spatial resolution is commonly reported in PET and SPECT studies. However, at the present time very few radiotracers selective for SERT, 5-HT1A, 5-HT1B, 5-HT2A, and 5-HT4 receptors are available.[87,134,136-140] The design of new radiopharmaceuticals for in vivo imaging is constrained by several criteria including brain penetrability, target selectivity, and the absence of troublesome radiometabolites.[141] Additionally, when using radiolabeled glucose analogs, PET and SPECT modalities provide information on blood flow and in some circumstances may reflect a local activity of nervous cells following a specific pharmaceutical treatment (eg, anxiolytics, antidepressants). Offering a better spatial and temporal resolution, phMRI represents another imaging method based on the hemodynamic response to changes

in neuronal activity induced by pharmacological manipulations. This emergent imaging modality providing an indirect measure of aggregated neuronal function could have an important impact on future 5-HT research in the living human brain.[133,135]

Despite the limited number of available radiotracers, in vivo imaging of 5-HT function gains more and more interest in basic research as well as in clinical medicine. For example, recent publications suggest a lateralization of 5-HT1A binding in language areas (auditory cortices) and sex differences in cortical and subcortical brain areas of healthy subjects.[142] A selective interrelation between 5-HT1A distribution, sex hormones, and aggression score in humans was also demonstrated by in vivo imaging and biochemical analyses.[143] More intriguingly, PET imaging studies clearly indicate that 5-HT2A receptor binding in the cortex is positively correlated to the body mass index[144] and the response in painful heat stimulation.[145] Furthermore, it was reported that an inverse relationship between 5-HT2A receptor and SERT BPs in the neocortex might be the result of interindividual differences in baseline 5-HT levels.[110] Mainly based on SERT binding, PET studies support a loss of serotonergic pathway integrity in ecstasy users[146] and patients suffering from schizophrenia, Alzheimer's and Parkinson's diseases, whereas they were more inconclusive for assessing human depression,.[137,147] Further, 5-HT dysfunction due to certain genetic variations in SERT and 5-HT receptor sequences is now detectable by functional neuroimaging.[87,148-150]

Although not quite completely understood, these recent data from living human brain imaging support and often greatly extend, previous data obtained by conventional postmortem investigations.

Serotonergic circuitries in function

Serotonergic circuitries chiefly include 5-HT-producing neurons, 5-HT-autoreceptors (ie, somatodendritic 5-HT1A receptors, 5-HT1B/1D receptors in terminal endings) and other neurotransmitter or hormone receptors including alpha-adrenoceptors, CRF receptors, tachykinin receptors, estrogen receptor beta and more recently demonstrated, oxytocin receptors[151] involved in neuronal firing and 5-HT release. Functionally connected neuronal elements bearing 5-HT-*heteroreceptors* (often called postsynaptic or perisynaptic receptors, see below) are obviously another major component of the serotonergic neurotransmission.[100,152,153] Additionally, classical neurotransmitters (eg, GABA, glutamate, dopamine, noradrenaline), peptidergic neuromodulators (eg, substance P), and endocannabinoid coexpression within 5-HT neurons also contribute to the serotonergic function.[154,155]

Considering that in several brain areas, including the neocortex and the hippocampus, 5-HT wired neurotransmission (WT) via true synapses coexists with volume transmission (VT), the terms pre- and postsynaptic should be used with caution. In fact, distances between release sites and receptors are not of the same magnitude, generally a few nm for WT vs up to 10 µm for VT. Thus, some authors consider that neuropsychoactive drugs act rather as volume transmission signals.[156]

Due to ethical and methodological limitations, our knowledge on neurotransmitter circuitries and their interconnections in human CNS largely benefits from that described with much detail in nonhuman primates and other species including cat and rodents. In laboratory animal species, the anatomical distribution of brain 5-HT neurons was often completed by other approaches such as transneuronal retrograde transport, selective lesions, microdialysis, electrophysiology associated with pharmacological manipulations, and more recently developed wireless fast-scan cyclic voltametry, a promising tool for the in vivo monitoring of 5-HT in the brain.[157] Therefore, the circuitries of serotonergic neurons in the human brain are mainly based on those known in other mammals. In spite of obvious species differences concerning the relative size and functional development of certain brain structures (eg, certain neocortical subdivisions, the olfactory system), behavioral effects of neurological lesions or other disease processes and neuroanatomopathological studies in human suggest that on the whole, serotonergic circuitries serve comparable basic functions among mammals. However, contemporary neuroimaging technologies mentioned above (especially functional and pharmacological MRI, and PET) combined with behavioral approaches, offer a variety of new opportunities for the investigation of the limbic system in the living human brain.[134,149,158,159] Thus, recent articles report the exploration of the corticolimbic circuitries in relation to emotion and cognition.[158,160,161] Multimodal in vivo imaging studies add new information on the medial prefrontal cortex and amygdala coupling,[160] providing an advanced knowledge on the brain mechanism of certain pathophysiological effects of social anxiety disorder.[134]

As described above, 5-HT neurons send axons and terminals throughout the entire brain and therefore can potentially interact with almost all the other neuronal systems via the diversity of 5-HT heteroceptors (ie, receptors expressed by neurons that do not synthesize 5-HT).[100] Recent investigations in mice indicate that other mechanisms could also contribute to the 5-HT signaling. Thus, it was demonstrated that local infusion of fluoxetine (a SSRI) in the dorsal raphe nucleus stimulates the secretion of the protein S100-beta by 5-HT neurons projecting to the locus cereuleus. This protein downregulates the microRNA miR-16 in noradrenergic neurons which in turn switch on serotonergic functions.[90]

Reciprocally, classical neurotransmitters, especially GABAergic, catecholaminergic, glutamatergic, cholinergic, and histaminergic systems, influence the serotonergic neurotransmission at different sites, including the raphe nuclei. It is well known that the raphe nuclei contain collections of non-5-HT neuronal elements (eg, GABAergic, glutamatergic, cholinergic, histaminergic, dopaminergic, noradrenergic) interacting with 5-HT cell bodies via their respective receptor subsets.[162,163] Moreover, the richness in heteroreceptors (eg, alpha2-adrenoceptors, glutamatergic, histaminergic receptors) expressed by 5-HT terminals and other local mechanisms (eg, vesicular-filling synergy) mentioned above illustrate the extent of the reciprocal chemocommunication between serotonergic circuitries and other neurotransmitter networks.

Other interactions of clinical importance concern the interaction between serotonergic neurotransmission and neuropeptidergic systems. It is well known that 5-HT influences the activity of the hypothalamo-pituitary-adrenal axis at multiple levels, playing a role in stress-related disorders. Thus, 5-HT1A, 5-HT1B, 5-HT2A, and 5-HT2C receptor agonists enhance CRH and ACTH secretion and, consecutively, cortisol and other hormone levels in the plasma.[164,165] In turn, corticosteroids attenuate the activity of 5-HT1A receptors in the dorsal raphe nucleus, the hippocampal formation and the frontal cortex. Many other interactions between the serotonergic and the peptidergic systems (eg, ACTH, cholecystokinin, CART peptide, neuropeptide Y, ghrelin) are implicated in the sleep-wake rhythm and feeding. Other factors known to locally influence 5-HT neurotransmission are neurosteroids (eg, progesterone in the hypothalamus),[166] lipids,[167] and neurotrophic factors (eg, BDNF in the hippocampus).[168]

Although not exhaustive, most all of the reciprocal interactions exemplified above involve specialized receptors.

Concluding remarks

It is conceivable that the list of molecular factors that act in 5-HT circuitries is still incomplete. The discovery of TPOH2 is less than 7 years old. Intriguingly, a very recent study in double (TPOH1/TPOH2) knockout mice mentioned a residual 5-HT synthesis, suggesting additional 5-HT synthetic pathway(s).[169] Further, it can reasonably be assumed that 5-HT receptor subtypes resulting from postranslational editing or alternative splicing mRNA are not restricted to 5-HT2C, 5-HT3, 5-HT4, and 5-HT7 receptor families. There is also a growing list of proteins playing a role in the regulation of SERT and 5-HT receptor activity. Beyond the diversity of 5-HT receptor subtypes, their crosstalk modalities, and their local ability for adaptation, volume transmission demonstrated in several brain regions adds to the complexity of the serotonergic circuitries. Such complexity may explain why small subpopulations of cell bodies sending axons throughout the entire brain may produce such a large spectrum of effects in brain functions. Molecular and cellular studies in laboratory animal models (mutant mice, *Caenorhabditis elegans*, cell lines) and postmortem human brain have enabled us to explore the serotonergic system and will certainly continue to do so.

Undoubtedly, improvement of the specificity and spatiotemporal resolution of in vivo imaging modalities coupled or not to pharmacological manipulations will also significantly contribute to a better knowledge of 5-HT circuitries, specifically in the living human brain. As already mentioned, human brain structures associated with emotional processing, attention, and some other cognitive functions, are currently being investigated by MRI. TEP modalities allow the visualization of receptors including 5-HT receptors. A next step in functional neuroimaging will be hybrid-scanner systems that combine both technologies.[170]

Finally, our reviewing on brain serotonergic circuitries has not taken into account the next level of complexity, ie, the fact that the role of other neurotransmitters is not limited to the modulation of 5-HT neuron activity.

Appendix—glossary

Autoreceptors/heteroreceptors

Autoreceptors are membrane receptors expressed by neurons that synthesize the neurotransmitter binding to these receptors, eg, 5-HT1A or 5-HT1B localized on 5-

HT neuronal elements. In contrast, heteroreceptors are membrane receptors born by neurons that do not produce the corresponding neurotransmitter, eg, alpha2-adrenoceptors on 5-HT neuronal elements.

Heterologous desensitization
A sustained stimulation of a receptor by one agonist results in a homologous desensitization of this receptor (eg, 5-HT1A receptor desensitization by buspirone). Heterologous desensitization occurs when the binding of one agonist to a receptor subtype induces the attenuation of another receptor signaling (eg, desensitization of hypothalamic 5-HT1A receptors following 5-HT2A activation, desensitization of 5-HT2A receptors by activation of 5-HT1A receptors in the same region).

Homodimerization/heterodimerization
Most membrane G protein-coupled receptors exist as dimers or oligomers. A complex formed by two identical receptors (eg, 5-HT2A/5-HT2A; 5-HT2C/5-HT2C receptors) is called a homodimer, whereas a complex formed by unrelated receptors is heterodimer (eg, 5-HT2A/Glutamate receptor 2; 5-HT2A/D2 receptors). Dimerization occurs during transport of newly formed receptors to the cell surface. The homo- or heterodimeric complexes influence the signaling and internalization of receptors.

MicroRNAs
MicroRNA are small noncoding RNAs mediating post-transcriptional gene regulation (mostly translational repression). Thus, it was recently demonstrated that fluoxetine infusion in the dorsal raphe nucleus increases the level of a microRNA called miR-16 and consequently downregulates the mRNA and protein expression of the membrane serotonin transporter.

Somatodendritic receptors
Somatodendritic receptors are localized on the membrane of the cell bodies (soma) and dendrites of neurons, eg, the somatodendritic 5-HT1A receptors in the dorsal raphe nucleus.

Symporters
A family of membrane molecules coupling the transmembrane movement of a transmitter (monoamine or amino acid) to the transport of ions (mainly Na^+, K^+ and Cl^-). Neurotransmitter transporters (also called neuronal or membrane transporters) play a major role in the regulation of neurotransmission by energy-dependent reuptake of the neurotransmitters from the extracellular space. The neurotransmitter is then recycled by a vesicular transporter (eg, monoamine vesicular transporters) or degraded.

Vesicular-filling synergy
Vesicular-filling synergy (or vesicular synergy) first reported in cholinergic neurons was also detected in 5-HT circuitries, especially in limbic areas (hippocampus, prefrontal cortex). The coexpression of a vesicular glutamate transporter (VGLUT3) and a vesicular monoamine transporter (VMAT2) on the same vesicles of 5-HT terminal subpopulations represents a local synergic mechanism between glutamate and 5-HT neurotransmitters. It was demonstrated that glutamate reuptake stimulates vesicular 5-HT accumulation by VMAT2. Thus, 5-HT transmission is locally tuned by glutamate.

Wiring/volume neurotransmission
In wiring neurotransmission the communication between neurons operates via specialized junctional complexes including synapses (intercellular space in the synaptic cleft around 20 nm). The interneuronal communication without junctional complexes is called diffuse (or volume) neurotransmission and was identified in serotonergic, catecholaminergic, cholinergic, and several other transmitter systems. The neurotransmitter released in the extracellular space reaches target receptors localized up to several µm from the source (axon varicosities or terminals). 5-HT volume neurotransmission is frequently observed in the neocortex, the hippocampus, and several other brain areas. For more details on the functional consequences see the references indicated in the text. ❑

Circuitos serotoninérgicos cerebrales

Los circuitos serotoninérgicos cerebrales interactúan con otros sistemas de neurotransmisión en una infinidad de diferentes niveles moleculares. En humanos, como también en otras especies de mamíferos, la serotonina (5HT) tiene un papel modulador en casi todas las funciones fisiológicas. Además se postula que la disfunción serotoninérgica participa en diversos trastornos psiquiátricos y neurodegenerativos. Se describe la neuroanatomía y la neuroquímica de los circuitos serotoninérgicos cerebrales. También se discute la contribución de novedosos métodos de imágenes in vivo para la localización regional de sitios de unión de receptores y ciertos aspectos de su conectividad funcional en relación con la conducta. Los cuerpos de 5-HT, localizados principalmente en los núcleos del rafe, envían axones a casi todas las regiones cerebrales. Se argumenta que la especificidad de la comunicación química local entre 5-HT y otros elementos neuronales depende principalmente de mecanismos que regulan la concentración extracelular de 5-HT, de la diversidad de receptores de membrana de alta afinidad y de sus modalidades de transducción específicas.

Circuits sérotoninergiques centraux

Les circuits sérotoninergiques centraux sont le théâtre d'une myriade d'interactions moléculaires dévolues à leur communication. Chez l'homme comme chez les autres espèces, la sérotonine (5-HT) joue un rôle modulateur dans la presque totalité des fonctions physiologiques. De plus, un dysfonctionnement des systèmes sérotoninergiques est présumé impliqué dans diverses pathologies psychiatriques et neurodégénératives. Nous décrivons en détail les circuits sérotoninergiques centraux à partir d'études neuroanatomiques postmortem. *La contribution des approches modernes* in vivo *permettant la localisation régionale de récepteurs et certains aspects de leur fonctionnalité corrélée à des comportements sont aussi discutées. Les corps cellulaires à 5-HT principalement localisés dans les noyaux des raphés projettent des axones dans la plupart des régions du cerveau. Ainsi la spécificité de la communication chimique locale établie entre les éléments neuronaux à 5-HT et les autres dépend de mécanismes régulant la concentration extracellulaire en 5-HT, de la diversité des récepteurs membranaires de haute affinité et de leurs modalités de transduction.*

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