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Modulation of firing and synaptic transmission of serotonergic neurons by intrinsic G protein-coupled receptors and ion channels

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Serotonergic neurons project to virtually all regions of the central nervous system and are consequently involved in many critical physiological functions such as mood, sexual behavior, feeding, sleep/wake cycle, memory, cognition, blood pressure regulation, breathing, and reproductive success. Therefore, serotonin release and serotonergic neuronal activity have to be precisely controlled and modulated by interacting brain circuits to adapt to specific emotional and environmental states. We will review the current knowledge about G protein-coupled receptors and ion channels involved in the regulation of serotonergic system, how their regulation is modulating the intrinsic activity of serotonergic neurons and its transmitter release and will discuss the latest methods for controlling the modulation of serotonin release and intracellular signaling in serotonergic neurons *in vitro* and *in vivo*.

Keywords: 5-HT system, GPCRs, auto-regulation, hetero-regulation, optogenetics

INTRODUCTION

The serotonergic system consists of a small number of neurons that are born in the ventral regions of the hindbrain (Deneris and Wyler, 2012). In the adult nervous system, serotonergic neurons [5-HT (5-hydroxytryptamine) neurons] are located in the nine raphe nuclei that are restricted to the basal plate of the midbrain, pons, and medulla (Dahlstrom and Fuxe, 1964). 5-HT neurons located in the rostral raphe nuclei, such as the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN), give rise to the majority of the serotonergic ascending fibers into the forebrain including cerebral cortex, limbic system, and basal ganglia (Jacobs and Azmitia, 1992). The activity of the serotonergic system is regulated via transmitter release from local interneurons and/or afferents to the raphe nuclei (hetero-regulation), via mechanisms arising from 5-HT neurons themselves (auto-regulation), and potentially via alterations in the extracellular milieu (e.g., increase in CO_2; Pineyro and Blier, 1999; Richerson, 2004). In this review, we will discuss G protein-coupled receptors (GPCRs) and ion channels located at somatodendritic and presynaptic regions of 5-HT neurons in the DRN and MRN that contribute to the modulation of 5-HT neuronal activity and 5-HT release (**Figure 1**).

The DRN and MRN are the primary nuclei of 5-HT projections to forebrain and provide the neural substrate to communicate between global forebrain and other neuromodulatory systems by sending a wide range of 5-HT projections and receiving a wide variety of afferents (Jacobs and Azmitia, 1992). The DRN is located right beneath the posterior part of cerebella aqueduct and contains about half of all 5-HT neurons in the central nervous system (CNS), which can be further divided into six regions: rostral, caudal, dorsomedial, ventromedial, interfascicular, and lateral parts. The MRN is located at the ventral expansion of the DRN or the midline of the pontine tegmentum where many 5-HT neurons are densely packed in the midline and some 5-HT neurons are scattered in the periphery. Within the DRN and MRN 5-HT neurons project to defined target area in brain (Adell et al., 2002; Lechin et al., 2006). For example, DRN 5-HT neurons innervate the prefrontal cortex, lateral septum, and ventral hippocampus, while MRN 5-HT neurons innervate the temporal cortex, medial septum, and dorsal hippocampus. Afferent projections to the raphe nuclei are diverse and include acetylcholine (ACh) from the laterodorsal tegmental nucleus, dopamine from the substantia nigra and ventral tegmentum area, histamine from tuberomammillary hypothalamic nucleus, noradrenaline (NA) from the locus coeruleus, serotonin itself from the raphe nuclei and several neuropeptides as well as excitatory glutamatergic and inhibitory GABAergic inputs. Glutamatergic inputs come from several nuclei including medial prefrontal cortex and lateral habenula nucleus (Aghajanian and Wang, 1977; Celada et al., 2001; Adell et al., 2002; Bernard and Veh, 2012). Some inputs make direct contacts with 5-HT neurons, while others project onto local GABAergic interneurons that provide feedforward inhibitory input to 5-HT neurons (Sharp et al., 2007). In addition to the local GABAergic interneurons located in the raphe nuclei and in the neighboring periaqueductal gray area, extrinsic GABAergic projections have been suggested (Gervasoni et al., 2000). The responsiveness of 5-HT neurons to each of the inputs differs between DRN and MRN, and also within the subnuclei of the DRN (Adell et al., 2002; Lechin et al., 2006). The differences in responsiveness most likely depend on differences in the strength of the afferent inputs for each of the nuclei and subnuclei and also on the expression and types of the ionotropic and metabotropic receptors in 5-HT neurons. Furthermore, the intrinsic membrane excitability of 5-HT neurons has been reported to differ in the distinct raphe nuclei (Beck et al., 2004; Crawford et al., 2010). Importantly, beyond the anatomical

and physiological differences, it has been reported that subpopulation of 5-HT neurons have distinct implications in specific physiological function and behavior (Abrams et al., 2004; Lechin et al., 2006; Hale and Lowry, 2010).

AUTO-REGULATION

The midbrain 5-HT neurons elicit spontaneous action potentials (APs), with a regular, slow firing pattern (1–5 APs/s; Aghajanian and Vandermaelen, 1982; Vandermaelen and Aghajanian, 1983). 5-HT released from 5-HT neurons act either on the 5-HT neuron itself or on the target circuits. There are several ways how 5-HT neurons may receive 5-HT. First, dendrodendritic synapses releasing 5-HT have been described in raphe nuclei between 5-HT neurons. Second, recurrent axonal collaterals have been suggested to back-propagate to the raphe nucleus itself to release 5-HT. Finally, 5-HT neurons between different raphe nuclei such as DRN and MRN communicate with each other via 5-HT (for review, see Adell et al., 2002; Harsing, 2006; Lechin et al., 2006). Indeed electrical stimulation in DRN slice preparations induces 5-HT_{1A} receptor-mediated slow inhibitory postsynaptic potentials (IPSPs) in 5-HT neurons (Pan et al., 1989; Morikawa et al., 2000), demonstrating 5-HT release in the proximity of 5-HT neurons.

Once 5-HT is released, 5-HT receptors will be activated. Seven subgroups of 5-HT receptors encoding ionotropic as well as metabotropic receptors have been described (5-HT_1–5-HT_7), with 15 total variants identified to date (Barnes and Sharp, 1999; Hoyer et al., 2002; Kroeze et al., 2002). The 5-HT GPCRs can be divided into three major subgroups depending on which G protein signaling pathway they activate. 5-HT_1 receptors couple mainly to the $G_{i/o}$ pathway; 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors couple to the G_s pathway; and 5-HT_2 receptors activate the $G_{q/11}$ pathway. The 5-HT_3 receptors are ligand-gated ion channels.

5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors are found on somatodendritic and axonal region of 5-HT neurons (McDevitt and Neumaier, 2011). All three receptors act as negative feedback effectors for 5-HT neuronal firing and 5-HT release (Andrade, 1998). Somatodendritically located 5-HT_{1A} receptors down-regulate the firing rate of 5-HT neurons via activation of G protein-coupled inwardly rectifying potassium channels (GIRK) leading to membrane hyperpolarization, and reduction or complete block of AP firing (Colino and Halliwell, 1987; Sprouse and Aghajanian, 1987; Blier et al., 1989; Hjorth and Sharp, 1991; Penington et al., 1993; Stamford et al., 2000). In addition, 5-HT_{1A} receptors inhibit voltage-gated Ca^{2+} channels of the N- and P/Q-type in 5-HT neurons, as application of a selective 5-HT_{1A} agonist diminishes somatic Ca^{2+} channel currents (Penington and Kelly, 1990; Penington et al., 1992; Bayliss et al., 1997b). The functional consequence of Ca^{2+} channel inhibition is an increase in the firing rate due to reduction in the afterhyperpolarization, which may involve Ca^{2+} activated-K^+ channel (Bayliss et al., 1997b). The physiological role of the differential effects of 5-HT_{1A} receptors on the AP firing has not been addressed so far, but may involve input specificity due to 5-HT_{1A}/GIRK and 5-HT_{1A}/Ca^{2+} channel colocalization in specific subcellular domains and/or differences in the regulatory properties of heterogeneous 5-HT neurons within and among different raphe nuclei (Calizo et al., 2011).

The predominant 5-HT receptors at the presynaptic terminal are the 5-$HT_{1B/1D}$ receptors. 5-$HT_{1B/1D}$ receptors have been shown to inhibit 5-HT release from the axonal varicosities as demonstrated with electrophysiological experiments (Sprouse and Aghajanian, 1987; Boeijinga and Boddeke, 1993; Morikawa et al., 2000), probably due to inhibition of Ca^{2+} influx through voltage-gated Ca^{2+} channels such as P/Q-type and N-type Ca^{2+} channel (Kimura et al., 1995; Harvey et al., 1996). The 5-HT_{1B} receptors have been shown to underlie presynaptic autoinhibition of 5-HT release in which 5-HT_{1A}-mediated slow IPSPs are reduced by previous released 5-HT activating presynaptic 5-HT_{1B} receptors (Morikawa et al., 2000). In addition, 5-HT_{1B} receptors have been suggested to up-regulate 5-HT reuptake by serotonin transporters (Xie et al., 2008; Hagan et al., 2012) and 5-HT synthesis by itself might be under the control of 5-HT_{1B} (Hjorth et al., 1995). Thus, 5-HT_{1B} autoreceptors may have the ability to control 5-HT release independently from the actual firing rate.

5-HT_{1F} receptor mRNA has also been detected in the raphe nuclei (Bruinvels et al., 1994). Since 5-HT_{1F} receptors have a high affinity for sumatriptan, a 5-$HT_{1B/1D}$ agonist, and the sumatriptan-induced reduction in 5-HT release (monitored by voltammetry in brain slices) could not be blocked by 5-$HT_{1A/1B/1D}$ receptor antagonists, 5-HT_{1F} had been suggested as a possible candidate of a serotonergic autoreceptor in the MRN (Hopwood and Stamford, 2001).

While the expression and function of 5-HT_1 receptors has been directly demonstrated in 5-HT neurons, involvement of other 5-HT receptors such as 5-HT_2, 5-HT_3, and 5-HT_{4-7} is less clear and may differ among species, the developmental stage of the animal and 5-HT neuron subtypes.

5-HT_2 receptors are functionally expressed in particular on GABAergic interneurons in the DRN, since activation of 5-$HT_{2A/2C}$ receptors increase fast inhibitory postsynaptic current (IPSC) frequency in 5-HT neurons and reduce 5-HT neuronal firing as electrophysiologically measured in brain slices (Liu et al., 2000; Leysen, 2004). 5-HT_2 receptor mRNA and proteins have been identified in the DRN and embryonic 5-HT neurons (Wright et al., 1995; Clemett et al., 2000; Wylie et al., 2010). Additionally, 5-HT_2 receptors have been postulated to increase 5-HT_{1A}-mediated responses in 5-HT neurons (Kidd et al., 1991). However, a direct modulatory effect of 5-HT_2 in 5-HT neurons has not been demonstrated.

The 5-HT_3 receptors have also been suggested to act as presynaptic autoreceptors in serotonergic nerve terminals. Although 5-HT_3 receptors have been shown to enhance 5-HT release in various brain areas including the raphe nuclei as monitored by [^{3}H]5-HT assays (Bagdy et al., 1998), there is no direct immunohistochemical and electrophysiological evidence of the presence of 5-HT_3 receptors in 5-HT neurons (van Hooft and Vijverberg, 2000).

For the G_s protein-coupled 5-HT_{4-7} receptors, mainly indirect evidence exists for an autoregulatory role of these GPCRs in 5-HT neurons.

5-HT_4 receptors seem to be located somatodendritically and presynaptically. A presynaptic potentiating effect of 5-HT_4 receptor on glutamate release, which can be counteracted by 5-HT_{1A} receptor-mediated inhibitory action, has been described in

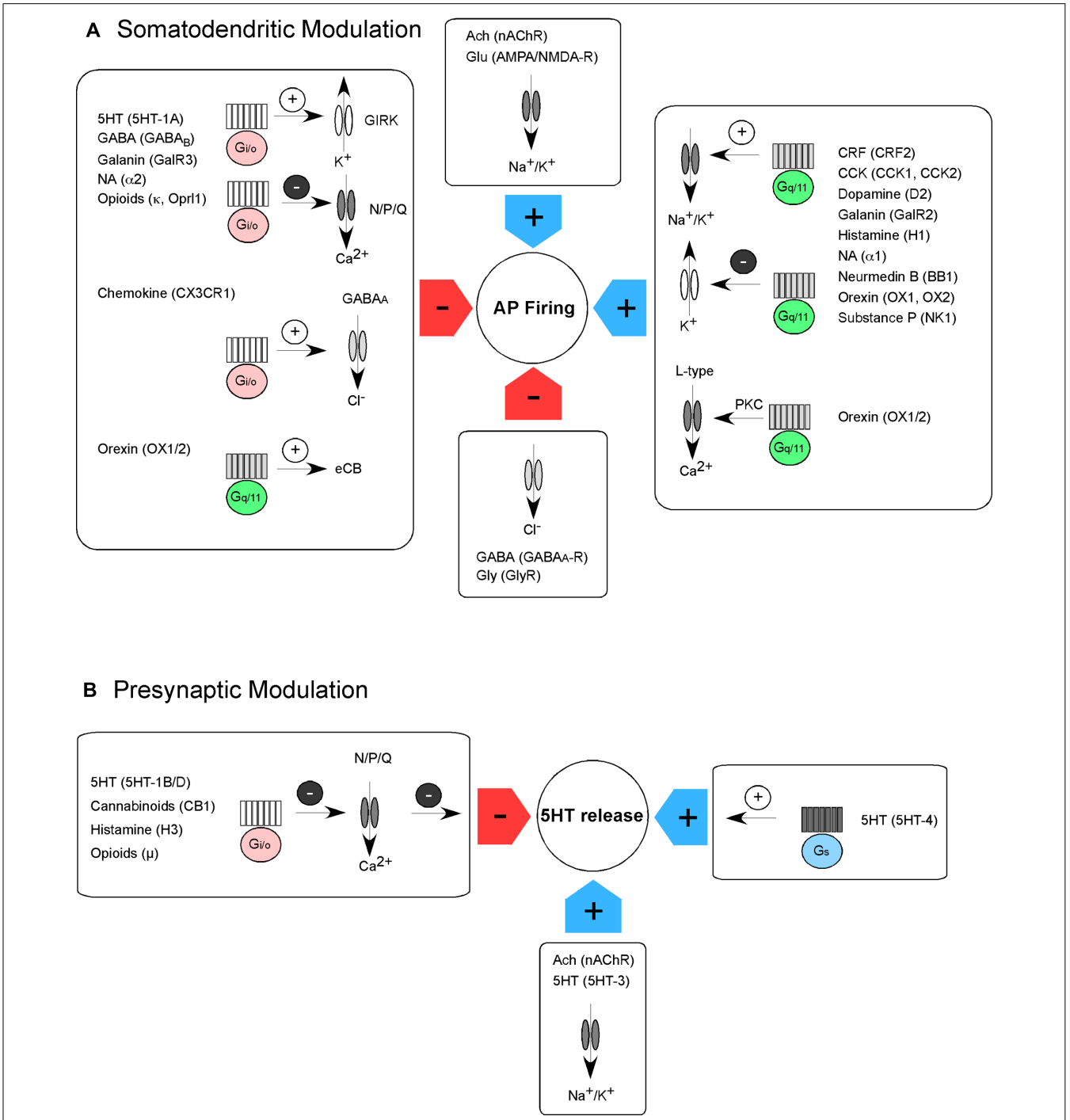

FIGURE 1 | Intrinsic somatodendritic and presynaptic modulation of action potential (AP) firing and transmitter release in 5-HT neurons. (A) AP firing of 5-HT neurons is modulated by GPCRs and ion channels. AP firing is reduced (red arrows) via activation of GPCRs coupling to the $G_{i/o}$ pathway or activation of inhibitory ligand-gated ion channels. $G_{i/o}$ pathway activation can lead to the activation of GIRK channels, inhibition of voltage-gated Ca^{2+} channels or increase in $GABA_A$ receptor currents. In addition, $G_{q/11}$ activation can lead to synthesis of endocannabinoids, which inhibit transmitter release onto 5-HT neurons. AP firing is increased (blue arrows) via activation of GPCRs coupling to the $G_{q/11}$ pathway or activation of excitatory ligand-gated ion channels. $G_{q/11}$ pathway activation can lead to the activation of non-selective cation channels or inhibition of K^+ conductance. In addition, the activation of L-type Ca^{2+} channels via protein kinase C (PKC) can most likely increase membrane depolarization and transcription. **(B)** 5-HT release of 5-HT neurons is also modulated by GPCRs and ion channels. 5-HT release is reduced (red arrow) via activation of GPCRs coupling to the $G_{i/o}$ pathway. $G_{i/o}$ pathway activation leads to the inhibition of presynaptic Ca^{2+} channels, reduction in Ca^{2+} influx and therefore reduction in transmitter release. 5-HT release is increased (blue arrows) via activation of GPCRs coupling to the G_S pathway and via opening of excitatory, ligand-gated ion channels.

hippocampal neurons (Kobayashi et al., 2008). Since neurotransmitter release of various transmitters including 5-HT is modulated by 5-HT_4 agonists, a presynaptic localization of 5-HT_4 receptors on 5-HT neurons seems possible (Mengod et al., 2010).

While the function of 5-HT_5 receptors in the CNS has not been thoroughly studied, two studies suggest a role of 5-HT_5 receptors for modulating 5-HT neurons. First, 5-HT_{5B} receptor mRNA, a receptor which is expressed in rodents but not in humans, is colocalized with the mRNA of 5-HT transporter in the DRN (Serrats et al., 2004). Second, block of 5-HT_{5A} receptors in the DRN attenuates the 5-carboxamidotryptamine (5-CT; non-selective agonist in particular for 5-$HT_{1A/1B/1D}$ receptors) induced reduction of 5-HT neuronal firing but fail to affect 5-HT release measured using fast cyclic voltammetry *in vitro* (Thomas et al., 2006). The data suggest an autoreceptor modulation of 5-HT neurons via 5-HT_{5A} receptors.

5-HT_6 and 5-HT_7 receptor protein and mRNA have been detected in cells in the raphe nuclei including the DRN (Ruat et al., 1993; To et al., 1995; Gustafson et al., 1996; Woolley et al., 2004; see also Gerard et al., 1997; Hamon et al., 1999), but a functional role as autoreceptors for modulating 5-HT neurons could not be demonstrated so far (Bourson et al., 1998; Roberts et al., 2001).

Thus, the auto-regulation of 5-HT neuronal firing is in particular regulated by 5-HT_{1A} receptors via activation of the $G_{i/o}$ pathway and opening K^+ and closing Ca^{2+} conductance. At the presynaptic terminal, 5-$HT_{1B/1D}$ receptor activation reduces 5-HT release most likely via $G_{i/o}$ protein-mediated inhibition of presynaptic Ca^{2+} channels. In addition, potentiation of 5-HT release by activating 5-HT_4 receptors via the G_s pathway seems possible. Since other 5-HT receptor mRNAs have been detected in 5-HT neurons, other autoregulatory mechanisms may exist in subgroups of 5-HT neurons or during different developmental stages of the serotonergic transmitter system. In particular, animal models for the selective activation of these GPCRs during development will further elucidate the modulatory role of other 5-HT receptors in the auto-regulation of 5-HT neuronal firing and 5-HT release.

HETERO-REGULATION

5-HT modulates various complex behaviors and therefore the serotonergic transmitter system receives feedback and feedforward information from other brain areas and networks involved in regulating the different behaviors (Adell et al., 2002; Lechin et al., 2006; Sharp et al., 2007). Thus, the hetero-regulation of the 5-HT neurons involves various transmitter systems.

Forty-nine different GPCRs belonging to all four GPCR subfamilies were identified in postmitotic embryonic 5-HT neurons using microarray expression profiling (Wylie et al., 2010). These GPCRs include adrenergic, calcitonin, cannabinoid, GABA, histamine, opioid, and serotonin receptors. For these transmitter systems, a postnatal modulatory role for 5-HT neurons has been described (see below). In addition, other GPCRs such as thrombin, chemokine, prostaglandin E, melanin-concentrating hormone, cadherin, and parathyroid hormone receptors as well as frizzled (FZD) and smoothened (SMO) homolog and the orphan receptors (GPR 19, 56, 85, 98, 125 135, 162, and 173) were also identified but a physiological role for their modulation of 5-HT neurons still needs to be defined and investigated (for a complete list, see Wylie et al., 2010). We will summarize recent findings on the intrinsic hetero-regulation of the serotonergic transmitter system.

GABA, GLYCINE, AND GLUTAMATE

5-HT neurons within the raphe nuclei receive in particular GABAergic but also glutamatergic input. As expected, the GABAergic input onto 5-HT neurons reduces the neuronal firing, while glutamatergic input increases the firing activity (Pan and Williams, 1989; Levine and Jacobs, 1992; Becquet et al., 1993a,b; for review, see Adell et al., 2002; Harsing, 2006). These effects have been mainly attributed to the expression of ionotropic GABA and glutamate receptors (GluRs) in 5-HT neurons (Tao and Auerbach, 2000; Gartside et al., 2007), which is in agreement with the expression of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA), *N*-methyl-D-aspartate (NMDA), kainate receptors [GluR 1,2; NMDA-R-2B, kainate receptor 5 (Grik5)] as well as $GABA_A$ receptor subunits ($GABA_A$ β1–3 and γ2) in embryonic 5-HT neurons (Wylie et al., 2010). Interestingly, the glycine receptor α1 and β subunits have also been identified in human brain and mice embryonic 5-HT neurons (Baer et al., 2003; Wylie et al., 2010). Indeed the DRN receives input from glycinergic fibers (Rampon et al., 1996, 1999) to inhibit 5-HT neuronal firing and 5-HT release (Gallager and Aghajanian, 1976; Wang and Aghajanian, 1977; Becquet et al., 1993a).

The modulation of 5-HT neurons by $GABA_B$ receptors has been addressed in various studies. In general, activation of $GABA_B$ receptor by selective agonists decreases 5-HT release (Becquet et al., 1993b). The decrease of 5-HT release by $GABA_B$ receptors located within 5-HT neurons is most likely mediated via activation of GIRK channels leading to a reduction in AP firing (Innis and Aghajanian, 1987; Williams et al., 1988; Bayliss et al., 1997a; Cornelisse et al., 2007). Within 5-HT neurons, $GABA_B$ receptors are located extrasynaptically, suggesting that spillover of GABA during high activity of GABAergic neurons would modulate 5-HT neuronal activity within raphe nuclei (Varga et al., 2002). On the other hand, there is little information about modulatory effects of metabotropic GluRs (mGluRs) in 5-HT neurons so far. Although administration of group II mGluR (mGluR2/3) antagonist has been reported to increase 5-HT neuronal activity, an indirect effect on presynaptic excitatory neurons seems to be involved (Kawashima et al., 2005).

CORELEASE OF GLUTAMATE OR GABA FROM 5-HT NEURONS

Previous reports have suggested the possibility of glutamate release from 5-HT neurons based on the presence of vesicular glutamate transporter type 3 in a subset of 5-HT neurons (Gras et al., 2002; Amilhon et al., 2010). Using optogenetic techniques, the corelease of glutamate and 5-HT from serotonergic terminals could be demonstrated in a serotonergic projection from the MRN to hippocampal GABAergic interneurons (Varga et al., 2009). The serotonergic fibers make direct synaptic contacts to the GABAergic neurons and exert fast synaptic transmission mediated by ionotropic GluRs and 5-HT_3 receptors. In addition to the glutamate transporters, GABA and its synthesizing enzyme, glutamic acid decarboxylase (GAD) have also been reported in subsets of 5-HT neurons, suggesting the corelease of GABA and 5-HT

(Nanopoulos et al., 1982; Belin et al., 1983; Fu et al., 2010; Hioki et al., 2010; Shikanai et al., 2012). However, vesicular inhibitory amino acid transporter which is necessary for filling synaptic vesicles with GABA was absent in 5-HT/GAD67 positive neurons and their projections, suggesting that GABA may be released by non-vesicular mechanisms such as a reverse transport through GABA transporters (Shikanai et al., 2012). Thus, 5-HT axonal projections have a potential to modulate the neuronal activity in target areas using at least three different transmitters, i.e., 5-HT, glutamate, and GABA. It is intriguing to speculate that the auto-regulation of 5-HT neurons itself might be modulated by corelease of glutamate and GABA.

ACETYLCHOLINE

The DRN also receives cholinergic input from the laterodorsal tegmental nucleus (Wang et al., 2000). Modulation of 5-HT neurons by ACh mainly involves nicotinic ACh receptors (nAChRs) and can increase 5-HT neuronal firing (Mihailescu et al., 2002) for example, via presynaptic modulation of glutamate release (Garduno et al., 2012) or via opening of nAChR expressed in 5-HT neurons (Galindo-Charles et al., 2008; Chang et al., 2011). Very limited information is available for the expression and function of muscarinic ACh receptors (mAChRs) in 5-HT neurons. mAChR-M_1 receptors ($G_{q/11}$) might be expressed on serotonergic projections into the hippocampus (Rouse and Levey, 1996) and application of mAChR antagonist, atropine into the DRN enhances antidepressant-like 5-HT_{1A} agonist effects (Haddjeri et al., 2004), which may involve M_2 ($G_{i/o}$) but not M_1 ($G_{q/11}$) receptors (Haddjeri et al., 2000). Nevertheless a detailed and direct demonstration of the involvement of mAChRs in 5-HT neurons is essential.

DOPAMINE

The 5-HT neurons of the DRN reciprocally interact with the dopaminergic mesencephalic transmitter system involving dopamine receptors. D_1-like receptors (D_1 and D_5) couple to the G_s pathway, while dopamine D_2-like receptors (D_{2-4}) have been described to couple to the $G_{i/o}$ pathway. In the DRN, D_2 and D_3 receptor expression has been detected so far, with very little or no expression of D_1-like receptors (Bouthenet et al., 1987; Dawson et al., 1988; Cortes et al., 1989; Wamsley et al., 1989; Yokoyama et al., 1994; Suzuki et al., 1998). Dopamine increases 5-HT neuronal firing in DRN *in vivo* and in slice electrophysiological recording, and also increases 5-HT release detected by *in vivo* microdialysis in DRN and other brain areas (Ferre and Artigas, 1993; Ferre et al., 1994; Matsumoto et al., 1996; Mendlin et al., 1998; Haj-Dahmane, 2001; Martin-Ruiz et al., 2001). The modulatory effects on 5-HT neurons have been shown to be mediated by D_1- and D_2-like receptors located outside the DRN (Martin-Ruiz et al., 2001) or by direct activation of D_2 receptors expressed in 5-HT neurons (Haj-Dahmane, 2001). Activation of D_2-like receptors in 5-HT neurons leads to membrane depolarization, involving activation of G proteins, phospholipase C, and a non-selective cation current, most likely mediated by a transient receptor potential (TRP) channel (Aman et al., 2007). The D_2-like receptor effects in 5-HT neurons suggest that D_2-like receptors may activate the $G_{q/11}$ rather than the $G_{i/o}$ signaling pathway. The $G_{q/11}$ protein coupling might be explained by the heterodimerization between D_1- and D_2-like receptors (Rashid et al., 2007; Hasbi et al., 2010). It has to be noted that the experiments suggesting the modulation of 5-HT neurons by intrinsic D_2-like receptors have been performed with relatively high concentrations of quinpirole and sulpiride in *in vitro* preparations. Therefore, the experiments have to be interpreted carefully.

NORADRENALINE

5-HT neurons in the raphe nuclei receive noradrenergic input in particular from the locus coeruleus (Adell et al., 2002; Lechin et al., 2006). α_1 and α_2 adrenergic receptor mRNA and protein have been detected in the DRN and MRN (Unnerstall et al., 1985; Rosin et al., 1993; Scheinin et al., 1994; Talley et al., 1996; Day et al., 1997; Strazielle et al., 1999). α_1 and α_2 adrenergic receptors couple to the $G_{q/11}$ and $G_{i/o}$ pathway, respectively. Therefore depending on pathway activation, an increase or decrease in 5-HT neuronal activity and 5-HT release can be postulated if adrenergic receptors are expressed in 5-HT neurons. Early studies revealed that NA causes an increase in 5-HT neuronal firing in DRN. This effect has been suggested to be mediated via activation of α_1 adrenoceptors (presumably α_{1B} adrenoceptor subtype) located on 5-HT neurons (Vandermaelen and Aghajanian, 1983; Day et al., 1997) and may involve the suppression of a 4-aminopyridine sensitive K^+ conductance (I_A; Aghajanian, 1985). *In vivo* experiments suggest that α_1 adrenoceptors are tonically activated by endogenous NA (Adell and Artigas, 1999; Pudovkina et al., 2003). In contrast, 5-HT release detected by voltammetry or [^{3}H]5-HT assay in the DRN slice preparation is inhibited by NA, an effect which has been attributed to α_2 adrenoceptors (involving α_{2A} adrenoceptor subtype) and also perhaps indirectly to α_1 adrenoceptors (Frankhuijzen et al., 1988; Hopwood and Stamford, 2001). Since α_2 receptors couple to the $G_{i/o}$ pathway, 5-HT release and 5-HT neuronal firing could be reduced via α_2 adrenoceptors located at the soma or presynaptic terminal of 5-HT neurons itself (Hopwood and Stamford, 2001), or via inhibition of NA release at noradrenergic synaptic terminals lowering the effective NA concentration for α_1 adrenoceptors/G_q signaling pathway activation. The direct effect of α_2 adrenoceptors in 5-HT neurons is supported by the fact that embryonic 5-HT neurons express α_{2A} adrenoceptors (Wylie et al., 2010).

HISTAMINE

There are four histamine receptors (H_{1-4}), which couple to different G protein pathways, i.e., H_1 ($G_{q/11}$), H_2 (G_s), H_3, and H_4 ($G_{i/o}$). Early studies suggested that histamine reduces the firing of 5-HT neurons in the DRN (Lakoski and Aghajanian, 1983) via H_2 receptors (Lakoski et al., 1984). Since H_2 couples to the $G_{q/11}$ pathway, the results suggest that H_2 receptors are localized on GABAergic terminals. Later findings showed that histamine increased 5-HT neuronal firing in the DRN via activation of H_1 receptors and the opening of a non-selective cation conductance through $G_{q/11}$ signaling pathways (Barbara et al., 2002; Brown et al., 2002). Expression profiling in mouse embryos suggested the expression of H_3 receptors in 5-HT neurons, which is consistent with high mRNA levels in the DRN (Lovenberg et al., 1999; Drutel et al., 2001; Pillot et al., 2002). However, the low binding

of a H_3 receptor selective radioligand in the DRN suggests that H_3 receptors are mainly functional at the presynaptic terminal of 5-HT projections (Pillot et al., 2002). Indeed increasing levels of histamine decrease 5-HT release detected by an *in vivo* electrochemical technique (Hashemi et al., 2011).

ENDOCANNABINOIDS

The cannabinoid receptor family consists of two subtypes, CB_1 and CB_2. Modulation of neuronal activity is mainly exerted via CB_1, which couples to the $G_{i/o}$ protein (Howlett et al., 1986) and probably also to the G_s pathway (Glass and Felder, 1997). CB_1 receptors are localized in particular at presynaptic terminals, where they inhibit presynaptic Ca^{2+} influx and reduce transmitter release via endocannabinoid (eCB)-mediated retrograde signaling (Maejima et al., 2001). CB_1 receptors are expressed in serotonergic fibers (Haring et al., 2007; Ferreira et al., 2012) and their mRNA is found early in development (Wylie et al., 2010). 5-HT release in projection areas from the DRN is reduced by activation of CB_1 as monitored by microdialysis (Egashira et al., 2002) and [^{3}H]5-HT assay (Nakazi et al., 2000; Egashira et al., 2002), and increased by inhibition of CB_1 *in vivo* and *in vitro* (Darmani et al., 2003; Tzavara et al., 2003; Aso et al., 2009). Studies in CB_1 knock-out animals and chronic activation of CB_1 receptors *in vivo* suggest that CB_1 may regulate the function and expression of 5-HT_{1A} receptors (Aso et al., 2009; Moranta et al., 2009; Zavitsanou et al., 2010). Interestingly, 5-HT neurons itself synthesize eCBs in an activity-dependent manner (Haj-Dahmane and Shen, 2009, 2011). eCB release from 5-HT neurons can be induced by orexin-B leading to the activation of orexin (OX) receptors via the $G_{q/11}$ pathway (Liu et al., 2002b; Haj-Dahmane and Shen, 2005). It has been therefore speculated that the activity-dependent activation of the $G_{q/11}$ pathway in general may lead to the production of eCBs in 5-HT neurons (Haj-Dahmane and Shen, 2011). Within the DRN eCBs mainly act on glutamatergic terminals and probably also on GABAergic terminals (Liu et al., 2002b; Haj-Dahmane and Shen, 2009; Mendiguren and Pineda, 2009; Tao and Ma, 2012), leading to a reduction in glutamate and GABA release onto 5-HT neurons and therefore changes the activity of the 5-HT neurons itself.

FRIZZLED RECEPTORS

Four frizzled receptors (FZD1–3 and SMO) have been detected in postmitotic embryonic 5-HT neurons (Wylie et al., 2010). These receptors mainly couple to the Wnt signaling cascade and are most likely involved in the development and maturation of 5-HT neurons (Simon et al., 2005; Song et al., 2012). However, the role of frizzled receptors in 5-HT neurons remains to be determined.

NEUROPEPTIDES

Dr. Hökfelt's laboratory demonstrated that various peptide transmitters are expressed in the DRN with species-specific differences between mice and rats (Fu et al., 2010). The identified neuropeptides are cholecystokinin (CCK), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), somatostatin, substance P, dynorphin, neurotensin, thyrotropin-releasing hormone (TRH), enkephalin, galanin, neuropeptide Y (NPY), and corticotropin-releasing factor (CRF). Among these, various peptide receptors have been identified and functionally described in 5-HT neurons.

BOMBESIN

To date three types of bombesin receptors have been described, i.e., the neuromedin B (NMB) receptor (BB_1 receptor), the gastrin-releasing peptide (GRP) receptor (BB_2 receptor) and the bombesin receptor subtype 3 (BRS-3 or BB_3 receptor; Jensen et al., 2008). The NMB receptors are functionally expressed in 5-HT neurons in the DRN (Pinnock et al., 1994; Woodruff et al., 1996). BB_1 receptor activation leads to an increase in 5-HT neuronal firing via suppression of K^+ current, involving most likely the activation of the $G_{q/11}$ pathway (Benya et al., 1992; Woodruff et al., 1996) and as a consequence, an increase in 5-HT release to projection site such as the hippocampus, which is monitored by *in vivo* microdialysis (Merali et al., 2006).

CALCITONIN

Calcitonin receptor (CalcR) mRNA has been localized in 5-HT neurons in the DRN (Nakamoto et al., 2000), which is in agreement with the expression profiling studies of postmitotic embryonic 5-HT neurons (Wylie et al., 2010). The CalcR couples to the G_s pathway (Hay et al., 2005) and $G_{q/11}$ pathway (Offermanns et al., 1996). Since high levels of amylin binding sites are detected in the DRN (Sexton et al., 1994) and mRNA for CGRP has been localized in 5-HT positive axon terminals in monkeys (Arvidsson et al., 1990), it is most likely that CalcR assemble with receptor activity-modifying proteins (RAMPs) in 5-HT neurons to respond to the various peptide transmitters (i.e., CGRP, adrenomedullin, and amylin; Tilakaratne et al., 2000). A direct function of CalcR in 5-HT neurons has not yet been demonstrated. However, injection of CGRP into rats induces anxiety-like behaviors and increases c-Fos expression in the DRN (Sink et al., 2011).

CHEMOKINE RECEPTORS

Two subtypes of the 18 identified chemokine receptors have been detected in microarray analyses from embryonic 5-HT neurons, i.e., Duffy antigen/chemokine (C-X-C motif) receptor 4 (CXCR4; Wylie et al., 2010). CXCR4 is expressed in the majority of 5-HT neuron outer membranes in the DRN (Heinisch and Kirby, 2010). So far only an indirect action of CXCR4 for modulation of 5-HT neuron has been demonstrated, since application of the CXCR4 ligands and antagonists modulate GABA and glutamate release onto 5-HT neurons (Heinisch and Kirby, 2010), which is in agreement with the described $G_{i/o}$ protein-mediated inhibition of Ca^{2+} channels via CXCR4 (Oh et al., 2002). In addition, CX_3CR1 is also expressed in 5-HT neurons in the DRN and MRN (Heinisch and Kirby, 2009). Here the CX_3CR1 specific ligand, fractalkine/CX_3CL1 increased evoked IPSC amplitude on 5-HT neurons (Heinisch and Kirby, 2009), an effect which is most likely mediated postsynaptically and not presynaptically. The effect is surprising, since CX_3CR1 has been described to couple to the $G_{i/o}$ pathway (Oh et al., 2002), which would inhibit synaptic transmitter release and induce paired-pulse facilitation (PPF) if activated on GABAergic terminals. Therefore, the CX_3CL1 could increase/modulate $GABA_A$ receptor trafficking and $GABA_A$

receptor currents in 5-HT neurons via activation of CX3CR1. A signaling function for Duffy antigen remains to be determined.

CHOLECYSTOKININ

The expression of CCK receptors in 5-HT neurons in the DRN has also been suggested. Application of CCK increases 5-HT neuronal firing, which is blocked by the CCK_1 antagonist L-364,718 (Boden et al., 1991). In addition, 5-HT release measured as outflow of [^{3}H]5-HT in cortical slices is increased by CCK-4, which involves CCK_2 receptors (Siniscalchi et al., 2001). Both CCK_1 and CCK_2 receptors mainly couple to the $G_{q/11}$ and G_s pathway (de Weerth et al., 1993; Lee et al., 1993; Ulrich et al., 1993).

CORTICOTROPIN-RELEASING FACTOR

Two CRF (CRF_1 and CRF_2) receptor subtypes have been described in brain and both seem to be localized in GABAergic neurons in the DRN as well as in 5-HT and non-5-HT neurons with differential subcellular localizations (for review, see Valentino et al., 2010). CRF_1 and CRF_2 couple to the G_s pathway (Chang et al., 1993; Perrin et al., 1993; Vita et al., 1993; Lovenberg et al., 1995; Liaw et al., 1996) and also the $G_{q/11}$ pathway in heterologous expression systems (Dautzenberg et al., 2004), leading to the assumption that stimulation of CRF_1 or CRF_2 will increase neuronal firing. Indeed, activation of CRF_1 on GABAergic neurons increases GABA release onto 5-HT neurons, while activation of CRF_2 elicits an inward current in 5-HT neurons (Kirby et al., 2008). Based on the differential localization of the CRF receptors within the DRN and its receptor type-specific action on 5-HT neurons, it has been suggested that at low concentrations of CRF, 5-HT neuronal activity is decreased, while at high concentrations, 5-HT neuronal activity is increased (Kirby et al., 2008; Valentino et al., 2010).

GALANIN

The neuropeptide galanin activates three types of galanin receptors ($GalR_{1-3}$). $GalR_1$ and $GalR_2$ are highly expressed in the DRN (Melander et al., 1988; Larm et al., 2003; Lu et al., 2005; Sharkey et al., 2008). Moreover, $GalR_1$ expression has also been described in 5-HT neurons from rats but not in mice (Xu et al., 1998; Larm et al., 2003). GalR activation in the DRN causes a K^+ conductance-mediated hyperpolarization in rat brain slices (Xu et al., 1998), most likely via GalR3-mediated $G_{i/o}$ activation of GIRK channels (Swanson et al., 2005). These effects are in agreement with *in vivo* microdialysis studies showing that injection of galanin into the DRN reduces 5-HT release via GalR activation in the hippocampus (Kehr et al., 2002). In contrast to the inhibitory action of galanin on 5-HT neuronal activity, a reduction in inhibitory input onto 5-HT neurons has also been described (Sharkey et al., 2008). Here, the pan $GalR_{1-3}$ agonist reduced GABA-mediated fast synaptic transmission accompanied by increase of PPF, suggesting that GalRs are expressed on GABAergic terminals and inhibit presynaptic Ca^{2+} channels via the $G_{i/o}$ pathway. On the other hand, $GalR_2$ agonist, galanin (2–11) reduced IPSP amplitude but did not cause PPF, suggesting a postsynaptic action (Branchek et al., 2000; Sharkey et al., 2008). Additionally, galanin (2–11) was demonstrated to increase 5-HT release in hippocampal tissue by immunofluorescence and high-performance liquid chromatography (HPLC) measurement (Mazarati et al., 2005). Therefore $GalR_2$ receptors may activate 5-HT neurons via reduction in GABAergic input onto 5-HT neurons and/or via $G_{q/11}$-mediated increase in 5-HT neuronal firing. Galanin also modulates $5\text{-}HT_{1A}$ autoreceptor responses *in vivo*. A possible mechanism of this modulation could be the heterodimerization of GalR with $5\text{-}HT_{1A}$ which has been observed in heterologous expression systems (for review, see Kuteeva et al., 2008; Borroto-Escuela et al., 2010).

HYPOCRETIN–OREXIN

The two hypocretin/orexin (OX1 and OX2) receptors are expressed in tryptophan hydroxylase-positive neurons in the DRN (Brown et al., 2002). Their intracellular signaling targets are rather complex involving activation of $G_{i/o}$, $G_{q/11}$, G_s, and other G proteins (Scammell and Winrow, 2010). Orexin positive fibers project onto GABAergic as well as 5-HT neurons in the DRN (Peyron et al., 1998). Application of the neuropeptides orexin-A and orexin-B causes a Na^+/K^+ non-selective cation current in 5-HT neurons (Brown et al., 2002; Liu et al., 2002b; Kohlmeier et al., 2008), suggesting that activation of OX1 and OX2 leads to the increase of 5-HT neuronal firing. The neuropeptides also induce GABA release onto 5-HT neurons at higher peptide concentrations (Liu et al., 2002b). In addition, orexin increases the somatic L-type Ca^{2+} current in 5-HT neurons in a protein kinase C-dependent manner (Kohlmeier et al., 2008). It has therefore been suggested that modulation of Ca^{2+} transients by orexin may be involved in the transcriptional regulation of long-term processes (Kohlmeier et al., 2008).

OPIOIDS

Raphe nuclei receive dynorphinergic, enkephalinergic, and β-endophinergic innervation (Adell et al., 2002). These transmitters activate μ, κ and δ opioid receptors, which primarily couple to the $G_{i/o}$ pathway. Injection of morphine into the DRN causes an increase in 5-HT release detected in forebrain microdialysis (Tao and Auerbach, 1994). The increase in 5-HT release is most likely mediated via $G_{i/o}$ protein-mediated inhibition of GABAergic interneurons in the DRN, involving μ opioid receptors located on GABAergic neurons (Jolas and Aghajanian, 1997). The modulation of 5-HT release in the DRN by κ and δ opioid receptors has also been described (Tao and Auerbach, 2002). Activation of δ receptors increased, while activation of κ receptors decreased 5-HT release measured with *in vivo* microdialysis. The κ receptors effects do not involve the modulation of GABAergic or glutamatergic inputs in the DRN (Tao and Auerbach, 2002), suggesting that κ receptors are expressed in 5-HT neurons. Likewise, opioid receptor-like1 (Oprl1) are most likely located and expressed in 5-HT neurons as follows. Oprl1 or nociceptin (NOP) receptors belong to the opioid receptor family but are activated by NOP (orphanin FQ), a neuropeptide derived from prepronociceptin protein. High levels of NOP receptor binding sites have been detected in the DRN (Florin et al., 2000) and Oprl1 receptor expression could be detected in embryonic 5-HT neurons (Wylie et al., 2010). NOP/orphanin FQ inhibits 5-HT release in the DRN via Oprl1 (Tao et al., 2007), suggesting a functional role of Oprl1 in 5-HT neurons early in development and in the adult brain.

SUBSTANCE P

Substance P belongs to the tachykinin family and has a high affinity for the three different neurokinin receptors (NK_{1-3}), in particular to NK_1 (Hokfelt et al., 2001). These GPCRs couple mainly to the $G_{q/11}$ pathway (Stratowa et al., 1995), but G_s pathway activation has also been reported for NK_1 in cell culture systems (Martini et al., 2002). Various histological studies have revealed extensive expression of NK_1 receptors in the DRN (Maeno et al., 1993; Saffroy et al., 1994; Vigna et al., 1994; Charara and Parent, 1998; Sergeyev et al., 1999; Froger et al., 2001). Most studies suggest that NK_1 receptors are not localized on 5-HT neurons (Froger et al., 2001; Santarelli et al., 2001), while others revealed NK_1 receptor expression in a subpopulation of 5-HT neurons (Santarelli et al., 2001; Lacoste et al., 2006, 2009). Interestingly, NK_1 receptors are found in the cytoplasm of the 5-HT neurons and in dendritic membranes of GABAergic neurons. After administration of NK1 antagonist or deafferentation of substance P releasing projections, the density of membrane bound NK_1 receptors is increased in the somatodendritic region of 5-HT neurons, suggesting that membrane trafficking of NK_1 receptors may be regulated by Substance P input. This mechanism may contribute to the modulation of 5-HT neuronal firing under certain physiological conditions (Lacoste et al., 2009). In addition, controversial results exist for the effect of NK_1 on 5-HT release and firing of 5-HT neurons. Inhibition of NK_1 in the DRN using antagonists or knock-out strategies leads to an increase in firing activity of 5-HT neurons *in vivo* (Haddjeri and Blier, 2001; Santarelli et al., 2001). In contrast, activation of NK_1 and NK_3 increases spontaneous excitatory postsynaptic currents (EPSCs) in DRN 5-HT neurons resulting in an increased firing of the 5-HT neurons as observed in brain slice recording (Liu et al., 2002a). These effects could be blocked by NK_1 and NK_3 antagonists (Liu et al., 2002a). Also, activation of NK_1 via intra-raphe injection of substance P in the DRN increases 5-HT release within the DRN, but decreases 5-HT release in frontal cortex as measured with *in vivo* microdialysis (Guiard et al., 2007). These effects and also the described increase in 5-HT firing in NK_1 knock-out mice involve changes in $5\text{-}HT_{1A}$ autoreceptor levels, suggesting at least a functional coupling between NK_1 and $5\text{-}HT_{1A}$ receptors. Further investigations to verify these interactions in 5-HT neurons are required.

In summary, the various heteroreceptors integrate incoming information via two main pathways, i.e., $G_{i/o}$ and $G_{q/11}$ leading to inhibition or activation of 5-HT neuronal firing and 5-HT release, respectively. Besides the "classical" $G_{i/o}$ protein-mediated, membrane-delimited modulation of GIRK and presynaptic Ca^{2+} channels, other ion channel targets have been identified in 5-HT neurons. For example, two-pore-domain K^+ channels [TWIK-related acid-sensitive K-1 (TASK-1) and TASK-3] have been described in dorsal and caudal raphe 5-HT neurons (Washburn et al., 2002). TASK channels are inhibited by GPCRs coupling to the $G_{q/11}$ pathway most likely in a membrane-delimited manner involving the direct binding of Gαq subunits (Chen et al., 2006). The existence of voltage-sensitive but not ATP-dependent K^+ channels in DRN neurons including 5-HT neurons have been proposed based on drug application studies (Harsing, 2006). In addition, TRP channels have been described to be modulated by D_2-like receptors (Aman et al., 2007). According to the microarray expression profiling studies, various ion channel targets of GPCRs are expressed in embryonic 5-HT neurons including TRP (Trpm4 and Trpm7), two-pore channels (TPCN1), cyclic nucleotide gate channels (Hcn3), and KCNQ (Kcnq2; Wylie et al., 2010). Therefore, more detailed studies have to be performed to determine the role of other ion channel targets and in particular long-term effects of GPCR modulation for the serotonergic system.

INTEGRATION AND SIGNAL PROCESSING OF MODULATORY INFORMATION BY SEROTONERGIC NEURONS: WHY SO MANY GPCRs?

The serotonergic transmitter system modulates many physiological functions such as mood, sexual behavior, feeding, sleep/wake cycle, memory, cognition, blood pressure regulation, breathing, and reproductive success (Mooney et al., 1998; Abrams et al., 2004; Lechin et al., 2006; Lerch-Haner et al., 2008; McDevitt and Neumaier, 2011). Because of the complexity and variety of the different behaviors modulated by serotonin, it is expected that modulatory signals from other brain areas including sensory information is integrated by GPCR signals in nuclei containing 5-HT neurons using a high diversity of GPCRs. While GABA and glutamatergic input into the raphe nuclei will adjust 5-HT neurons to the current inhibitory/excitatory state of the brain, other transmitter systems will inform 5-HT neurons more directly about the serotonin-associated behavior. For example, dopamine is involved in reward-driven learning; ACh modulates arousal and reward; NA and CRF are involved in stress responses; histamine is involved in sleep regulation and sexual function; bombesin and CCK regulate eating behavior; eCBs modulate memory, appetite, stress, social behavior, anxiety, and sleep; galanin has been implicated in the regulation of sleep–wake cycle, cognition, emotion, and blood pressure; hypocretin–orexin modulate arousal, wakefulness, and appetite; and opioids and substance P are involved in pain perception and mood (White and Rumbold, 1988; Woodruff et al., 1996; Greenough et al., 1998; Bear et al., 2001; Merali et al., 2006; Monti, 2010; Haj-Dahmane and Shen, 2011). Since all different behavioral responses can be integrated in nuclei containing 5-HT neurons, regulation of serotonin release will affect similar behaviors as stated above. Thus there is a tight interaction and signaling exchange between the different transmitter systems to precisely modulate behavioral output. Consequently, long-term changes in serotonin release can involve changes in the auto-regulation involving 5-HT receptor or hetero-regulation involving the above mentioned GPCRs and can cause neuropsychiatric disorders, most notably depression, anxiety, schizophrenia, and dementia (Lucki, 1998; Davidson et al., 2000; Mann et al., 2001; Nelson and Chiavegatto, 2001). The modulation of the different behaviors is even more complex since other GPCRs, such as orphan GPCRs, with so far unknown function are also expressed in 5-HT neurons. Therefore, new strategies and techniques have to be applied and developed to understand complex behaviors related to the serotonergic system.

NEW APPROACHES TO CONTROL AND UNDERSTAND SEROTONERGIC G PROTEIN-COUPLED RECEPTOR SIGNALING PATHWAYS

Recently, several new approaches to manipulate the activity of 5-HT neurons in a cell type-specific manner have been developed. Various promoter/enhancer sequences have been isolated and characterized, which allow for the expression of proteins of choice within at least subsets of 5-HT neurons. These DNA sequences include the promoter or enhancer sequences of Pet-1/Fev transcription factor, the serotonin transporter (SLC6A4), and the tryptophan hydroxylase 2 (TPH2; Scott et al., 2005). Using these different promoter/enhancer sequences, different mouse lines and virus approaches have been developed to activate and/or silence/delete reporter genes such as green fluorescent protein (GFP) or tdTomato, Cre or Flip recombinases, tetracycline inducible systems, tetanus toxin light chain, and genes of interest (Scott et al., 2005; Kim et al., 2009; Madisen et al., 2010, 2012; Richardson-Jones et al., 2010; Zhao et al., 2011). Using the tetracycline inducible system, for example, the genetic ablation of 5-HT_{1A} from the majority of 5-HT neurons could be achieved (Richardson-Jones et al., 2010, 2011).

For the investigation of the modulation and function of neuronal circuits in general and for the serotonergic system in particular, chemical and optogenetic techniques have been developed in recent years (Herlitze and Landmesser, 2007; Masseck et al., 2010). For control of neuronal activity in various neuronal circuits including the 5-HT system, the light-gated non-selective cation channel, ChR2 has been used and allows for the dissection of 5-HT-mediated behavioral effects in different raphe nuclei (Li et al., 2005; Varga et al., 2009; Zhao et al., 2011; Madisen et al., 2012; see also Kim et al., 2009). For the investigation of GPCR signals, various chemically and light-activated GPCRs have been developed (Masseck et al., 2010). For example vertebrate rhodopsin (vRh) has been used to regulate $G_{i/o}$ signaling pathways in neurons by light (Li et al., 2005). Exogenously expressed vRh inhibits neuronal firing and neurotransmitter release *in vitro* and *in vivo* most likely via activation of GIRK and inhibition of presynaptic Ca^{2+} channels (Li et al., 2005; Oh et al., 2010; Gutierrez et al., 2011). Since vRh belongs to class A or rhodopsin like group of GPCRs, like serotonergic autoreceptors 5-$HT_{1A/1B/1D}$, the idea arose to generate light-activated chimeric receptors which couple to intracellular signaling pathways in 5-HT_1 receptor domains. The feasibility of receptor domain swapping has been demonstrated by Dr. Khorana's group (Kim et al., 2005). They replaced the intracellular loops of vRh with that of the β_2-adrenergic receptor and turned vRh into a light-activated G_s protein-coupled receptor, Opto-β_2AR. The approach however was not suitable for the exchange of vRh intracellular peptide loops by the 5-HT_{1A} intracellular receptor domains, since this chimeric receptor revealed altered activation and deactivation kinetics in respect to GIRK channel modulation (Oh et al., 2010). However, it could be shown that the C-terminus (CT) of the 5-HT_{1A} receptor was sufficient to target vRh into expression domains of 5-HT_{1A} receptor and functionally substitute for $G_{i/o}$ pathway activation. The CTs of GPCRs contain peptide signal domains for subcellular targeting and G protein interaction. The CT of 5-HT_{1A} had been shown to be necessary for trafficking of the receptor to dendritic domains via interaction with trafficking protein Yif1B (Carrel et al., 2008). Fusion of the 5-HT_{1A} receptor CT onto vRh was therefore sufficient to target the chimeric construct Rh-$CT_{5\text{-}HT1A}$ into somatodendritic regions of hippocampal neurons and 5-HT neurons in the DRN and exclude the expression of the chimeric receptor from the axons. The Rh-$CT_{5\text{-}HT1A}$ receptors were able to functionally substitute for intracellular 5-HT_{1A} signals in DRN neurons of 5-HT_{1A} knock-out mice, i.e., light illumination induced K^+ conductance (most likely GIRK) reduced the firing rate of spontaneously active 5-HT neurons. Thus, the adjustment of for example light-activated or chemically activated GPCRs and their coupling and anchoring to and in intracellular signaling domains will allow for the dissection of multiple GPCR pathways within the serotonergic system and their interaction *in vitro* and *in vivo*.

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