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Cellular and network mechanisms of neuromodulation in the olfactory cortex

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Cortical modulation by serotonin

The main challenge in revealing the role of serotonin (5-HT) in brain function is the large number of its receptors expressed both pre- and post-synaptically with some evidence of cell-type specificity combined with the lack of tools to induce physiological 5-HT release. Pharmacological application of serotonin can result in various effects, but whether such effects are also observable when serotonin is physiologically released was questionable. To this end we applied combined optogenetic and electrophysiological techniques in mice to reveal the function of serotonin in cortical function.

a) *Effect on spontaneous neuronal activity*

When serotonergic neurons were specifically stimulated using optogenetic techniques the spontaneous action potential output of most neurons recorded in the olfactory cortex was significantly altered. Of these, the majority decreased its firing, but a minority increased its firing. Importantly, the action potential half width of the latter group of neurons was significantly narrower suggesting cell-type specific differences in the effects observed. These results have been published in the Journal of Neuroscience (Lőrincz, Lottem and Mainen, 2016).

b) *Effect on sensory evoked neuronal activity*

In stark contrast to the prominent effects of serotonergic photostimulation on cortical spontaneous activity neuronal activity evoked by sensory stimulation was less affected. Specifically, the neuronal responses during odorant stimulation in the olfactory cortex are mildly affected by serotonergic photostimulation. However, when the effect on spontaneous activity is subtracted from these responses, the resulting pure odor responses are identical in control and photostimulated trials. This surprisingly specific effect could have important implications for serotonergic involvement in sensory coding. These results have been published in Journal of Neuroscience (Lőrincz, Lottem and Mainen, 2016).

c) Effect on performance

We developed a custom behavioral training and testing device based on a Raspberry Pi microcomputer. The system can be coupled to various devices delivering sensory stimuli and

can control an optogenetic device delivering light pulses and can be utilized for the training and testing of simple behaviors like Pavlovian conditioning and Go/NoGo tasks in head fixed mice. When mice learned the tasks (stable reaction time, 80% correct) we attempted optogenetic stimulation of the DRN. The preliminary results seem to be more complex than initially anticipated, more control animals will need to be tested to prove that the results obtained are genuine. We plan to perform these experiments using cues of various sensory modalities and compare the results obtained. We will publish these results at the beginning of 2018.

d) Mechanisms of the observed effects

To confirm the local release of serotonin upon photostimulation and reveal the putative cell-type specific features of the effects seen *in vivo* we performed *in vitro* whole cell patch clamp recordings from pyramidal cells and interneurons in various cortical areas of SERT-cre mice previously (2 weeks-1 year) infected with the light sensitive cation channel Channelrhodopsin. In agreement with the *in vivo* data a small number of fast spiking neurons (2/20) were depolarized upon the photostimulation of local serotonergic axons. This effect was fully blocked by an antagonist of 5-HT2a receptors. In a minority of regular spiking neurons (3/95) a small hyperpolarization could be detected upon the photostimulation of local serotonergic axons. This effect was fully blocked by an antagonist of 5-HT1a recptors. The experiments were repeated with viruses of various serotypes (AAV2/1 and AAV9) and also in slices obtained from TPH-ChR2 mice of various ages (3 weeks to 6 months) with similarly unreliable effects. The unreliable nature of the serotonergic effects *in vitro* do not allow us to draw any conclusions regarding the mechanisms of the serotonergic effects in the cortex,

however, these do demonstrate that local release of 5-HT can occur following local photostimulation of ChR2 expressing axons of 5-HT neurons.

In a different set of experiments we aimed to reveal the network mechanisms of serotonin photostimulation. To this end, SERT-cre mice previously (4 weeks) infected with ChR2 in the dorsal raphe nucleus (DRN) were implanted with multi-site laminar Si electrodes and optical fibers in the olfactory cortex. Local field potentials and multiunit activity was recorded from all cortical layers and current source density analysis performed. The main results are the following: i) local photostimulation leads to different responses compared to photostimulation of serotonergic neuron somata in the DRN and ii) responses evoked by intracortical microstimulation (i.e. associational stimulation) are substantially affected by 5-HT photostimulation, but responses elicited by the electrical stimulation of the olfactory bulb (i.e. afferent stimulation) is unaffected. These results are fully compatible with our previously published observations and provide a mechanism for the 5-HT effects in the olfactory cortex. These results will be be submitted for publication by the end of the present year.

Cortical cholinergic modulation

a.) Effect on rhythmic cortical activity

In the absence of sensory input, the mammalian brain exhibits a wide array of structured, spontaneous activity including the slow oscillation characterizing sleep and anesthesia. UP and DOWN states, the cellular substrates of EEG slow waves are synaptically generated events, but the mechanisms of their generation and pronounced rhythmicity have remained elusive. To reveal the role played by muscarinic cholinergic receptors in UP and DOWN states we locally blocked these receptors while monitoring the neocortical local field

potential and action potential output of neurons of lightly anesthetized mice. We found that the duration of both UP and DOWN states was shortened, the amplitude of high frequency oscillations reduced and the firing of neurons reduced. Hence, the cholinergic tone present under light anesthesia plays a role in shaping UP and DOWN states.

We found that when the cholinergic tone present in vivo is reinstated in neocortical slices rhythmic UP and DOWN states can be recorded and these closely resemble those recorded *in vivo*. Using this physiologically validated *in vitro* model we show that the initiation of UP states is driven by an electrophysiologically distinct subset of morphologically identified layer 5 neurons, which exhibit intrinsic rhythmic low-frequency burst firing at 0.2–2 Hz. This low-frequency bursting is resistant to the block of glutamatergic and GABAergic transmission but is absent when slices are maintained in a low Ca^{2+} medium (an alternative, widely used model of cortical UP/DOWN states), thus explaining the lack of rhythmic UP states and abnormally prolonged DOWN states in this condition. This study was published in the Journal of Neuroscience (Lőrincz et al, 2015) and identifies an important role for cell-type-specific neuronal activity in driving cortical UP states.

b.) Effect on thalamocortical activity

We investigated the effect of optogenetically stimulating the brainstem cholinergic system. To this end, we photostimulated the brainstem cholinergic nuclei (PPT and LDT) nuclei of ChAT-cre x Ai32 mice while recording spontaneous and sensory evoked single unit activity in the lateral geniculate nucleus of awake head-fixed mice. In 14 neurons tested we could not detect a significant effect of photostimulation. These results, combined with the very week $ChR2^{+}$ expression in the thalamus in these mice led to the conclusion that ChR2 expression was not sufficiently strong to activate brainstem thalamic projecting cholinergic neurons. We will attempt similar experiments using a different ChAT-cre line.

Publications

[*] equal contribution

Lőrincz ML, Gunner D, Bao Y, Connelly WM, Isaac JT, Hughes SW, Crunelli V (2015) A distinct class of slow (~0.2-2 Hz) intrinsically bursting layer 5 pyramidal neurons determines UP/DOWN state dynamics in the neocortex. *J Neurosci* 35(14): 5442-58

Lőrincz ML[*], Lottem E[*], Mainen ZF (2016) Optogenetic Activation of Dorsal Raphe Serotonin Neurons Rapidly Inhibits Spontaneous But Not Odor-Evoked Activity in Olfactory Cortex. *J Neurosci* 36(1):7-18

Lőrincz ML, Adamantidis AR (2016) Monoaminergic control of brain states and sensory processing: Existing knowledge and recent insights obtained with optogenetics. *Prog Neurobiol* 151:237-53

Lőrincz ML[*], Crunelli V[*], Connelly WM, David F, Hughes SW, Lambert R, Leresche N and Errington AC. Dual function of thalamic low-vigilance state oscillations: information transfer and plasticity. *Nat Rev Neurosci* (accepted for publication)

McCafferty C, David F, Venzi M, Orbán G, Lambert RC, Leresche N, Di Giovanni G, **Lőrincz ML** and Vincenzo Crunelli. A novel cortico-thalamo-cortical network oscillation lacking widespread thalamic post-inhibitory rebound bursts. *Nat Neurosci* (minor revision requested)