Mechanisms of serotonergic neuromodulation in the olfactory cortex

PhD Thesis

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Szeged, 2023

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1. LIST OF PUBLICATIONS

1.1. Publications related to the subject of the thesis

- **Ildikó Piszár** and Magor L. Lőrincz (2022) Differential serotonergic modulation of principal neurons and interneurons in the anterior piriform cortex. *Front Neuroanat* 16:821695. DOI: 10.3389/fnana.2022.821695
- **Ildikó Piszár** and Magor L. Lőrincz (2023) Differential serotonergic modulation of synaptic inputs to the olfactory cortex. *International Journal of Molecular Sciences* 24(3):1950. DOI: 10.3390/ijms24031950

1.2. Other publications

- Nóra Faragó, Ágnes Katalin Kocsis, Csilla Braskó, Sándor Lovas, Márton Rózsa, Judith Baka, Balázs Kovács, Katalin Mikite, Viktor Szemenyei, Gábor Molnár, Attila Ozsvár, Gáspár Oláh, **Ildikó Piszár**, Ágnes Zvara, Attila Patócs, Pál Barzó, László G. Puskás and Gábor Tamás (2016) Human neuronal changes in brain edema and increased intracranial pressure. *Acta Neuropathologica Communications* 4(1):78. DOI: 10.1186/s40478-016-0356-x

2. List of Abbreviations

5-HT: 5-hydroxytryptamine, serotonin

ACh: acetylcholine

ADHD: attention deficit hyperactivity disorder

AP: action potential

EPSP: excitatory post-synaptic potential

IPSP: inhibitory post-synaptic potential

FR: firing rate

GABA: gamma-aminobutyric acid

LC: locus coeruleus

NMDA: N-methyl-D-aspartate (receptor)

OB: olfactory bulb

OCD: obsessive compulsive disorder

PB: phosphate buffer

PFA: paraformaldehyde

PV: parvalbumin

Vm: membrane potential

Additional abbreviations in figures are explicated in their corresponding captions.

3. INTRODUCTION

3.1. Neuronal heterogeneity and neuromodulation

Diverse individual cells of living organisms can perform a variety of complex operations. In the nervous system, cells characterized by defined spatial position, morphological, neurochemical, genetic and physiological heterogeneity engage in a plethora of close- and long-range dynamic communications enabling them to fulfill diverse functions. The cerebral cortex contains an abundant variety of neurons, particularly amongst inhibitory interneurons. These neurons show differences in their morphology (Szentágothai, 1978), electrophysiology (McCormick et al., 1985) and neurochemistry (Tremblay et al., 2016), but the recently described plethora of cell types defined by transcriptomics (Zeisel et al., 2015) does not always correlate with classical morphological or intrinsic electrophysiological properties of neurons (Gouwens et al., 2020). A recent study has elegantly shown that the physiological parameter that best correlates with these numerous transcriptomic classes is brain state (Bugeon et al., 2022). This suggests that the extensive array of organized spontaneous activity patterns carry meaningful information beyond noise. As the electrical activity of neurons synthetizing and releasing various neuromodulator substances is brain state dependent (Reimer et al., 2016) the concentration of these neuromodulators varies depending on the state of vigilance (de Saint Hilaire et al., 2000; Marrosu et al., 1995; Portas et al., 2000) and is thought to be the major regulator of state dependent differential neuronal communication.

3.2. Neuromodulation of brain state dependent neuronal activity

Brain states refer to the patterns of neural activity and physiological parameters associated with different mental states such as wakefulness, sleep, attention, and emotional states. These patterns are largely controlled by the activity of different brain regions, such as the neocortex, thalamus, and brainstem, and involve changes in the levels of various neurotransmitters, hormones, and neuromodulators. Brain states can fluctuate rapidly, for example, in response to sensory stimuli or shifts in attention, or more slowly, such as during sleep-wake cycles or changes in mood. Understanding how brain states are generated and regulated is a central

question in neuroscience and has important implications for the study of cognitive and emotional processes, as well as for the development of treatments for brain disorders.

Neuromodulation refers to the regulation of the activity of neurons by chemical substances, such as neurotransmitters and neuromodulators, that are produced and released by neurons or other cells in the nervous system. Neuromodulation plays a crucial role in regulating brain state-dependent neuronal activity by changing the excitability, synaptic strength, and plasticity of neurons, and thus, the overall network activity (Marder et al., 2014). This can result in changes in behavior, sensation, perception, and cognition, depending on the specific brain regions and neurotransmitter systems involved.

The main neuromodulators are serotonin, acetylcholine, dopamine, and noradrenaline. Serotonin is a neurotransmitter involved in regulating mood, appetite, and sleep, among other functions, its release is modulated by various factors, including stress and antidepressant medications (Dayan & Huys, 2009). Acetylcholine is another major neurotransmitter that is involved in attention, learning, and memory (Thiele & Bellgrove, 2018). Dopamine is a neurotransmitter involved in motivation, reward, and attention (Schultz, 2007). Noradrenaline is a neurotransmitter involved in regulating the "fight or flight" response, mood and attention (Aston-Jones & Cohen, 2005).

Neuromodulators play a crucial role in shaping neuronal excitability and activity, and they can do so through multiple mechanisms targeting various aspects of neuronal function. Neuromodulators can regulate the opening and closing of ion channels, which in turn affects the membrane potential of neurons. This can lead to changes in the excitability of the neuron, including changes in its resting potential, threshold for firing, and the speed and duration of its action potentials. Neuromodulators can also bind to neurotransmitter receptors and modulate their activity, which can lead to changes in the responsiveness of neurons to neurotransmitters. Some neuromodulators can modulate synaptic transmission by regulating the release and reuptake of neurotransmitters and the number of available neurotransmitter receptors. Finally, some neuromodulators can also modulate gene expression, which can result in changes in the functional properties of neurons over longer time scales.

The release of neuromodulators is influenced by the state of the animal; thus, the brain concentration of neuromodulators depends on the current state of the brain. The release of these neurotransmitters is regulated by various factors such as arousal, sensory input, attention, learning, memory, and emotions, which can all influence the overall state of the brain. In the

pathogenesis of some brain disorders neuromodulation plays a role as is the case of depression, anxiety, OCD and ADHD.

One notable aspect of neuromodulation is that it operates on various timescales, ranging from rapid and transient changes to longer-lasting alterations. At the rapid end of the spectrum, neuromodulation can occur within milliseconds or seconds, as when a neurotransmitter released by one neuron rapidly affects the activity of another. Of particular interest, the neuromodulatory tone during wakefulness is thought to cause a decreased excitatory/inhibitory ratio in sensory responses (Haider et al., 2013). On a longer timescale, neuromodulation can influence the expression of genes and the structural organization of neural circuits, which can persist for hours, days, or even longer (Bibb et al., 2001). Indeed, most known neuromodulator substances can effect active behaviors on a rapid (seconds) timescale, but also affect brain states on a slower (minutes) timescale (Figure 1).

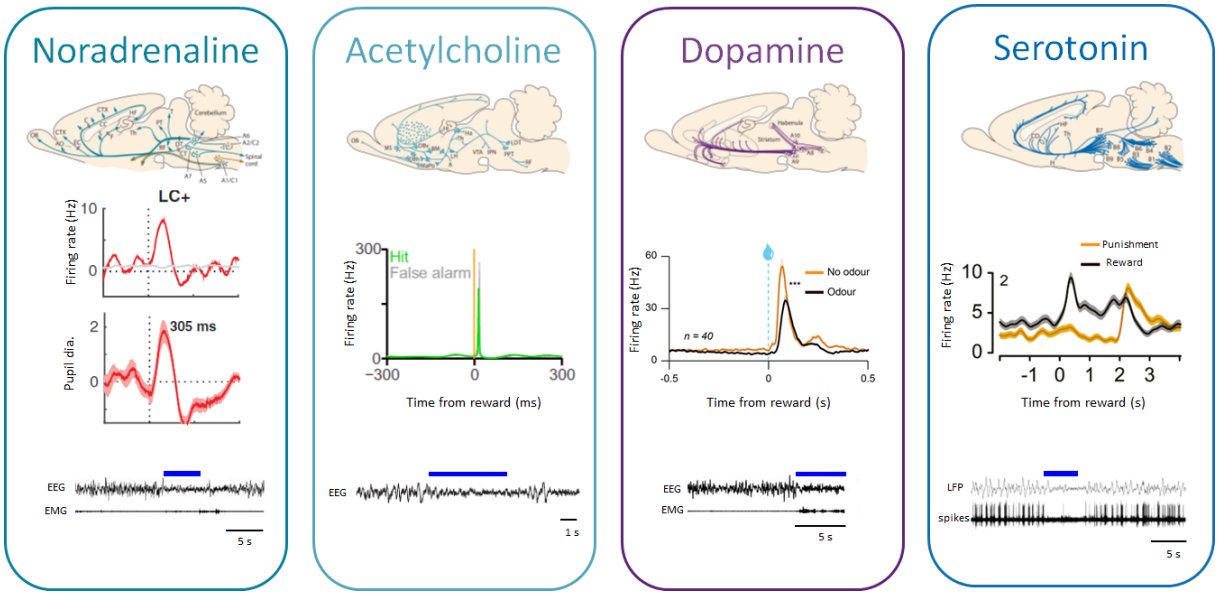

Figure 1. *Neuromodulation acts on various timescales.* Anatomical and physiological properties of the four major neuromodulatory systems (noradrenaline, acethylcholine, dopamine and serotonin). The top row shows the projections of the four neuromodulatory nuclei in rodents. The middle row shows examples of neuronal correlates of identified neuromodulatory neurons showing the correlation of the activity of a noradrenergic neuron to pupil diameter (Joshi et al., 2016), differential activity of a basal forebrain cholinergic neuron to tones in a behavioral task for hits and false alarms (Hangya et al., 2015), reward related activity of a VTA dopaminergic neuron (Eshel et al., 2015) and reward and punishment related activity of a raphe serotonergic neuron (Cohen et al., 2015). The bottom row shows the effects of selective stimulation of noradrenergic (Carter et al., 2010), basal forebrain cholinergic (Pinto et al., 2013), VTA dopaminergic (Eban-Rothschild et al., 2016) and dorsal raphe serotonergic (Crunelli et al., 2017) photostimulation and the resulting suppression of EEG sleep slow waves and transitions to wakefulness.

3.3. The olfactory system

Olfaction is a chemical sense with several unique morphological features paramount for its function. It consists of multiple types of olfactory receptor neurons (ORNs) located in the sensory epithelium expressing a single unique odorant receptor (OR) protein (300 in humans, 1000 in rodents) (Buck & Axel, 1991). ORNs distributed throughout the sensory epithelium converge to form a segregated, ordered spatial map in the olfactory bulb (OB) where each glomerulus receives projections from ORNs expressing the same receptor protein. The OB is thus a highly specialized extrathalamic relay station for olfactory information in which axons of ORNs make excitatory synapses with the dendrites of the two types of projection neurons in the OB, mitral cells (M) and tufted cells (T). Each M and T neuron sends a primary dendrite to a single glomerulus. In the mouse OB, an individual OR is represented by a pair of glomeruli (Mombaerts et al., 1996; Mori & Sakano, 2011). The OB has a number of unique morpho-functional features including its laminar structure, the presence of specialized input structures, the glomeruli, the presence of reciprocal dendrodendritic synapses between the dendrites of M/T cells and granule cells, respectively (Shepherd, 2004). The axons of M/T neurons form the lateral olfactory tract (LOT) that terminates in a variety of brain regions including the primary olfactory or "piriform" cortex (PirC), amygdala, hypothalamus, orbitofrontal cortex, (Shepherd, 2004). The highly ordered spatial map of odorant receptors in the OB is discarded in the piriform cortex; M/T axons from individual glomeruli project randomly to the PirC without apparent spatial preference (Sosulski et al., 2011). The PirC is a three layered paleocortical region characterized by a surprisingly high level of inputs originating from of intracortical sources. Importantly the afferent and intracortical inputs are segregated both spatially, physiologically, pharmacologically and functionally. Specifically, the LOT inputs contact the distal dendrites of pyramidal and semilunar neurons, the main principal neurons of the PirC and also local interneurons in layer 1a, whereas synapses originating from intracortical sources (including other regions of the PirC, orbitofrontal cortex, amygdala and entorhinal cortex) (associative input) are formed more proximally in layer 1b, layer 2 and layer 3 (Price, 1973a). In addition to this spatial separation, intracortical, but not afferent synapses are sensitive to cholinergic (Hasselmo & Bower, 1992) and noradrenergic (Hasselmo et al., 1997) neuromodulation and are subject to a presynaptic control by $GABA_B$ receptors (A. C. Tang & Hasselmo, 1994). Both afferent and associational fiber pathways are susceptible for long- and short-term plasticity although with some differences (Hasselmo & Bower, 1990; Kanter & Haberly, 1993).

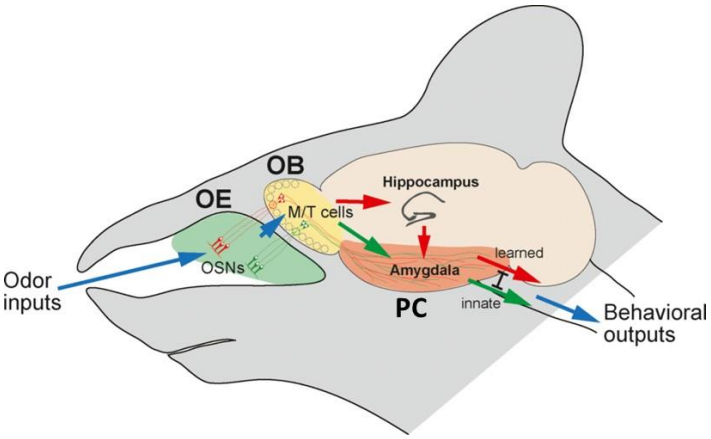

Figure 2. *The mouse olfactory system.* Odorant molecules are detected by odorant receptors in the olfactory epithelium. Individual sensory neurons in the nasal cavity express a single type of OR ("one cell -one receptor" phenomenon) project to the same glomerulus at the surface of the olfactory bulb (OB) (called "glomerular convergence rule"), the first relay place in the central olfactory system. Odor information is then transferred by M/T cells to various regions in the olfactory cortex and higher association areas (Sakano, 2020).

Due to its phylogenetically old, relatively simple 3 layered structure and its spatially and pharmacologically distinct feed-forward and feed-back inputs, the primary olfactory cortex represents an ideal model system to study the serotonergic modulation of cortical circuits.

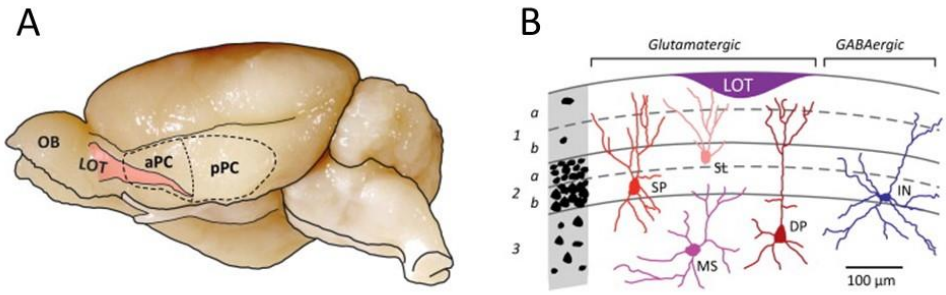

Figure 3. *The piriform cortex (PC).* (A) Location of the olfactory bulb (OB), lateral olfactory tract (LOT, pink) and the anterior (aPC) and posterior PC (pPC) in the rat brain. (B) Schematic coronal view of the main neuronal types (colored shapes) and their relative distribution (black shapes on the left) in the different layers of the aPC. Semilunar (SL) and superficial pyramidal (SP) cells` somata are located in layer 2a and 2b, respectively and create a dense layer. Deep pyramidal (DP) and multipolar spiny (MS) cells are sparsely distributed in layer 3. GABAergic interneurons (INs) are found across all layers (Bekkers & Suzuki, 2013).

3.4. The serotonergic system

Located at the brainstem raphé nuclei (RN), serotonergic neurons project to various forebrain areas and release serotonin (5-hydroxytryptamine, 5-HT) throughout the entire neuraxis. 5-HT is implicated in a variety of physiological functions, including the regulation of sensory and motor responses (Davis et al., 1980; Dugué et al., 2014; Liu et al., 2014; Lottem, Lőrincz, et al., 2016), brain states (Gazea et al., 2021; Jacobs & Fornal, 1991; Oikonomou et al., 2019), learning and reward processing (Cohen et al., 2015; Liu et al., 2014; Matias et al., 2017) and social interactions (Wu et al., 2021). Dysfunctions of the serotonergic system are implicated in several neurological and psychiatric disorders, including depression (Pehrson et al., 2022) and epilepsy (M. Lörincz et al., 2007).

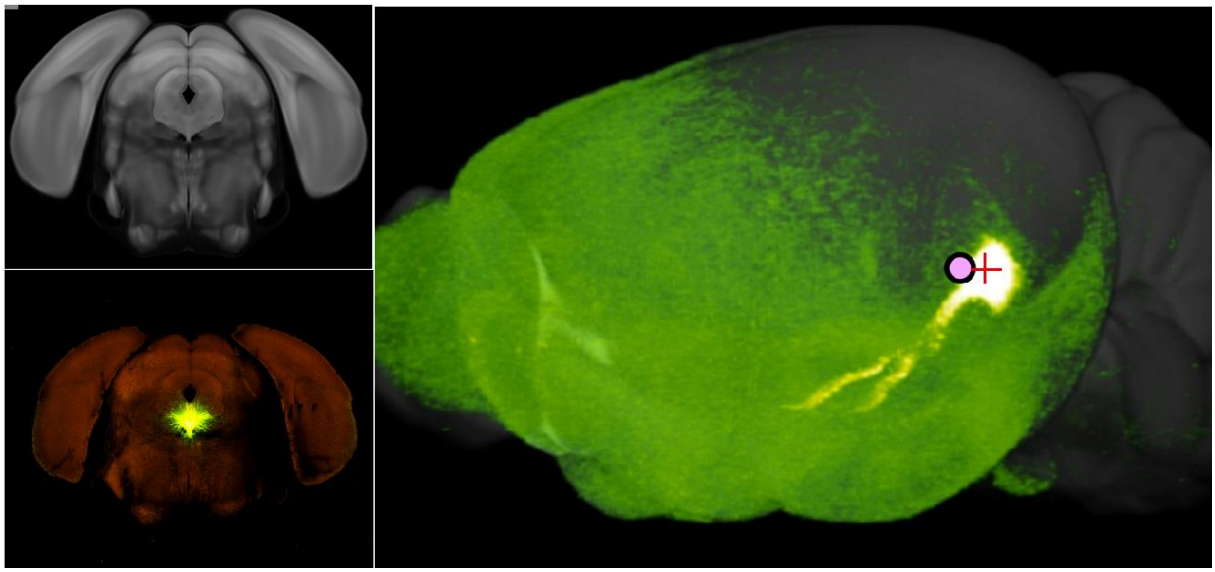

Figure 4. *Coronal and sagittal view of the dorsal raphe nucleus.* Green channel: serotonin immunoreactive cells/fibers (source: The Allen Mouse Brain Atlas).

Several structural and functional aspects of the serotonergic system make it a likely contributor to the neuromodulation of sensory functions. At the same time, some of these features make deciphering its exact functions and their mechanisms difficult. These include its widespread and diffuse projections, the extreme neurochemical and anatomical heterogeneity of its source neurons within the raphé nuclei, the existence of one ionotropic and multiple types of metabotropic receptors, expressed both pre- and post-synaptically, and in a cell-type specific manner in neurons and astrocytes, some of them mediating antagonistic effects, resulting in a

pronounced heterogeneity of 5-HT effects that influence complex cellular and network phenomena.

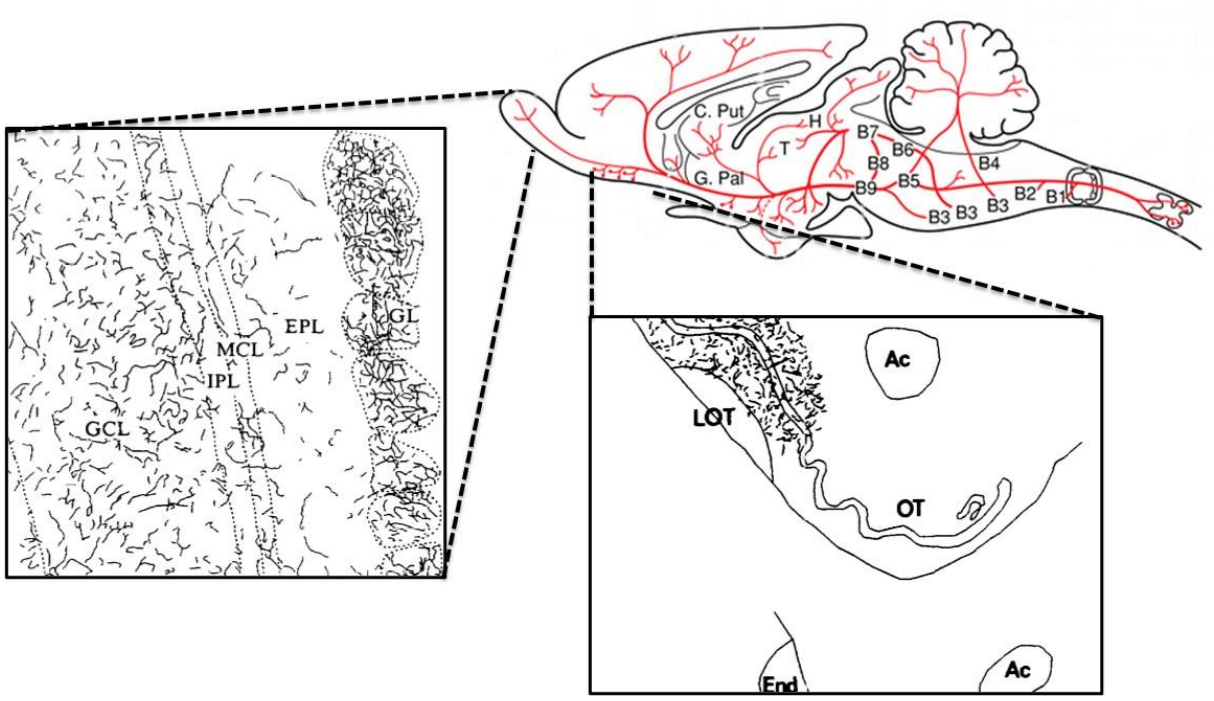

Figure 5. *Sagittal view of serotonin-immunopositive cell groups* (B1-B9: according to the terminology of Dahlstrom and Fuxe) *in the rodent brain.* Higher magnification images show the serotonergic axonal innervation of the olfactory bulb (left) and anterior piriform cortex (right) respectively. Modified from (Dahlström & Fuxe, 1964).

Beyond sensory representations, the olfactory system is intimately linked to affective functions that are important for social interactions (Brennan & Kendrick, 2006), including the regulation of mood (Canbeyli, 2022) and maternal behavior (Corona & Lévy, 2015), among others. Interestingly, several neuropsychiatric disorders are accompanied by impaired olfactory functions and reduced volume of the olfactory bulb (Fomin-Thunemann & Garaschuk, 2022). The link between depression and olfactory function seems particularly strong. Specifically, depressed patients are characterized by impaired olfactory function, including altered sensitivity to odors (Lombion-Pouthier et al., 2006), odor identification and discrimination (Zucco & Bollini, 2011). Boosting olfactory function with training ameliorates depression (Sabiniewicz et al., 2022), whereas olfactory bulbectomy results in depressive-like symptoms,

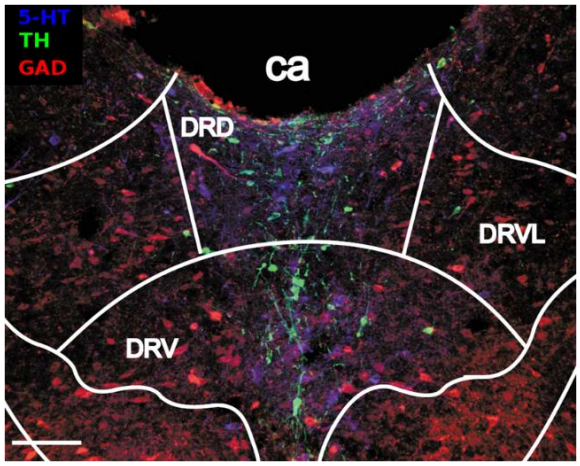

Figure 6. *Neurochemical heterogeneity of the dorsal raphe nucleus (DRN).* Serotonergic (blue), dopaminergic (green) and GABAergic (red)neurons have different distributions within the DRN (*Allers and Sharp, 2003).*

making it a validated rodent model for depression (Song & Leonard, 2005).Because of its prominent projections targeting various olfactory areas including the olfactory bulb (OB) and the primary olfactory or piriform cortex (PirC), 5-HT is ideally suited to influence olfaction, which may lead to changes in both sensory perception and regulation of higher brain functions. However, the mechanisms by which 5-HT shapes olfactory information processing remains to be elucidated.

3.5. Cellular effects of serotonin

Within the OB, serotonergic fibers mostly innervate the glomeruli, the external plexiform layer, internal plexiform layer, and granule cell layer (McLean & Shipley, 1987). DRN neurons preferentially target the granule cell layer and avoid the glomerular layer, while MRN neurons mostly target neurons in the glomerular layer (Steinfeld et al., 2015).

The cellular effects of 5-HT have been identified under *in vitro* conditions primarily using pharmacological tools. Bath application of 5-HT evokes a $5HT_{2C}$ receptor-mediated depolarizing current in short axon (SA) cells and excites external tufted cells (TC) by activating $5HT_{2A}$ receptors. This excitation was suggested to lead to subsequent inhibition of mitral/tufted cells (M/T cells; the primary projection neurons in the OB) by increasing the excitatory drive onto inhibitory interneurons. Additional inhibitory effects on M/T cells may arise from the depolarization of juxtaglomerular neurons via $5\text{-}HT_{2c}$ receptors (Hardy et al., 2005), and from the synchronization of granule cell-mediated IPSCs (Schmidt & Strowbridge, 2014).

RN 5-HT fibers densely innervate the PirC as well, with both DRN and MRN neurons contributing to this projection (Datiche et al., 1995). The most prominent effect of exogenously applied 5-HT in PirC is a $5HT_{1A}$ receptor-mediated hyperpolarization of principal neurons (Araneda & Andrade, 1991), and a $5HT_2$ and $5HT_3$ receptor-mediated depolarization of local

interneurons (Férézou et al., 2002; Gellman & Aghajanian, 1994; S. Lee et al., 2010; Marek & Aghajanian, 1994, 1996), which coincides with an increase of IPSPs in principal neurons (Sheldon & Aghajanian, 1990).

However, care must be taken when interpreting the results of exogenously applied 5-HT or 5-HT receptor agonists, as a mismatch between a certain receptor function and neuronal transmission can occur. Specifically, the expression of a particular receptor does not necessarily imply that it is a functional part of the endogenous neurotransmission of a given system, if a transmitter-receptor location mismatch exists (Rao et al., 2000). This has been documented in the case of widely-expressed substance P receptors in the hippocampus (Acsády et al., 1997), even in the absence of substance P fibers around them (Seress & Leranth, 1996).

Accordingly, the effects of endogenously-released 5-HT probed with specific optogenetic activation of 5-HT neuronal somata or axon terminals are, generally speaking, more subtle compared to exogenous application of 5-HT or agonists of its receptors (Sengupta et al., 2017; Z.-Q. Tang & Trussell, 2015; Varga et al., 2009). Photostimulation of ChR2 expressing axons leads to endogenous transmitter release even in the absence of their somata (Petreanu et al., 2009). Thus, exogenous activation of 5-HT receptors and endogenous 5-HT release may have very different effects. Even in a state-of-the-art experiment using optogenetics, determining the cellular and network effects of 5-HT is a challenge. The unfolding effects will depend on several factors, including the identity of the 5-HT receptors expressed (with possible nonlinear summations of multiple, antagonistic effects), their pre- or post-synaptic location, the number of 5-HT fibers around the neuron(s) recorded, and the possibility of co-release/co-transmission (Sengupta et al., 2017; Varga et al., 2009). A recent study has revealed that specific stimulation of 5-HT axons can reduce the excitability of principal neurons in the PirC (Wang et al., 2020a).

Numerous studies have investigated the role of 5-HT signaling in the modulation of sensory responses of different modalities. An examination of auditory processing in bats has shown that iontophoretically applied 5-HT increased response latencies in the auditory system, independent of overall changes in response strength (Hurley & Pollak, 2005). Modulatory effects of 5-HT were also observed in the visual system. It was found that similarly applied 5-HT decreased visual response and increased latencies in the primate primary visual cortex (Seillier et al., 2017). A similar effect was observed in the visual system of rodents, where optogenetic activation of DRN neurons resulted in the suppression of both stimulus-evoked and spontaneous V1 activity (Azimi et al., 2020). Interestingly, the inhibitory effect on the evoked and spontaneous activities was mediated by different types of serotonin receptors (5-$HT_{1A/2A}$ respectively) (Azimi et al., 2020). Such effects were also observed in the somatosensory system, where electrical DRN stimulation resulted in an increase in response latency and a decrease in response magnitude of barrel cortex neurons following whisker deflections (Sheibani & Farazifard, 2006), and optogenetic DRN stimulation resulted in decreased behavioral responsivity to hind paw stimulation (Dugué et al., 2014). In a two alternative forced choice task, some DRN neurons show rapid, odor specific modulations of activity (Figure 7).

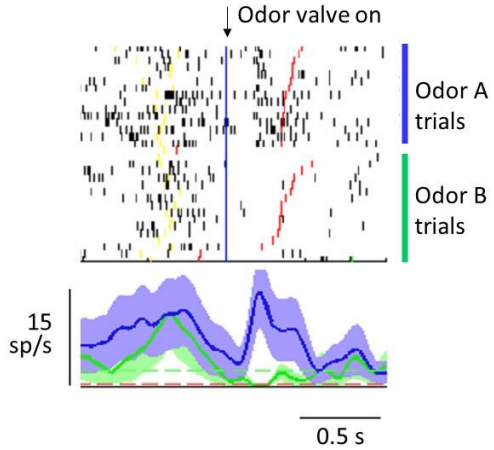

Figure 7. *Neuronal activity of a DRN neuron of a rat performing an odor cued two alternative forced choice task.* The neuron shows rapid, odor specific modulations of its activity (*Modified from Ranade and Mainen, 2009).*

Several studies examined the role of the 5-HT system in odor processing and demonstrated its complex modulatory effects on sensory input. Similar to the 5-HT suppressive effects observed in other sensory modalities, activation of DRN neurons resulted in an attenuation of olfactory receptor neurons' synaptic activity and reduced glomerular sensory input. This effect was mediated by the activation of 5-HT_{2C} receptors on GABAergic periglomerular cells, causing them to increase their GABA release both during rest and in response to odors, and was dependent on the strength of the glomerular odor responses (Petzold et al., 2009).

Another finding regarding the role of the serotonergic system in olfactory processing is the distinct effect of serotonergic modulation on different cell types in the OB. It was shown that

brief activation of OB inputs from the DRN enhances the responsiveness of periglomerular and SA cells to both ambient air and odorant inhalation. This enhancement was strongly related to increased glutamatergic input to these neurons, suggesting a dual release of 5-HT and glutamate from raphé terminals. The effect observed on OB principal neurons was heterogeneous. These results indicate that the serotonergic system dynamically regulates olfactory processing through the innervation of multiple OB targets (Brunert et al., 2016).

OB principal neurons, namely MCs and TCs were differentially modulated by the activation of DRN neurons (Kapoor et al., 2016). While TC odor responses were generally potentiated and became more correlated, MC odor responses were bidirectionally modulated and further decorrelated. Such differential modulation of TCs and MCs may be behaviorally relevant for the detection and identification of the input, and to the generation of accurate distinct outputs. Furthermore, additional optogenetic experiments have revealed the modulation of TCs and MCs is mediated through a dual release of glutamate and 5-HT from raphé terminals (Kapoor et al., 2016). The interplay between glutamate and 5-HT release contributes to the complexity of the net effect of the serotonergic system on olfactory processing.

Testing the effect of 5-HT on the spontaneous and odor-evoked activity of the PirC has led to contrasting results. Selective stimulation of DRN 5-HT neurons resulted in the divisive suppression of spontaneous, but not odor-evoked, spiking activity of anesthetized mice (Lottem, Lörincz, et al., 2016).

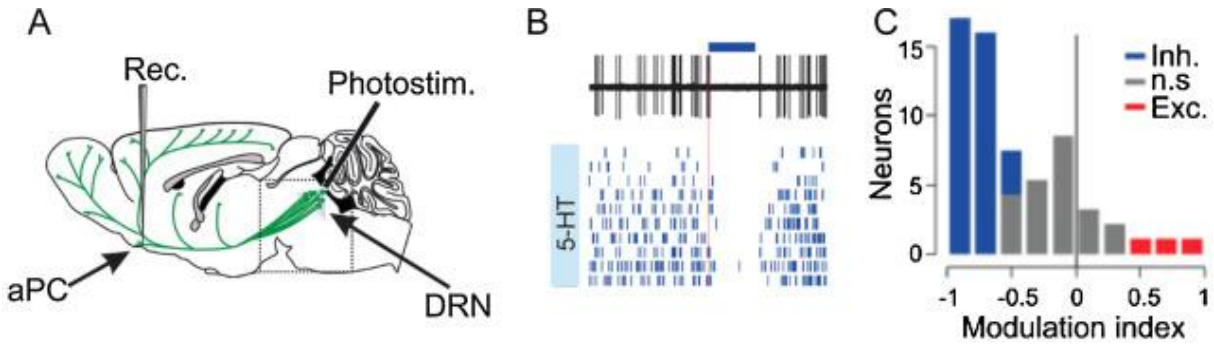

Figure 8. *DRN 5-HT neurons can rapidly affect the activity of aPC neurons.* (A) Recording configuration: a recording electrode (Rec.) was inserted in the aPC to monitor single-unit activity and its modulation by photostimulation (Photostim.) of ChR2 expressing DRN 5-HT neurons. (B) Example baseline activity of an aPC unit and its response to DRN photostimulation. Top, Single-trial raw data (blue bar marks photostimulation, red line its onset), bottom, raster plot in which each tic is a single-unit spike and each row a single trial. (C) Modulation indices for all recorded neurons. Blue bars: significantly inhibited units (Inh.), red: significantly excited (Exc.), and gray: not significantly modulated. *Adopted from Lőrincz and Adamantidis, 2017.*

However, odor-evoked activity was decreased and spontaneous activity remained unaltered during similar manipulation of 5-HT neurons, when monitoring the population Ca^{2+} dynamics of PirC principal neurons using fiber photometry in awake mice (Wang et al., 2020a). Thus, the inhibition of sensory responses seems to be an important feature of 5-HT across multiple sensory modalities, whereas its effects on spontaneous activity may vary as a function of brain region and state of vigilance.

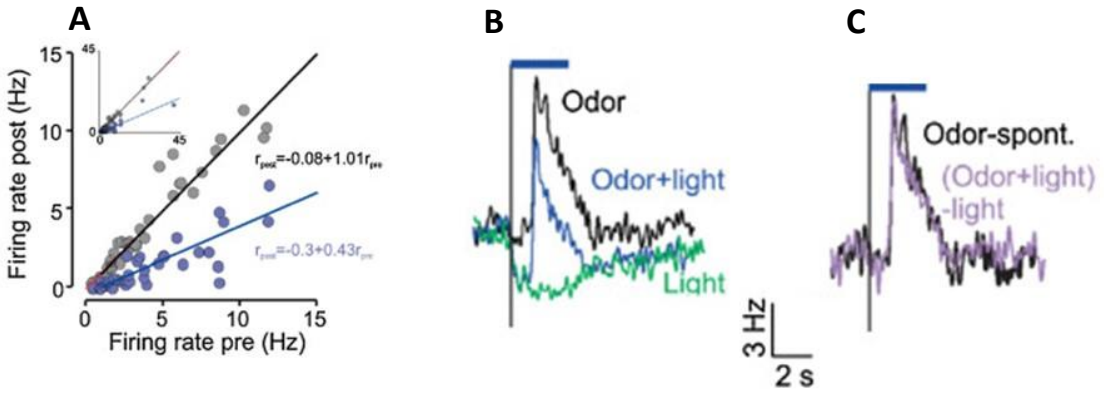

Figure 9. (A) Scatter plot comparing firing rates under control and photostimulated trials for all significantly suppressed units indicates divisive inhibition, linear regression fit superimposed, equations indicated. (B) Averaged PSTHs of all neurons with significant odor responses and light modulations in the absence (black line) and presence (blue line) of DRN photostimulation, green line: average PSTH after DRN photostimulation in the absence of odorant presentation. (C) Mean PSTHs of all neurons showing significant odor responses and light modulations in the absence (black line) and presence (purple line) of DRN photostimulation after subtraction of the corresponding baselines (spontaneous PSTHs). *Modified from Lottem et al., 2016*

4. AIMS

To reveal the cellular and network mechanisms of serotonergic neuromodulation and its effects on the function of the primary olfactory cortex we used a combination of *in vitro* and *in vivo* electrophysiology, optogenetics, pharmacology and immunohistochemistry. Our specific aims were the following:

I. To explore the cellular effects of serotonin on the principal neurons of the primary olfactory cortex

II. To explore the cellular effects of serotonin on various interneurons of the primary olfactory cortex

III. To reveal the effect of serotonin on the afferent and intracortical synaptic inputs to the primary olfactory cortex

IV. To reveal the receptors involved in cortical serotonergic neuromodulation

V. To test whether the cortical effects of serotonergic stimulation are due to local 5-HT release or are the reflection of modulation at another station of the olfactory system

5. MATERIALS AND METHODS

All experimental procedures were performed in accordance with the European Union Directive (86/609/EEC) and approved by the local ethical committees. An effort was made to minimize the number and suffering of animals used.

5.1. Viral injections

For the selective stimulation of DRN serotonergic neurons adult male heterozygous SERT-cre mice (Zhuang et al., 2005) were injected with 0.5-1 µl of AAV2/1-Flex-ChR2-YFP (AV1-20298P, University of Pennsylvania, 10^{13} GC/mL) in the DRN [coordinates: anteroposterior (AP), -4.7 mm; dorsoventral (DV), 3.1-3.6 mm] leading to prominent and specific ChR2 expression in DRN 5-HT neurons (Dugué et al., 2014; Lottem, Lörincz, et al., 2016) and axons in the aPC. 8-16 weeks following the viral infections mice were used for electrophysiological experiments.

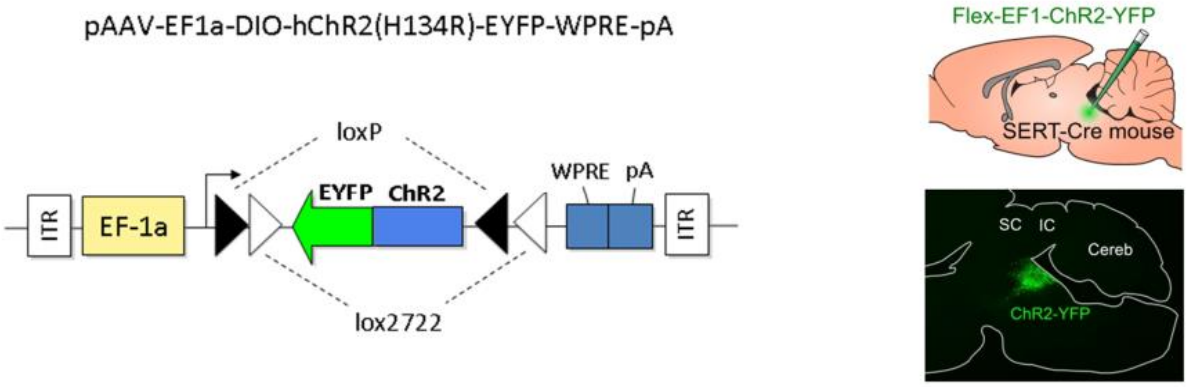

Figure 10. *Viral targeting of DRN 5-HT neurons*. (Left) The construct used to express ChR2 in DRN 5-HT neurons. (Right, top) Schematics of the experimental design. A pulled glass capillary is filled with the viral construct and positioned in the DRN of a SERT-cre mouse. (Right, bottom) ChR2 expression. *Modified from Lottem et al., 2016.*

5.2. Slice preparation

Mice were deeply anesthetized with ketamine and xylazine (80 and 10 mg/kg, respectively), and perfused through the heart with a solution containing (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 N-acetyl-L-cysteine, 5 Na-ascorbate, 3 Na-pyruvate, 10 $MgSO_4$, and 0.5 $CaCl_2$. The same solution was used to cut 320 µm coronal slices containing the aPC at 4°C and for the initial storage of slices (32°C-34°C for 12 min) following which the slices were stored in a solution containing the following (in mM): 30 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.3 $NaHCO_3$, 20 HEPES, 25 glucose, 5 N-acetyl-L-cysteine, 5 Na-ascorbate, 3 Na-pyruvate, 3 $CaCl_2$, and 1.5 $MgSO_4$.

5.3. In vitro electrophysiology

For recording, slices were submerged in a chamber perfused with a warmed (34°C) continuously oxygenated (95% O2, 5% CO2) ACSF containing the following (in mM): 130 NaCl, 3.5 KCl, 1 KH_2PO_4, 24 $NaHCO_3$, 1.5 $MgSO_4$, 3 $CaCl_2$, and 10 glucose. Whole-cell patch clamp recordings were performed in either current-clamp or voltage clamp mode using 4-6 MOhm. For whole-cell current clamp the pipettes contained (in mM): 126 K-gluconate, 4 KCl, 4 ATP-Mg, 0.3 GTP-Na2, 10HEPES, 10 creatine-phosphate, and 8 Biocytin, pH 7.25; osmolarity, 275 mOsm. For whole-cell voltage clamp the pipettes contained (in mM): 130 Cs-gluconate, 5 NaCl, 3 ATP-Mg, 0.3 GTP-Na2, 10 EGTA, 10 HEPES, 12 creatine-phosphate, and 8 Biocytin, pH 7.25; osmolarity, 275 mOsm. Neurons were visualized using DIC imaging on an Olympus BX51WI microscope (Tokyo, Japan). Membrane potentials and currents were recorded using a Multiclamp 700B amplifier (Molecular Devices, USA). The liquid junction potential (-13 mV) was compensated for. Series resistance was continuously monitored and compensated (80%) during the course of the experiments; recordings were discarded if the series resistance changed more than 25%. The recorded neurons were classified as principal neurons based on somatic morphology under DIC (presence of a prominent apical dendrite, pyramidal shaped somata in layer 2 or layer 3), membrane responses to hyperpolarizing and depolarizing current steps including a relatively low input resistance or axodendritic arborizations following post hoc fluorescent imaging following Streptavidin immunoreactions. The recorded neurons were classified as interneurons based on somatic morphology under DIC

(absence of a prominent apical dendrite, small, round or oval somata in layers 1–3), membrane responses to hyperpolarizing and depolarizing current steps including a relatively high input resistance and presence of EPSPs or axodendritic arborizations following post hoc fluorescent imaging following Streptavidin immunoreactions.

For synaptic stimulation two concentric bipolar stimulating electrodes (FHC, Germany) were positioned in the lateral olfactory tract (LOT) and layer 2 (L_2) for afferent, and associational fiber stimulation, respectively. A recording pipette filled with ACSF (resistance: 4 MΩ) was then positioned above L_2. Stimulation consisted of brief (0.1 ms) current pulses (10-100 µA). Afferent and associational stimulation was separated by 0.5 seconds. After obtaining a baseline of field excitatory postsynaptic potentials (fEPSPs), EPSCs or EPSPs serotonin was applied to the recording chamber. ChR2 expressing axons in the aPC were photostimulated through the microscope objective using the epifluorescent illumination via a LED light source (Thorlabs, Germany). Light intensity was set to 0.5 mW. Photostimulation consisted of a 3 second train of 10 ms pulses at 10 Hz. Control and photostimulation trials were intermingled.

Concentration of the drugs used: ketanserin: 10 µM, WAY100635 1 µM, NBQX 10 µM, 5-HT 10 µM, 5-carboxamidotryptamine maleate (5-CT) 50 nM, SB224289 10 µM, CP93129 10 µM, GR127935 10 µM.

5.4. Focal drug application

For the focal application of 5-HT patch pipettes were loaded with 100 µM 5-HT dissolved in ACSF, positioned near (40-60 µm) the soma of the neuron recorded and connected to a Picospritzer III (Parker Hannifin, USA). 5-HT was ejected using a 2 s long (~200 mbar) pulse.

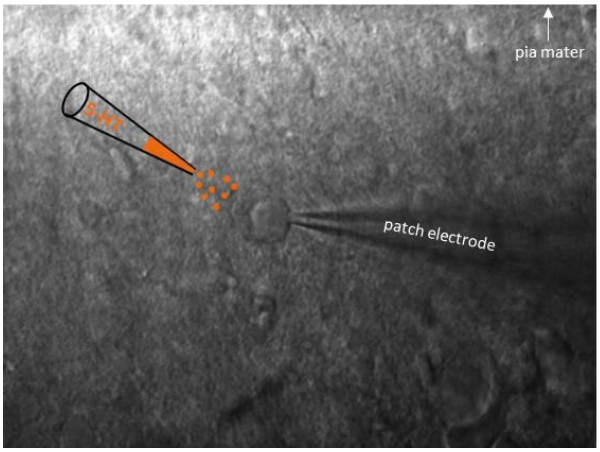

Figure 11. *Schematics of the experimental design for the focal HT application.* A layer 1 interneuron is recorded in the whole cell current configuration while a second pipette loaded with 100 µM 5-HT is located near the soma and 5-HT is pressure ejected.

5.5. In vivo electrophysiology

For the selective stimulation of DRN 5-HT neurons *in vivo* we used similar protocols to the ones in (Lottem, Lörincz, et al., 2016). Briefly, SERT-cre mice previously (4-8 weeks) injected with 0.5–1 µl of AAV2/1–Flex–ChR2–YFP (AV-1-20298P, University of Pennsylvania, 10^{13} GC/mL) in the DRN were anesthetized with Urethane (1.2 g/kg), mounted in a stereotaxic frame and small holes drilled above the target areas (OB: AP, +6.0-7.0 mm; lateral, 2.2 mm; DV, 2.5–4.0 mm, aPC: AP, +2.3 mm; lateral, 2.5 mm; DV, 2.9–3.6 mm) and recording microelectrodes lowered into the OB and aPC, respectively. The DRN was photostimulated using an optical fiber (200 µm diameter; numerical aperture 0.38, positioned at a 32° angle at the following coordinates: AP, −4.7 mm; DV, 3.0–3.6 mm) coupled to a 470 nm laser (Laserglow Technologies). OB and aPC neurons were recorded simultaneously with glass electrodes (impedance: 8-20 MOhm) filled with saline and connected to a DC amplifier (Axoclamp 2B, Axon Instruments, USA). Electrophysiological data were acquired using a Power 1401 and Spike2 software (Cambridge Electronic Design, UK) at 30 kHz sampling rate and stored on a personal computer for offline analysis. Spike sorting was performed using Spike2 software (Cambridge Electronic Design, UK). The 5-HT_{1B} receptor antagonist GR127935 (3 mg/kg, dissolved in saline) was administered intraperitoneally.

5.6. Immunohistochemistry

After *in vitro* recordings the slices were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at 4°C overnight. Slices were cryoprotected using 20% sucrose in 0.1 M PB, freeze-thawed with liquid nitrogen, washed thoroughly with PB and re-sectioned to 50 µm thickness. After blocking with 10 % NHS in TBS, slices were incubated with the primary antibody Rb-α-5-HT (Immunostar, Hudson, WI, United States, Rb-α-5-HT polyclonal, 1:1000) overnight. Following several TBS washes, the slices were incubated with the secondary antibody Alexa488-conjugated Donkey-α-Rb (1:400) for 2 hours and mounted in Vectashield-DAPI medium for microscopy.

Depolarizing current pulses employed during in vitro electrophysiological recordings resulted in an adequate filling of neurons by Biocytin. Following recordings, the slices were placed between two Millipore filters to avoid deformations and were immersed in a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH=7.4) at 4°C for at least 12 h. After several washes in 0.1 M PB, slices were cryoprotected in 10%, then 20 % sucrose in 0.1 M PB. Slices were freeze-thawed in liquid nitrogen, then embedded in 10 % gelatine. 320 µm thick slices embedded in gelatine blocks were resectioned at 60 µm thicknesses and washed in PB, then in triton X-100 (0.4%) in tris buffered saline (1X TBS). The sections were incubated with streptavidin-conjugated Alexa 488 (1:500) and triton X-100 (0.4%) for 2 hrs at room temperature to identify the Neurobiotin-labeled neuron by fluorescent microscopy. Fluorescent images were acquired with a confocal microscope (Olympus FV1000).

5.7. Data analysis

Data were analyzed using Spike2 (Cambridge Electronic Design), Clampfit (Molecular Devices) and Origin Pro (Microcal) software. Data are presented as mean ± s.e.m. Statistical significance was considered at p values below 0.05.

6. RESULTS

6.1. Differential effect of 5-HT PS on OB and aPC neurons

The suppression of baseline neuronal activity in the aPC (Lottem et al., 2016) could be due to the effect of 5-HT release on OB neurons. Specifically, if the activity of M/T neurons in the OB would be decreased by 5-HT PS this could lead to a weaker OB input to the aPC and hence a suppression of aPC neuronal activity. To certify that the suppression of baseline firing by 5-HT PS is not caused by a decreased OB input we simultaneously recorded the activity of OB M/T neurons and aPC neurons and scrutinized the effect on the baseline electrical activity of these neurons in the absence and presence of 5-HT PS, respectively. We recorded the activity of single OB and aPC units either simultaneously (n=8 pairs of simultaneously recorded OB and aPC neurons) or individually (OB: 6 units, aPC: 55 neurons) in SERT-cre mice previously (4-12 weeks) infected with a cre-dependent AAV construct. We compared the spontaneous activity of recorded aPC and OB neurons in the absence and presence of serotonergic photostimulation (5-HT PS) using two statistical approaches: (1) directly comparing firing rates before and during 5-HT PS, and (2) calculating a ROC-based modulation index (MI = 2*(AUC-0.5); where AUC is the area under the ROC curve, positive MI values indicate excitation following PS, and negative values indicate inhibition). We found that while the activity of aPC neurons was significantly suppressed by 5-HT PS (control: 5.42±0.76 Hz, 5-HT PS: 3.25±0.62 Hz; n=63; $p<0.001$, Wilcoxon's signed-rank test; average MI: -0.49±0.05), OB neurons were unaffected (control: 10.00±0.95 Hz, 5-HT PS: 11.37±1.31 Hz; n=14; $p=0.07$, Wilcoxon's signed-rank test; average MI: 0.17±0.09, data not illustrated). The differential effect of 5-HT PS held true when considering simultaneously recorded pairs of aPC and OB neurons (aPC average MI: -0.36±0.19, OB average MI: 0.75±0.16; n=8 pairs; $p<0.05$, Wilcoxon's signed-rank test, Figure 12).

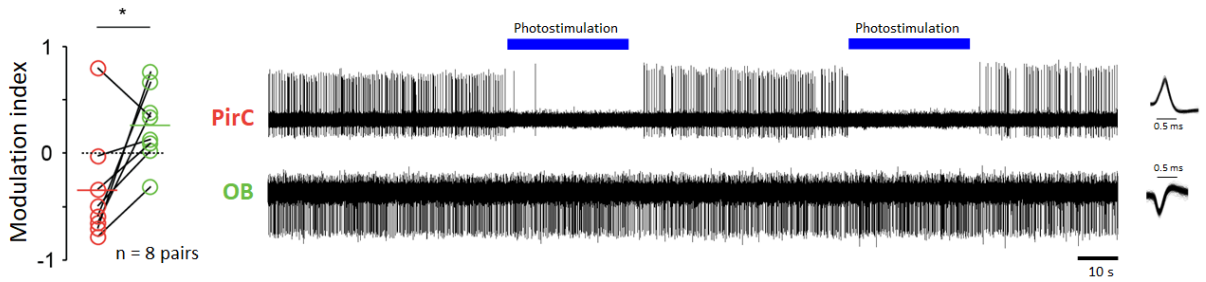

Figure 12. *Selective stimulation of DRN neurons suppresses the ongoing activity of PirC, but not OB neurons.* Simultaneous single-unit recording of a layer 3 neuron in the PirC and an M/T cell in the OB of an anesthetized SERT-cre mouse previously infected with ChR2 in the DRN. (Right) Multiple action potentials from both recording sites are overlayed on a fast timebase to confirm that they originate from a single unit. (Center) Photostimulation of the DRN (blue bars, 10 ms pulses at 30 Hz) results in cessation of action potential output of the aPC neuron and an increase in the activity of the OB neuron. (Left) Population data from 8 simultaneously recorded PirC/OB neuron pairs (red circles: PirC neurons, green circles: OB neurons) shows modulation indices following DRN photostimulation (positive modulation values correspond to an increase in neuronal firing following photostimulation; negative values indicate suppression).

6.2. Serotonergic innervation of the primary olfactory cortex

To characterize the extent of serotonergic innervation of the aPC we performed immunohistochemical experiments by staining the 5-HT fibers with an antibody against 5-HT in coronal brain slices containing the aPC. The results revealed dense 5-HT fibers in the aPC with subregion and layer specific features (Fig 13). Specifically, the aPC contained relatively dense 5-HT fibers in all its layers, most fibers were observed in layer 1 and 3.

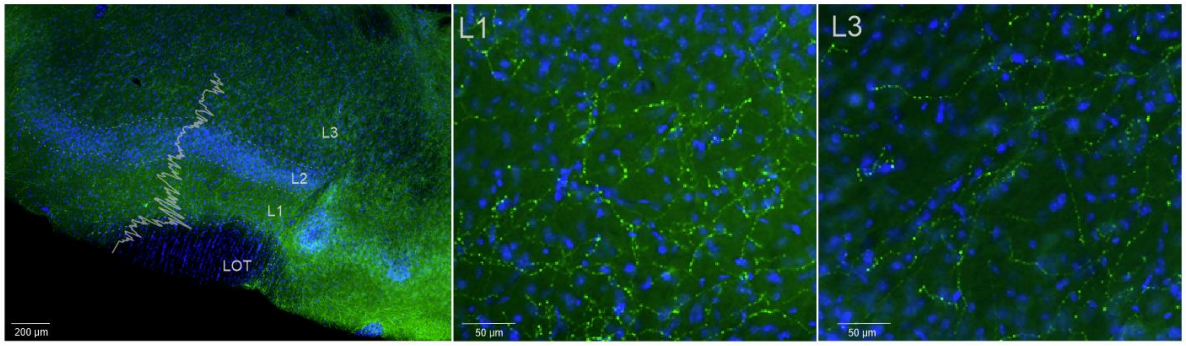

Figure 13. *Serotonergic innervation of the aPC (green channel: serotonin immunopositive fibers, blue channel: DAPI).* Coronal section of a mouse brain at the level of the aPC. The lateral olfactory tract (LOT) and the three layers of the aPC are indicated. The overlayed histogram shows the distribution of 5-HT fibers in various aPC layers. Higher magnification images of layers 1 and 3, respectively are shown on the right side.

6.3. Effect of focally applied 5-HT on principal neurons and interneurons of the aPC

To test the effect of 5-HT on various aPC neurons we focally applied 5-HT while monitoring their membrane potential (Figure 14). Morphologically and physiologically identified pyramidal neurons were hyperpolarized by 5-HT (control: -70.47±0.79, 5-HT: -73.38±1.07, $p<0.05$, Wilcoxon signed-rank test, n=5) (Figure 14A right top). When the neurons were held around the threshold for action potential generation by injecting steady depolarizing current via the recording electrode, the application of 5-HT suppressed their firing (Figure 14A right middle). Both the hyperpolarization (5-HT: -2.64±0.47 mV, WAY100635 + 5-HT: 0.05±0.14 mV, $p<0.05$, Wilcoxon signed-rank test, n=5) and the suppression of action potential firing could be prevented by the bath application of the 5-HT1a receptor antagonist WAY 100635 (Figure 14A right bottom) (5-HT: 1.13±0.78%, WAY100635 + 5-HT: 96.10±2.82%, $p<0.001$,Wilcoxon signed-rank test n=5) suggesting the effect of 5-HT on pyramidal neurons is mediated by 5-HT1 receptors. When 5-HT was focally applied near the somata of various identified interneurons while monitoring their membrane potential, the cells were depolarized (5-HT: 7.30±3.24 mV, n=5) and this depolarization led to action potential firing in all the recorded interneurons (mean firing rate: 2.66±1.40 Hz, n=5) (Figure 14D top right). Both the membrane depolarization and the action potential firing could be prevented by the bath application of the 5-HT_2 receptor antagonist ketanserin (Figure 14D right bottom) suggesting the effect of 5-HT on interneurons is mediated by 5-HT_2 receptors.

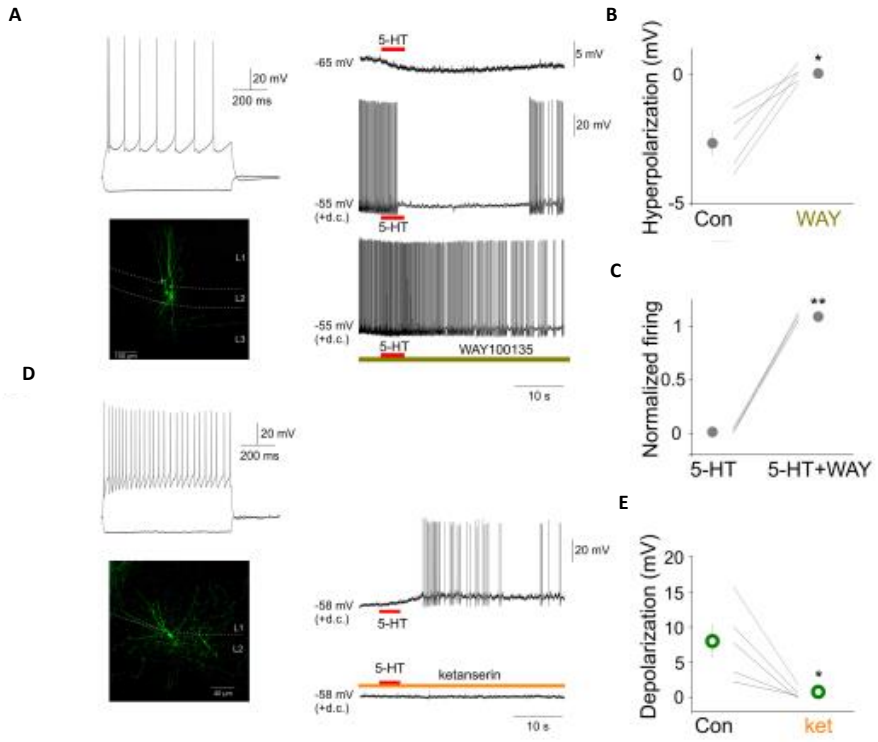

Figure 14. *Focal 5-HT application suppresses the activity of principal neurons and increases the activity of interneurons in the aPC.* (A) Membrane responses to hyperpolarizing and depolarizing current steps (top left), morphology (bottom left) and effect of focally applied 5-HT (100 µM, middle) of a principal neuron at resting membrane potential and during periods of action potential firing (+d.c.). The 5-HT1a antagonist WAY100635 (1 µM) prevented the suppressive effects of 5-HT. (B) Effects of focally applied 5-HT in ACSF (Con) and WAY 100635 (WAY) on all recorded aPC principal neurons (n = 5). (C) Normalized firing rates of all recorded principal neurons (n = 5) during focal 5-HT application in the absence and presence of WAY 100635 (WAY), respectively. (D) Membrane responses to hyperpolarizing and depolarizing current steps (top left), morphology (bottom left) and effect of focally applied 5-HT (100 µM, middle) of an aPC interneuron. The 5-HT2 antagonist ketanserin (10 µM) prevented the depolarizing effects of 5-HT. (E) Effects of focally applied 5-HT in ACSF (Con) and ketanserin (ket) on all recorded aPC interneurons (n = 5). *p < 0.05, **p < 0.01.

6.4. Postsynaptic effects of endogenously released 5-HT in the aPC

Since the effects of exogenously applied 5-HT can differ from the effects of endogenous 5-HT, we next tested the effects of endogenously released 5-HT on various aPC neurons by monitoring their membrane potential and selectively stimulating local 5-HT axons in SERT-cre mice previously (8-15 weeks) infected with ChR2 in the DRN (Dugue et al., 2014), which led ChR2 expression in the somata of DRN 5-HT neurons and prominent axonal ChR2-YFP expression (Figure 15B). In 20 morphologically and/or physiologically identified pyramidal neurons the local photostimulation (PS) of 5-HT axons did not lead to an apparent membrane potential hyperpolarization (Figure 15C, right) (mean Vm change following 5-HT PS: -0.03±0.04 mV,

p>0.05, Wilcoxon signed-rank test, n=20). When we photostimulated 5-HT axons while monitoring the membrane potential of various morphologically and/or physiologically identified interneurons we observed a membrane potential depolarization in 5 out of 8 (62%) interneurons (mean Vm change following 5-HT PS: 1.11±0.42 mV, n=8). 5-HT axonal PS in the aPC could lead to action potential firing in 4 out of 8 (50%) interneurons recorded. Both the depolarization and the effect on action potential firing could be prevented by bath application of the 5-HT2 receptor antagonist ketanserin (10 µM) (Figure 15E, bottom) suggesting aPC interneurons can be excited by endogenously released 5-HT originating from the axons of DRN 5-HT neurons via 5-HT_2 receptors.

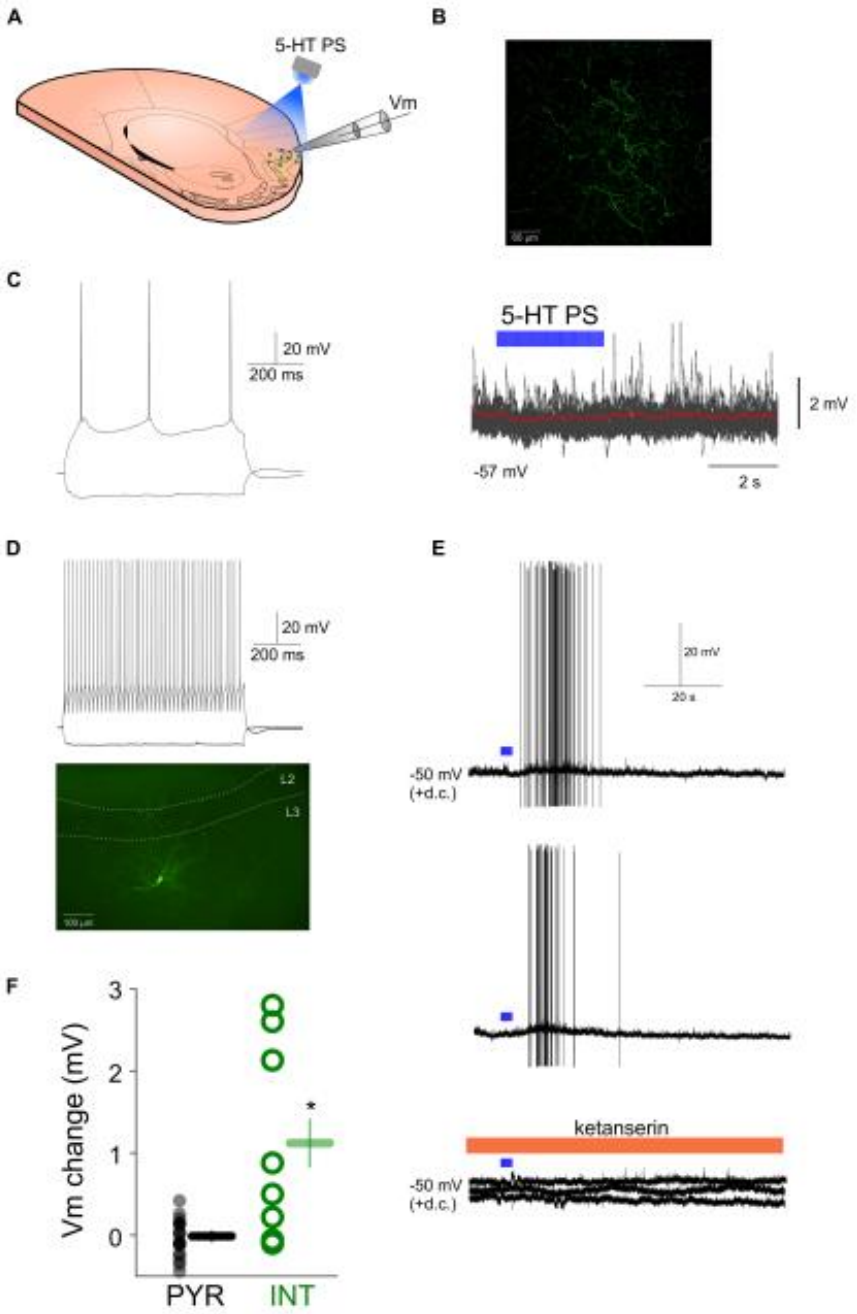

Figure 15. | *Effects of endogenously released 5-HT on aPC principal neurons and interneurons.* (A) Schematic of the experimental design. (B) Confocal image of ChR2-YFP expressing fibers in the aPC of a SERT-cre mouse previously injected with AAV-DIO-ChR2-eYFP in the DRN. (C) Membrane potential responses of an aPC pyramidal neuron to hyperpolarizing and depolarizing current steps (left) and lack of effect of local photostimulation (10 ms pulses, 20 Hz, 5 mW, 3 s) of DRN 5-HT axons in the aPC on the membrane potential (left, 20 sweeps overlayed, red trace: average). (D) Membrane responses to hyperpolarizing and depolarizing current steps (top) and morphology (bottom) of an aPC fast spiking interneuron. (E) Effect of local photostimulation (10 ms pulses, 5 mW, 20 Hz, 3 s) of DRN 5-HT axons in the aPC on the membrane potential of the neuron shown in (D) (two consecutive sweeps). The depolarizing effect is blocked by ketanserin (bottom, three consecutive sweeps). (F) Effects of 5-HT photostimulation on all recorded aPC pyramidal neurons (PYR, gray circles, n = 20) and interneurons (INT, green circles, n = 8). *$p < 0.05$.

6.5. Effect of endogenously released 5-HT on the evoked firing of aPC principal neurons and interneurons

Based on the presence of a direct membrane effect on interneurons and the lack of effect on pyramidal neurons, the endogenous release of 5-HT indicates that it can regulate the rate and timing of action potential output in aPC pyramidal neurons and interneurons, consistent with previous findings (Wang et al., 2020a). In order to investigate this, we applied suprathreshold depolarizing current steps (ranging from 50-200 pA) and compared the firing rate of different aPC neurons under two conditions: the absence and presence, respectively of 5-HT phostimulation (Figure 16). Our findings demonstrate that pyramidal neuron firing rates were significantly reduced in the presence of 5-HT phostimulation (normalized mean firing rate during 5-HT phostimulation: 30.96±9.51%, $p<0.01$, Wilcoxon signed-rank test, n=16) (Figure 16A and D). Conversely, when we repeated the same procedure with aPC interneurons, we observed that the firing rates of 7 out of 10 interneurons (70%) increased in the presence of 5-HT phostimulation, although this change was not statistically significant for the entire group (normalized mean firing rate during 5-HT phostimulation: 133.73±28.63%, $p>0.05$, Wilcoxon signed-rank test, n=10, Figure 16B, C and D). These results suggest that endogenously released 5-HT can modulate the activity of aPC neurons in a cell type-specific manner, enhancing the activity of interneurons while suppressing the firing of pyramidal neurons.

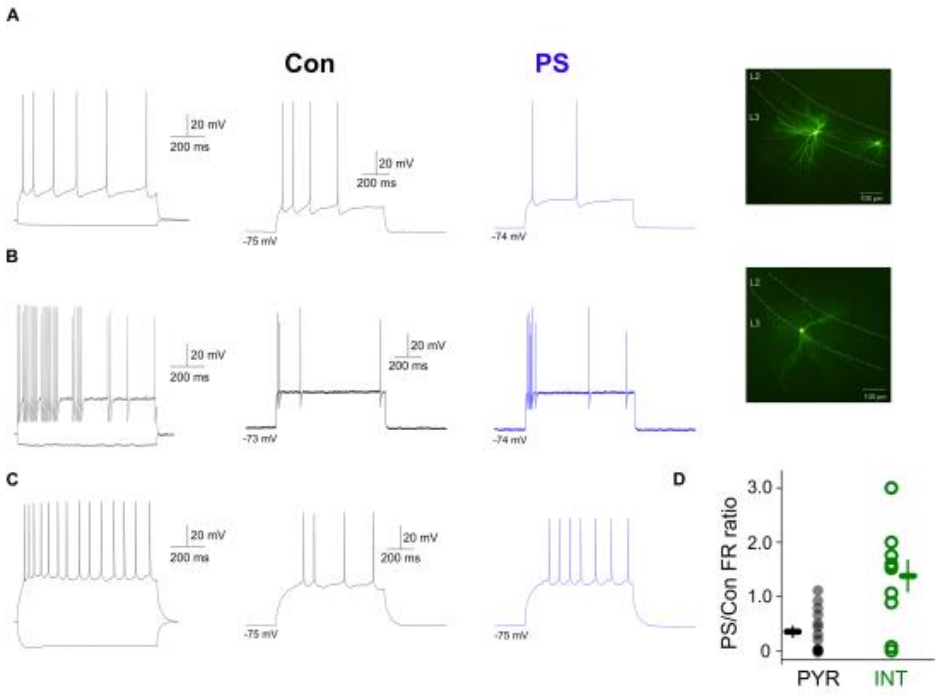

Figure 16.| *Effects of endogenously released 5-HT on the evoked firing of aPC principal neurons and interneurons.* (A) Membrane potential responses and morphology of an aPC pyramidal neuron to hyperpolarizing and depolarizing current steps in the absence (Con, black trace) and presence (PS, blue trace) of 5-HT photostimulation, respectively. (B) Membrane potential responses and morphology of an aPC interneuron to hyperpolarizing and depolarizing current steps in the absence (Con, black trace) and presence (PS, blue trace) of 5-HT photostimulation, respectively. (C) Same as panel (D) for a regular firing aPC interneuron. (D) Firing (normalized to control) of all photostimulated pyramidal neurons (PYR, gray circles, n = 16) and interneurons (INT, green circles, n = 10).

6.6. Effect of bath applied 5-HT on afferent and intracortical synaptic inputs

To examine the effects of 5-HT on synaptic inputs to the aPC, we conducted *in vitro* experiments where the spatially segregated afferent fibers originating from the OB and associational fibers originating from various intracortical sources were separately stimulated and the resulting responses compared before and after bath application of 5-HT (Figure 17A). Electrical stimulation of the LOT resulted in feed-forward (FF) field excitatory postsynaptic potentials (fEPSPs), while stimulating aPC L_2 produced feedback (FB) fEPSPs (Figure 17B left), consistent with previous studies (Haberly & Price, 1978; Luskin & Price, 1983; Price, 1973b). Both types of fEPSPs could be blocked by the AMPA/KA glutamate receptor blocker NBQX (10 µM), while the axonal volley persisted (Figure 17B right; n = 3 slices). We normalized individual FF and FB fEPSPs to the fiber volley magnitude and examined the effects

of bath-applied 5-HT (10 µM) on the fEPSPs. The peak of FF fEPSPs was enhanced by 5-HT (5-HT: +37 ± 0.13%; n = 8 slices; $p < 0.05$, Figure 17C), whereas FB fEPSPs were suppressed (5-HT: -19 ± 0.04% of control; n = 8 slices; $p < 0.01$, Figure 17C), indicating that 5-HT has a pathway-specific influence on aPC synaptic responses. Since 10 µM 5-HT produced nearly maximal effects (Figure 17D), we used this concentration throughout the study. Figure 17E depicts the time course of 5-HT effects on FF and FB inputs in a single experiment. Figure 17F demonstrates the average time course of fEPSP slope changes by 5-HT for FF and FB pathway stimulation. Additionally, the 5-HT_1 receptor agonist 5-carboxamidotryptamine maleate (5-CT, 50 nM) could suppress FB fEPSPs (-29.71 ± 2.37%, $p = 0.006$, data not shown) while simultaneously increasing the paired pulse ratio (control: 1.07 ± 0.06%, 5-CT: 1.25 ± 0.09%, $p < 0.05$), suggesting that presynaptic 5-HT1A or 5-HT_{1B} receptors might mediate these observed effects.

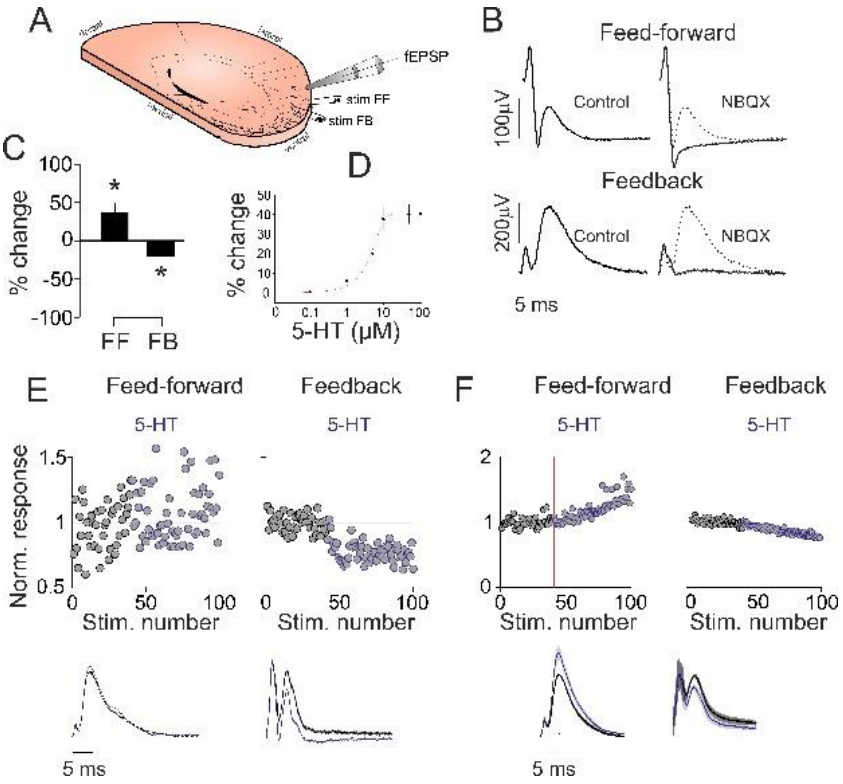

Figure 17. *5-HT suppresses intracortical but increases afferent synaptic activity in vitro.* (A) Schematics of the experimental design: tilted, angled coronal section of the brain showing the placement of the stimulating and recording electrodes. (B) Stimulation of the LOT (top, left) and aPC layer 2 (bottom, left) results in both volley spikes and field excitatory postsynaptic potentials (fEPSPs). The fEPSPs are blocked by the AMPA/KA glutamate receptor blocker NBQX (right). (C) Bar graph illustrating the 5-HT-induced changes in LOT and layer 2 stimulation-evoked fEPSPs in 8 recordings (values correspond to average peak responses during the last 20 trials following 5-HT application). (D) Dose–response curve of the layer 2 evoked fEPSPs. (E) (Top) Single experiment time course of the fEPSP slope changes by 5-HT for feed-forward and feedback pathway stimulation. Application of 5-HT (blue traces,

10 µM in the perfusing solution) increasesed LOT stimulation-evoked (left, feed-forward), but suppressed layer 2 evoked fEPSPs (right, feedback). Average fEPSPs recorded from aPC layer 2b following either LOT (left, feed-forward) or layer 2 stimulation (right, feedback). (F) (Top) Grand-average (n = 8 recordings) time course of the fEPSP slope changes by 5-HT for feed-forward and feedback pathway stimulation. The application of 5-HT resulted in the increase of LOT stimulation-evoked, but suppressed layer 2 evoked fEPSPs. (Bottom) Grand-average fEPSPs recorded from aPC layer 2b following either LOT (left, feed-forward) or layer 2 stimulation (right, feedback) from n = 8 experiments. * indicates $p<0.05$.

6.7. Effect of bath applied 5-HT on aPC neuronal firing evoked by synaptic stimulation

We next studied the effect of 5-HT on the synaptic stimulation effects of individual aPC neurons. To this end, we recorded various aPC neurons in whole-cell current clamp mode and set the FB and/or FF stimulation intensity to evoke moderate action potential firing. Figure 18B shows an example layer 3 fast spiking neuron that ceased FB pathway-evoked firing following 5-HT application. The suppression of FB stimulation-induced firing by 5-HT was significant for the population of neurons recorded (number of spikes evoked in control: 0.94 ± 0.10, number of spikes evoked in 5-HT: 0.06 ± 0.04, $p < 0.001$). This corresponded to a reduction in firing to 5.57 ± 3.44% of the control (n = 5, $p < 0.0001$, Figure 18B right). The effect was reversed by washing out 5-HT from the perfusion chamber. To reveal the receptor type involved in mediating the suppression of firing, we blocked 5-HT_{1B} receptors by bath application of 10 µM SB224289 and scrutinized the effect of 5-HT on aPC neuronal firing evoked by FF and FB synaptic stimulation, respectively. In the presence of 5-HT_{1B} receptor blockers, 5-HT failed to suppress FF (spikes evoked by FF stimulation in SB: 0.84 ± 0.05, spikes evoked FF stimulation in SB + 5-HT: 0.9 ± 0.044, n = 5, $p > 0.05$), corresponding to a reduction in firing of 7.57 ± 3.134%, n = 5, $p > 0.05$, Figure 18C) or FB stimulation-induced firing (spikes evoked by FB stimulation in SB: 0.90 ± 0.07, spikes evoked FB stimulation in SB + 5-HT: 0.88 ± 0.073, n = 5, $p > 0.05$), corresponding to a change in firing of 2.33 ± 2.22% (n = 5, $p > 0.05$, Figure 18C bottom).

The 5-HT_{1B} receptor agonist, CP93129 (10 µM), replicated the suppressive effects of 5-HT for FB (spikes evoked by FB stimulation in control: 4.3 ± 3.22, spikes evoked by FB stimulation in CP93129: 0.04 ± 0.02, n = 5, $p < 0.00001$), leading to a reduction of 97.24% of control (n = 5, $p < 0.00001$, Figure 18D bottom) but not FF stimulation (spikes evoked by FF stimulation in control: 3.68 ± 2.68, spikes evoked by FF stimulation in CP93129: 4.16 ± 3.11, n = 5, $p > 0.05$),

leading to an increase of 7.22 ± 3.14% of control (n = 5, $p > 0.05$); see Figure 18D top. These results suggest 5-HT can suppress FB responses by acting on 5-HT_{1B} receptors.

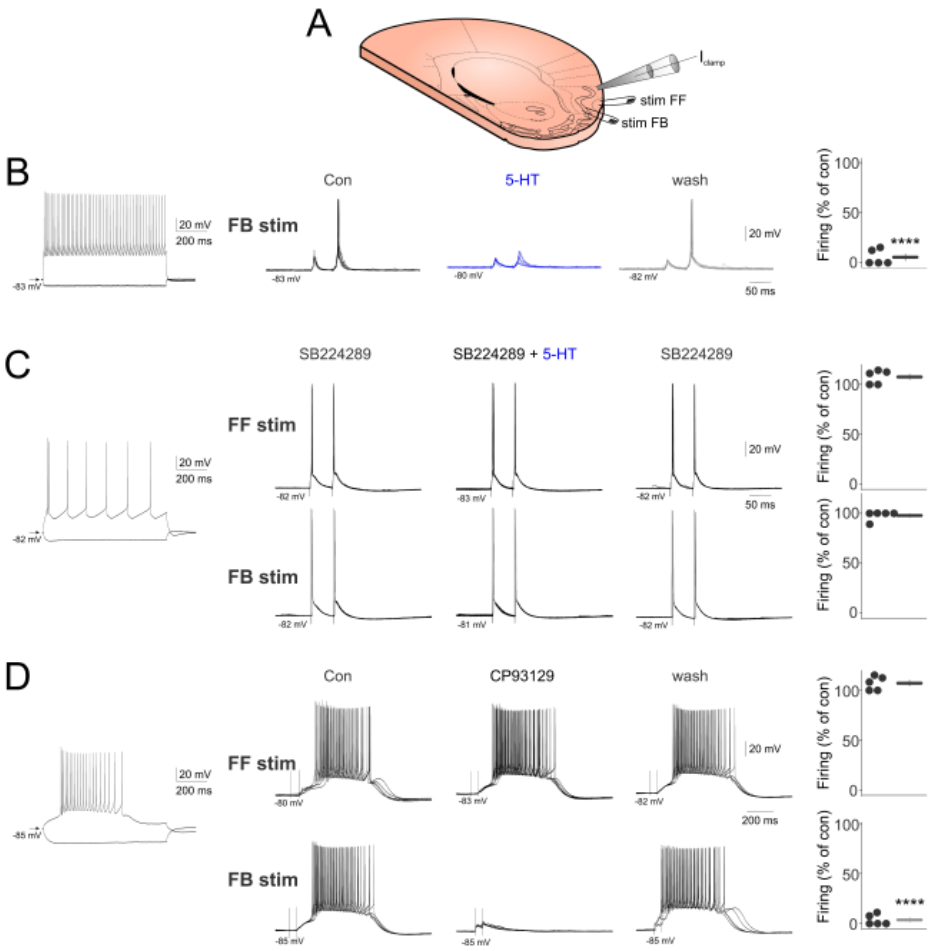

Figure 18. *5-HT suppresses FB stimulation-induced firing via 5-HT_{1B} receptors.* (A) Schematics of the experimental design. (B) (Left) Membrane responses to hyperpolarizing and depolarizing current pulses of a layer 3 fast spiking neuron. (Middle) Bath applied 5-HT suppresses the FB stimulation-evoked firing. (Right) Quantified effects of 5-HT on FB stimulation-induced firing for all neurons recorded (n = 5). (C) (Left) Membrane responses to hyperpolarizing and depolarizing current pulses of a layer 2 regular spiking neuron. (Middle) Bath applied 5-HT fails to suppress the FF and FB stimulation evoked firing in the presence of the 5-HT_{1B} receptor blocker SB224289 (10 µM). (Right) Quantified effects of 5-HT on FF and FB stimulation-induced firing in the presence of the 5-HT_{1B} receptor blocker SB224289 for all neurons recorded (n = 5). (D) (Left) Membrane responses to hyperpolarizing and depolarizing current pulses of an L1 interneuron. (Middle) Bath applied CP93129 (10 µM) suppresses the FB but not FF stimulation-evoked firing. (Right) Quantified effects of CP93129 on FF and FB stimulation-induced firing for all neurons recorded (n = 5). **** indicates $p < 0.0001$.

6.8. Effect of bath applied 5-HT on aPC synaptic currents

We next revealed the effects of 5-HT on excitatory postsynaptic currents (EPSCs) evoked by layer 2 electrical stimulation in various aPC neurons in whole-cell voltage clamp mode. In aPC principal neurons (Figure 19B), two brief electrical stimuli (0.1 ms, 20–100 µA) delivered to layer 2 evoked clear EPSCs that were reduced in amplitude following the bath application of 10 µM 5-HT ($EPSC_1$ control: 158.75 ± 5.35, $EPSC_1$ 5-HT 131.5 ± 3.48, $p < 0.001$, $EPSC_2$ control: 192.87 ± 7.09, $EPSC_2$ 5HT: 167.0 ± 5.15, n = 5, $p < 0.001$, Figure 19C, D). The paired pulse ratio (PPR, $EPSC_2/EPSC_1$) was significantly increased following 5-HT application (PPR control: 1.22 ± 0.05, PPR 5HT: 1.27 ± 0.06, n = 5, $p < 0.01$, Figure 19E). In aPC interneurons (Figure 19F), EPSCs were also reduced in amplitude following bath application of 5-HT ($EPSC_1$ control: 205.2 ± 9.92, $EPSC_1$ 5-HT 157.4 ± 12.98 ($p < 0.001$), $EPSC_2$ control: 230.2 ± 22.18, $EPSC_2$ 5HT: 191 ± 19.70, n = 5, $p < 0.001$, Figure 19G, H). The PPR showed a tendency of increase following 5-HT application, but this was below significance level (PPR control: 11.12 ± 0.08, PPR 5HT: 1.23 ± 0.13, n = 5, $p > 0.05$, Figure 19I)

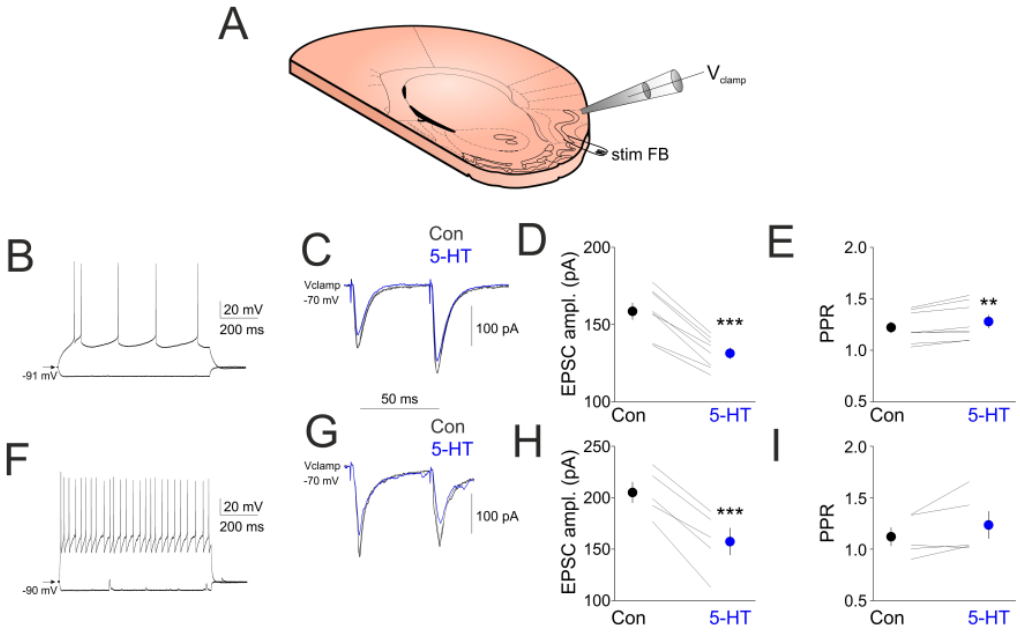

Figure 19. *5-HT suppresses FB stimulation induced synaptic currents.* (A) Schematics of the experimental design. (B) Membrane responses of a layer 2 pyramidal neuron. (C) Synaptic currents evoked by electrical stimulation of layer 2 in control conditions (black line) in the presence of 10 µM 5-HT (blue line) and following washout of 5-HT (gray line). (D) Quantification of $EPSC_1$ amplitude changes following bath application of 5-HT in all principal neurons recorded (n = 8). (E) Changes in paired pulse ratios following 5-HT application in all principal neurons recorded (n = 8). (F) Membrane responses of a layer 3 interneuron. (G) Synaptic currents evoked by electrical stimulation of layer 2 in control conditions (black line) in the presence of 10 µM 5-HT (blue line) and following washout of 5-HT (gray line). (H) Quantification of $EPSC_1$ amplitude changes following bath application of 5-HT

in all interneurons recorded (n = 5). (I) Changes in paired pulse ratios following 5-HT application in all interneurons recorded (n = 5). ** indicates p<0.01, *** indicates p<0.001.

6.9. Effect of endogenously released 5-HT on aPC synaptic currents

To reveal the effect of endogenously released 5-HT on EPSCs evoked by local synaptic stimulation of the FB pathway, we electrically stimulated layer 2 in the absence and presence of local photostimulation of ChR_2 expressing 5-HT axons (5-HT PS) while recording aPC neurons in whole-cell voltage clamp mode (Figure 20A). This evoked clear EPSCs that were reduced in amplitude following 5-HT PS ($EPSC_1$ control: 234.2 ± 21.99 pA, $EPSC_1$ 5-HT PS 180.4 ± 14.54, $p < 0.01$; $EPSC_2$ control: 222.6 ± 34.35, $EPSC_2$ 5HT PS: 207.4 ± 25.77, n = 5, $p > 0.05$, Figure 20B, C). In the presence of the selective $5\text{-}HT_{1B}$ receptor blocker GR127935, 5-HT PS failed to decrease the amplitude of the FB stimulation-evoked EPSCs ($EPSC_1$ ampl GR127935: 213.6 ± 25.38, $EPSC_1$ ampl 5-HT PS + GR127935: 211.2 ± 26.25, n = 5, $p > 0.05$, $EPSC_2$ ampl GR127935: 210.6 ± 34.45, $EPSC_2$ ampl 5-HT PS + GR127935: 208.4 ± 33.98, n = 5, $p > 0.05$, Figure 20B, C). The PPR was significantly increased following 5-HT PS (PPR control: 0.92 ± 0.07, PPR 5HT PS: 1.13 ± 0.06, n = 5, $p < 0.01$, Figure 20E). In the presence of GR127935, 5-HT PS failed to increase the PPR (PPR GR127935: 0.96 ± 0.05, PPR GR127935 + 5-HT PS: 0.97 ± 0.05, n = 5, $p > 0.05$, Figure 20F).

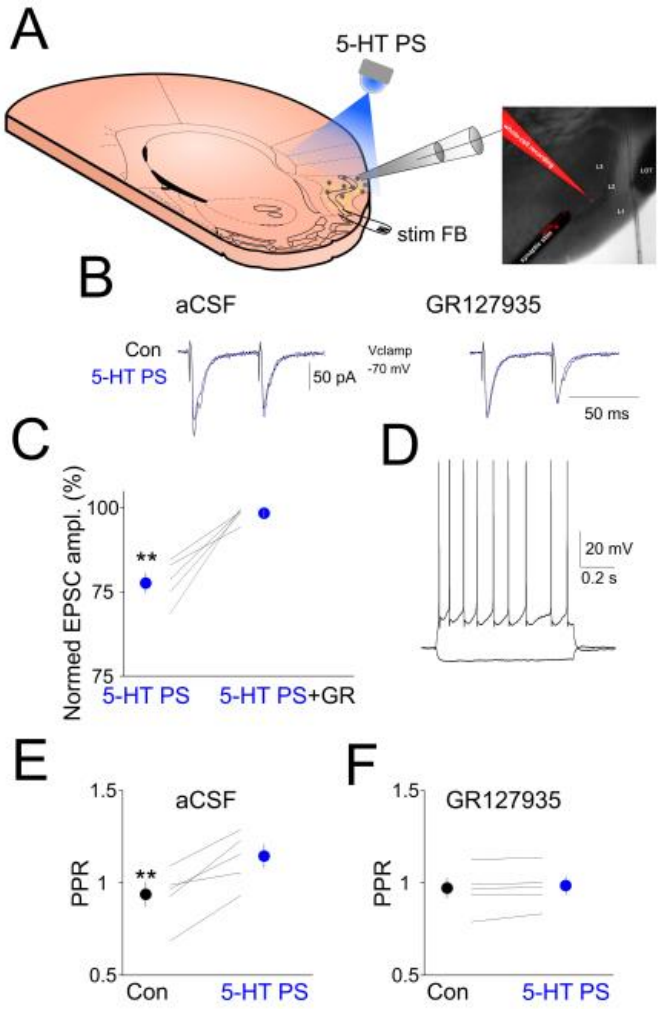

Figure 20. *Local photostimulation of ChR2-expressing DRN axons in the aPC suppresses FB stimulation-induced synaptic currents.* (**A**) Schematics of the experimental design. (**B**) (Left) Synaptic currents evoked by electrical stimulation of layer 2 are suppressed by the local photostimulation of ChR2 expressing 5-HT axons (5-HT PS, blue line) compared to control (Con, black line). (Right) The 5-HT_{1B} receptor antagonist GR127935 (10 µM) blocks the effect of 5-HT fibers photostimulation. (**C**) Quantification of the EPSC suppression by 5-HT PS in the absence and presence of 5-HT_{1B} receptor antagonist GR127935. (**D**) Membrane responses of the layer 3 pyramidal neuron shown in A and B to hyperpolarizing and depolarizing current pulses. (**E**) Changes in paired pulse ratio (PPR, EPSC1/EPSC2) following 5-HT PS in all the neurons recorded (n = 5). (**F**) Changes in paired pulse ratio (PPR, EPSC1/EPSC2) following 5-HT PS in the presence of 5-HT_{1B} receptor antagonist GR127935 in all the neurons recorded (n = 5). ** indicates $p<0.01$.

6.10. Effect of blocking 5-HT$_{1B}$ receptors on aPC activity suppression by 5-HT in vivo

To investigate the involvement of 5-HT$_{1B}$ receptors in mediating the effects of 5-HT *in vivo*, we conducted recordings of spontaneous firing in aPC neurons in anesthetized SERT-cre mice expressing ChR$_2$ in their DRN 5-HT neurons (Figure 21A). Consistent with previous findings (Lottem et al., 2016), 5-HT phostimulation led to a significant suppression of baseline firing in all recorded aPC neurons (modulation index control: -0.83 ± 0.07, n = 5, $p < 0.01$, Figure 21B, D). However, this suppressive effect of 5-HT phostimulation was abolished following systemic administration of the 5-HT$_{1B}$ receptor antagonist GR127935 (modulation index GR127935: -0.18 ± 0.01, n = 5, $p > 0.05$, Figure 21C, D). Furthermore, blocking 5-HT$_{1B}$ receptors resulted in an increase in baseline firing rate in the absence of 5-HT phostimulation (FR control: 1.24 ± 0.14, FR GR127935: 1.67 ± 0.21, n = 5 neurons, $p < 0.05$, Figure 21E). These findings provide evidence that 5-HT$_{1B}$ receptors play a role in mediating the suppressive effects of 5-HT on aPC neuronal activity *in vivo*, and their blockade leads to an elevation in baseline firing rate even in the absence of 5-HT phostimulation.

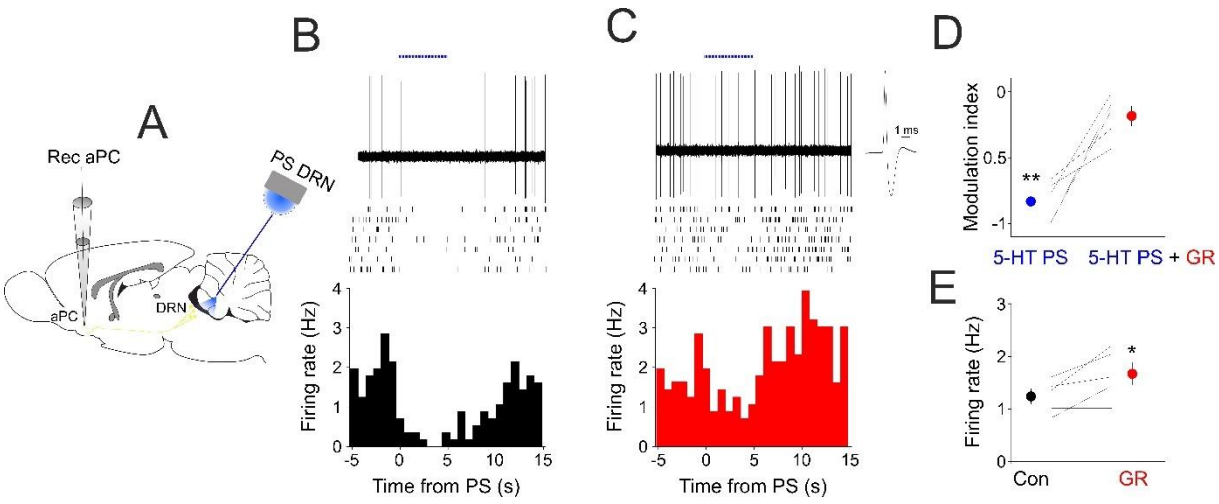

Figure 21. *Blocking 5-HT$_{1B}$ receptors reduces the suppressive effect of DRN photostimulation in vivo.* (A) Schematics of the experimental design. (B) DRN photostimulation suppresses aPC baseline activity. Raw single trial example recording of an aPC neuron shows prominent suppression of its baseline action potential firing following DRN photostimulation (blue bars, 10 ms pulses at 30 Hz, 5 mW). Rasters of 7 consecutive trials are shown below. (Bottom) Peri/event time histogram of the photostimulated aPC neuron. Time zero marks the start of the photostimulation. (C) Same neuron as in B, but following systemic administration of the 5-HT$_{1B}$ receptor

antagonist GR127935 (3 mg/kg). The action potential average is shown on a faster time-base on the right. (D) Modulation index in control conditions (Con) and following GR127935 administration of all aPC neurons recorded (n = 4). (E) Baseline firing in control conditions (Con) and following GR127935 administration of all aPC neurons recorded (n = 5). * indicates $p<0.05$.

7. DISCUSSION

In order to provide comprehensive insights into the effects of 5-HT on the neurons of theaPC, we conducted a series of experiments utilizing *in vitro* and *in vivo* electrophysiological approaches combined with, optogenetic, pharmacological, and immunohistochemical techniques. Our findings collectively demonstrate the following key observations:

(i) By performing simultaneous OB and aPC recording during 5-HT PS we excluded the possibility that the prominent suppression of aPC neuronal activity upon 5-HT PS is originating from the OB as the neuronal activity in the two regions showed a differential effect: increased activity in OB neurons and suppression of most aPC neurons.

(ii) We identified that aPC interneurons, including perisomatic inhibitory fast-spiking interneurons, are excited by 5-HT, whereas principal neurons are inhibited. This differential response highlights the specific modulation of distinct neuronal populations within the aPC by 5-HT.

(iii) Additionally, we observed that 5-HT can exert differential effects on synaptic inputs to the aPC. Specifically, it suppresses intracortical inputs while increasing afferent inputs. This suggests a dual role of 5-HT in modulating synaptic transmission within the aPC, with distinct effects on local circuitry and incoming sensory information.

(iv) Further investigation into the mechanisms underlying the suppression of feedback inputs revealed that 5-HT primarily acts through a 5-HT_{1B} receptor-dependent pathway to reduce glutamate release. This specific receptor-mediated modulation provides mechanistic insight into the observed synaptic alterations induced by 5-HT.

(v) *In vivo* experiments employing targeted stimulation of 5-HT neurons originating from the dorsal raphe nucleus (DRN) and subsequent systemic application of 5-HT_{1B} receptor antagonists revealed that the suppression of baseline aPC neuronal activity induced by DRN 5-HT stimulation can be effectively blocked. This highlights the significance of 5-HT_{1B} receptors in mediating the suppressive effects of 5-HT on aPC neuronal activity in a broader physiological context.

These comprehensive results have important implications for understanding the network mechanisms underlying cortical neuromodulation, particularly in the context of olfactory coding within the central nervous system. By elucidating the specific effects of 5-HT on

different neuronal populations and synaptic inputs in the aPC, our results contribute to a more detailed understanding of how neuromodulation influences sensory processing and information flow within cortical circuits.

7.1. Local effects of 5-HT PS

While optogenetics enables specific and precise stimulation of genetically targeted neuronal populations in intact preparations, care must be taken when interpreting the results obtained by photostimulating a brain area and examining its effects in another area. As neurons can have axon collaterals, it is possible that stimulating area A can lead to changes in neuronal activity in area B, which may in turn impact area C. In our study, the suppressive effects of 5-HT photostimulation on aPC neuronal activity (Lottem et al., 2016) could theoretically be due to DRN fibers affecting the OB, which subsequently impacts aPC neuronal activity. However, we provided evidence for a local effect of endogenously released 5-HT in the aPC. Firstly, simultaneous OB/aPC recordings did not show a decrease in putative M/T neurons, suggesting that the suppressive effects observed in the aPC are unlikely caused by changes in OB activity. Secondly, our in vitro experiments, where long-range connectivity is disrupted, demonstrated that local photostimulation of DRN 5-HT axons expressing ChR2 led to changes in aPC neuronal activity, providing evidence of local 5-HT release within the aPC. The local release of classical neurotransmitters from ChR2-expressing axons can easily be probed by blocking voltage-gated Na+ and K+ channels (Gazea et al., 2021). However, given the small and slow effects of 5-HT, this was not possible in the present experiments. Nevertheless, the lack of suppression in OB M/T neurons in vivo, the small but significant effects of local 5-HT photostimulation in vitro, and the blockade and prevention of these effects by specific antagonists of 5-HT receptors suggest that 5-HT is indeed released in the aPC upon an increase in firing in DRN 5-HT neurons. However, the effects of 5-HT photostimulation could overestimate the effects of 5-HT for several reasons. Firstly, the synchronous firing rate increase in all DRN 5-HT neurons expressing ChR2 that are in the proximity of the optical fiber is unlikely to occur similarly in vivo. Secondly, in our in vivo anesthetized and in vitro conditions, the baseline 5-HT levels are low, so the optogenetic stimulation likely has more pronounced effects than it would in a behaving animal, where the DRN neuronal activity and resulting 5-HT release are more pronounced. Nevertheless, the mechanisms revealed are

grounded in genuine physiological activity and most probably contribute to the complex effects of 5-HT.

7.2. Serotonergic modulation of various cell-types in the olfactory cortex

The results of 5-HT focal application show a prominent hyperpolarization of aPC principal neurons in accordance with previous studies using bath applied 5-HT (Gellman & Aghajanian, 1994; Marek & Aghajanian, 1994, 1996; Sheldon & Aghajanian, 1990). Interestingly, endogenously released 5-HT from ChR_2 expressing DRN 5-HT axons had no prominent hyperpolarizing effect on the membrane potential of aPC principal neurons. While this could be caused by ineffective release of 5-HT from DRN axons in the aPC, an alternative explanation is that the exogenously applied 5-HT can activate receptors that are far from any 5-HT terminal as in the case of dopamine (Rosen et al., 2015), while the endogenous release of a neuromodulator only binds to the receptors situated proximal to its axon terminals . In line with this, endogenous 5-HT release did affect the membrane potential of some aPC interneurons and the evoked firing of both principal neurons and interneurons. Taken together these results argue against the possibility of a failure in 5-HT release from ChR_2 expressing DRN terminals *in vitro* and suggest that 5-HT has a subtle cell-type specific effect on aPC neurons.

These results are in line with our previous observations showing a suppressive effect of 5-HT photostimulation on the spontaneous activity of most aPC neurons and an increase in firing in a minority of aPC neurons *in vivo* (Lottem et al., 2016). In addition to the direct suppressive effects of 5-HT on principal neurons, the increase in activity in various interneurons, including perisomatic targeting fast spiking interneurons could be an additional mechanism by which 5-HT can potently control the activity of aPC principal neurons. Whether the same DRN neurons target multiple types of aPC neurons including principal neurons and interneurons or whether the connectivity itself possesses cell-type specific features remains to be established. As the activity of DRN neurons is modulated on relatively rapid timescales in a behaviorally relevant manner (Cohen et al., 2015; Fonseca et al., 2015; Liu et al., 2014; Lottem et al., 2018; Ranade & Mainen, 2009) it is interesting to speculate on the potential consequences of rapid and cell-type specific 5-HT effects on aPC neurons. In addition to potential effects on sensory information processing 5-HT could also control the timing of various aPC neurons in relation to gamma oscillations originating in the olfactory bulb which are known to be important in

widespread gamma oscillations in the limbic system (Becker & Freeman, 1968). Interestingly, fast spiking interneurons, the more strongly affected neuronal population in our 5-HT PS experiments are key players in the generation and maintenance of cortical gamma oscillations (Cardin et al., 2009). As limbic gamma oscillations are important in the maintenance of a healthy mood and are affected in major depression (Scangos et al., 2021) the effects of 5-HT described here could be also relevant for the (patho)physiology of higher brain functions.

7.3. Serotonergic modulation of synaptic inputs to the olfactory cortex

The results of the experiments aiming to elucidate the effects of serotonergic neuromodulations of synaptic inputs to the aPC have revealed that 5-HT can have a pathway specific effect suppressing intracortically evoked, but boosting afferent input evoked activity. These results provide a synaptic mechanism for our previous observations that 5-HT can suppress spontaneous but not odor-evoked activity *in vivo* (Lottem et al., 2016) and complement our observations concerning the direct effect of 5-HT on single aPC principal neurons and interneurons (Piszár & Lőrincz, 2022). Thus, 5-HT can directly suppress the activity of aPC principal neurons, increase the activity of aPC GABAergic interneurons and increase feed-forward synaptic inputs originating from the olfactory bulb while suppressing feedback inputs originating from various cortical sources including the aPC.

Similar to acetylcholine and noradrenaline (Hasselmo and Bower, 1992; Hasselmo et al., 1997), 5-HT can suppress feedback synaptic responses originating from cortical sources but not afferent (feed-forward) inputs originating from the OB. This differential effect on various inputs is in line with a synapse-specific effect of 5-HT, which is a general feature of this neuromodulator in various brain regions such as the olfactory tubercle (Hadley & Halliwell, 2010), the hippocampus (Schmitz et al., 1998), and the nucleus accumbens (Christoffel et al., 2021).

Testing the effect of 5-HT on the spontaneous and odor-evoked activity of the aPC has led to contrasting results. The selective stimulation of DRN 5-HT neurons resulted in the divisive suppression of spontaneous, but not odor-evoked, spiking activity of anesthetized mice (Lottem et al., 2016). However, odor-evoked activity was decreased, and spontaneous activity remained unaltered during similar manipulation of 5-HT neurons when monitoring the population Ca^{2+} dynamics of aPC principal neurons using fiber photometry in awake mice (Wang et al., 2020b).

The contrasting results might be due to different techniques used (bulk imaging vs. single neuron recordings).

Our study has also shed light on the identity of 5-HT receptors involved in the modulation of feedback inputs to the aPC. Both endogenous and exogenous application of 5-HT decreased FB stimulation induced fEPSPs and EPSCs in single neurons. In addition to the reduction in EPSCs in single aPC neurons, bath-applied 5-HT or the local photostimulation of ChR2-expressing DRN axons in the aPC led to an increase in the paired pulse ratio, suggesting a presynaptic site of action. Presynaptic 5-HT_{1B} receptors are key players in decreasing the release of glutamate in various brain areas (Choi et al., 2012; Guo et al., 2017; Hwang & Chung, 2014; K. S. Lee et al., 2008; Nagata et al., 2019; Nishijo et al., 2022; Pickard et al., 1999). The mechanisms of 5-HT mediated modulation of feed-forward inputs will need to be revealed by future studies.

7.4. Functional implications

The differential effect of 5-HT on feed-forward and feedback inputs can have important functional implications. As the afferent inputs to the aPC originate in the OB, these results argue for a locus-specific effect, where 5-HT can differentially modulate neurons and/or synapses located at various levels of the sensory pathway. 5-HT can thus regulate the relative weights of synaptic inputs to aPC influenced by the multiple synaptic input sources of the DRN (Pollak Dorocic et al., 2014). One of the major sources of inhibitory and excitatory inputs to DRN neurons is the lateral hypothalamus (Gazea et al., 2021; Sere et al., 2021) that can broadcast information related to energy balance and arousal to the olfactory system. Another source of excitatory inputs to DRN neurons are the projections from the frontal cortex, namely the medial prefrontal and orbitofrontal cortices. These brain areas most likely orchestrate the activity of DRN neurons in conjunction based on either higher order cortical or autonomous information.

The activity of sensory neurons may be broadly subdivided into ongoing, or spontaneous, and sensory-evoked activities. While the relationship between sensory-evoked activity and the various properties of sensory stimuli has been extensively researched, the role of spontaneous neuronal activity in stimulus representation has received less attention. One salient feature of cortical activity is the variety of network states expressed as a wide array of structured, state-dependent spontaneous activities (Lőrincz et al., 2015). This variety is most pronounced when

comparing sensory responses during periods of natural sleep (or anaesthesia) and wakefulness (Livingstone & Hubel, 1981; Massimini et al., 2005). However, state changes during wakefulness have also been identified (McGinley et al., 2015), and are thought to influence profoundly sensory processing in both humans (Bradley et al., 2008) and rodents (McGinley et al., 2015), as well as neuronal responses to sensory stimuli of different modalities. The mechanisms generating these state changes are thought to rely on neuromodulatory influences on various stages of different sensory modalities, ranging from peripheral (Lőrincz et al., 2008; Schröder et al., 2020) through thalamic-relay (McGinley et al., 2015; Molnár et al., 2021; Nestvogel & McCormick, 2022) and cortical (McGinley et al., 2015; Vinck et al., 2015) sites.

Information processing in PirC is characterized by a particularly powerful state-dependent gating of odor information. Odor-evoked responses were significantly different between two states: during a slow-wave state, odorants elicited weak neuronal responses, whereas during a fast-wave (desynchronized) state, odor stimuli resulted in robust spiking (Murakami et al., 2005). These findings suggest the existence of a state-dependent switchover of signal processing modes in the olfactory cortex, as part of a sensory gating mechanism that may be common to all sensory systems and does not involve the thalamus (since the olfactory system lacks a specialized thalamic relay). One modulatory system that can affect the level of sensory sensitivity is the serotonergic one.

As discussed above, the DRN exerts potent effects on both the OB and the PirC, enhancing activity in the former while suppressing activity in the latter. Electrophysiological recordings from DRN neurons in rats performing an odor-guided decision task found that these neurons encoded diverse sensory and motor events, with some even encoding specific odor identities (Ranade & Mainen, 2009). However, since the DRN receives diverse inputs from multiple cortical as well as subcortical brain regions (Pollak Dorocic et al., 2014; Weissbourd et al., 2014) and in turn projects widely throughout the brain, its effects on sensory information processing are unlikely to correspond to fine-grained, low-level modulation of sensory responses, and may instead reflect a more general role of 5-HT in regulating information processing and behavior control.

A number of recent studies have proposed that a significant role of the 5-HT system is encoding uncertainty levels in the environment. Surprising events, such as an unexpected reward or the omission of an expected reward result in elevated 5-HT activity (Matias et al., 2017; Ranade & Mainen, 2009), and more generally, 5-HT neurons were shown to continuously track

uncertainty levels in tasks where action-outcome relations' stability changes (Grossman & Cohen, 2022). Furthermore, in reversal learning tasks, in which cue-reward contingencies change abruptly (thus leading to high uncertatinty levels), chemogenetic inhibition of DRN 5-HT neurons in mice (Matias et al., 2017), and prefrontal destruction of the ascending serotonergic projections in monkeys (Clarke et al., 2004), impaired learning so that animals kept following old behaviors even though these were no longer rewarding.

Predictive processing theory postulates that in sensory systems, an incoming sensory input is combined with expectations (also known as priors) to form internal representations of the environment (Keller & Mrsic-Flogel, 2018). Since the serotonergic system affects spontaneous activity, its functional role regarding sensory processing relies on the interpretation of such activity. In the context of predictive processing theory, it was proposed that spontaneous activity represents samples from priors, in the absence of a sensory stimulus (Berkes et al., 2011; Fiser et al., 2010). In the same context, unexpected events that strongly violate priors would lead to increased 5-HT release. In turn, in the PirC, this would lead to an enhanced input from the OB and the suppression of ongoing activity. The combination of both these processes may result in a decrease in the weight of priors vs. input in forming the olfactory percept. At that time, new priors that are better matched to the current statistics of the environment could be learned. And indeed, the 5-HT system has been implicated in facilitating the neuronal plasticity needed for this updating (Branchi, 2011; Carhart-Harris & Nutt, 2017).

In summary, our findings provide valuable insights into the serotonergic modulation of the anterior piriform cortex. By elucidating the specific effects of 5-HT on distinct neuronal populations and synaptic inputs, we contribute to a deeper understanding of how neuromodulation influences sensory processing and information flow within cortical circuits, particularly in the context of olfactory coding in the central nervous system.

8. SUMMARY

Serotonin (5-hydroxytryptamine, 5-HT) is an important monoaminergic neuromodulator involved in a variety of physiological and pathological functions. It has been implicated in the regulation of sensory functions at various stages of multiple modalities, but its cellular mechanisms and functions in the olfactory system have remained elusive. Specific optogenetic stimulation of serotonergic neurons results in the divisive suppression of spontaneous, but not sensory evoked activity in the majority of neurons in the primary olfactory cortex and an increase in firing in a minority of neurons. To reveal the mechanisms involved in this dual serotonergic control of cortical activity we used a combination of *in vivo* electrophysiology, *in vitro* whole-cell current and voltage clamp recordings from identified neurons in the primary olfactory cortex, synaptic stimulation, optogenetics, pharmacology and immunohistochemistry. We found that 5-HT suppressed the activity of principal neurons, but excited local interneurons. In addition, afferent (feed-forward) pathway-evoked synaptic responses are boosted, whereas feedback responses are suppressed by presynaptic 5-HT_{1B} receptors in the olfactory cortex *in vitro*. Blocking 5-HT_{1B} receptors also reduces the suppressive effects of serotonergic photostimulation of baseline firing *in vivo*. By differentially affecting various cell types and regulating the relative weights of synaptic inputs to the olfactory cortex, 5-HT finely tunes sensory information processing the olfactory cortex.

9. ÖSSZEFOGLALÓ

A szerotonin egy fontos monoaminerg neuromodulátor, amely számos fiziológiás és patológiás folyamatban kiemelt szerepet játszik. Különböző modalitások perifériás és centrális stációjában is képes a szenzoros funkciók szabályozására, de ennek sejtes mechanizmusai és szerepei a szaglórendszerben kevésbé tisztázottak. A szerotoninerg neuronok specifikus stimulációjának hatására a a szaglókérgi neuronok spontán aktivitása csökken, de szagingerekre adott válasza változatlan marad, illetve a sejtek egy része aktivitás fokozódással válaszol. Ennek a kettős szerotoninerg hatás mechanizmusainak tisztázása érdekében *in vitro* és *in vivo* elektrofiziológiai, optogenetikai, farmakológiai és immunhisztokémiai kísérleteket kombináltunk. Azt találtuk, hogy a szerotonin csökkentette a szaglókéreg principális neuronjainak aktivitását, de fokozta a gátlósejtekét. Ugyanakkor fokozta az afferens, de csökkentette az asszociációs rostok által kiváltott szinaptikus aktivitást preszinaptikus 5-HT_{1B} receptorok közreműködésével, amelyek blokkolása jelentősen csökkentette a szerotonerg fotostimuláció okozta aktivitás-csökkenést *in vivo*. Sejt- és útvonal-specifikus hatásai által a szerotonin fontos szerepet játszhat a szaglókérgi információfeldolgozás finomhangolásában.

10. ACKNOWLEDGEMENTS

The completion of this study could not have been possible without the expertise and help of my scientific supervisor Dr. Magor L. Lőrincz. I am very grateful for his patience, motivation and enthusiasm.

I would like to express my gratitude to Prof. Gábor Tamás for his help during my undergraduate project. I am also thankful to my former colleagues in Gábor' s lab for the inspiring environment, lots of help and all the fun we had in those years. It was a great time.

I would also like to thank Prof. József Toldi and Dr. Levente Gellért and all the members of the Department of Physiology, Anatomy and Neuroscience of the University of Szeged.

A special thanks goes to Sándor Lovas and Judit Herédi.

And to whom I owe the most are my parents and my sisters. There are no words to express how grateful I am for your constant support throughout my life. Thank you for always believing in me, even when I don't and for your endless love, from which I can always draw strength to move forward. I know how proud you would be of me, my beloved Dad.

I am deeply thankful to the Kreider family, and I am very lucky to have you in my life my Michèl. Your love and encouragement helped me to decide on finishing my PhD.

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