Serotonin 5-HT1A Receptors Modulate Neural Rhythms in Prefrontal Cortex and Hippocampus and Prefronto-Hippocampal Connectivity in Alert Mice

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Abstract. The serotonergic system plays a crucial role in cognition and is a target of many psychiatric treatments. In particular, serotonin 5-HT_{1A} receptors 5-HT_{1A} receptors (5-HT_{1A}R) in the prefrontal cortex and hippocampus play key roles in learning, memory, behavioural flexibility and response inhibition. Here, we investigated how 5-HT_{1A} receptors influence neural network dynamics in the prefrontal cortex and hippocampus and prefronto-hippocampal functional connectivity in alert mice. We found that pharmacological stimulation of 5-HT_{1A}R with 8-OH-DPAT markedly reduces theta, beta and high gamma oscillations in both areas and weakens prefronto-hippocampal phase synchronization at theta and beta frequencies. Pharmacological inhibition of 5-HT1A receptors with WAY-100635 reduces theta and high gamma oscillatory activity but increases beta and delta oscillations. It also weakens prefronto-hippocampal phase synchronization at theta frequencies. These results reveal that prefronto-hippocampal neurodynamics are highly sensitive to 5-HT_{1A} manipulation and may be relevant for understanding the actions of psychiatric medication targeting the serotonergic system.

Keywords: Neural circuits · Neural networks · Neuropharmacology

1 Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized by serotonergic neurons of the midbrain raphe nuclei whose axons reach almost every brain structure. This widespread innervation allows a powerful modulation of brain activity and function, including cognition. The prefrontal cortex (PFC) and hippocampus (HPC) are two core structures for cognition and express densely 5-HT_{1A}R [1], [2]. Serotonin 5-HT_{1A}R are $G_{i/0}$-protein-coupled receptors that hyperpolarize neuronal membranes inhibiting neural spiking. They are expressed by both excitatory pyramidal neurons and fast-spiking inhibitory interneurons where they influence network activity [2]. Pharmacological manipulations of 5-HT transmission in the PFC and HPC have

highlighted a crucial role of 5-HT in cognition [3], [4], 5-HT_{1A}R being the receptors more thoroughly investigated. Excessive or insufficient 5-HT_{1A}R activation in PFC increases impulsivity and cognitive inflexibility [5] whereas abnormal hippocampal 5-HT_{1A}R activation causes learning and memory deficits [6], [7]. Due to its anatomical and functional organization, the serotonergic system has become the target of many pharmacological interventions to treat brain disorders. For example, many antipsychotic drugs are 5-HT_{1A}R agonists [8], 5-HT_{1A}R agonists display anxiolytic/antidepressant activity in animal models [9], whereas 5-HT_{1A}R antagonists reverse drug-induced cognitive deficits [7]. Here, we investigated the influences of a selective activation or inhibition of 5-HT_{1A}R with 8-OH-DPAT and WAY-100635, respectively, on network dynamics of the PFC and HPC and fronto-hippocampal functional connectivity in alert mice.

2 Materials and Methods

2.1 Experimental Subjects

C57BL/6 male mice (*n = 10*) were obtained from the local colony at the Barcelona Biomedical Research Park (PRBB) Animal Facility. Mice were 2-3 months old and weighed 20-25 g at the beginning of the experiment. Animals were housed in individual cages over the course of the experiments to avoid damage to their implant. Cages were maintained under controlled atmosphere (humidity of 55 ± 7 % and temperature of 22 ± 1 °C) and with a 12:12-h light–dark cycle. Food and water were available *ad libitum*. All procedures outlined in this work had authorisation granted by the PRBB Animal Research Ethics Committee and were carried out in accordance with the guidelines of the European Union Council (2003/65/CE) and Spanish regulations (BOE 252/34367-91, 2005).

2.2 *Surgical Procedure*

Mice were anesthetized (induction with ketamine/xylacine and maintenance with isofluorane 0.5-4%) and placed in a stereotaxic apparatus. Three tungsten electrodes 25 μm wide were lowered down into the medial PFC (AP: 1.5-2.0 mm; ML: ± 0.4 mm; DV: -1.7 mm from bregma) and three into the dorsal HPC (AP: -1.8-2.5 mm; ML: 1.3-2.3 mm; DV: -1.5 mm). Several micro-screws were screwed into the skull to stabilize the implant and the one on top of the cerebellum was used as a general ground. Electrodes were implanted with dental cement. During the 7-10 day recovery period, animals were extensively monitored and received both analgesia (buprenorphine) and anti-inflammatory (meloxicam) treatments.

2.3 *Electrophysiological Recordings and Pharmacology in Alert Mice*

Following post-surgical recovery, we recorded local field potentials (LFPs) in freely-moving mice exploring their own home cage. Recordings were implemented with the Open Ephys system (http://www.open-ephys.org/) at 0.1-6000 Hz and a sampling rate of 30 kHz. 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; 5-HT_{1A}R agonist) and WAY-100635 (5-HT_{1A}R antagonist) were obtained from Sigma/Aldrich. Drugs were diluted in saline and the pH corrected to be between 6-8. Drugs were first prepared in a concentrated solution and frozen at -20C. The day of the experiment, the solution was thawed and diluted before intraperitoneal administration.

2.5 *Histology*

Animals were sacrificed and the brains immediately extracted and frozen at -80º. Serial coronal sections of 30 μm thickness were cut through the entire brain using a criostat at −20 °C. The sections were stained with cresyl violet for the reconstruction of the electrode tracks.

2.6 *Data Analyses*

LFP signals were detrended, Notch filtered to remove 50 Hz artifacts and down-sampled to 1000 Hz with custom-written scripts in Python. Signals were then bandpass filtered at 0.1-600 Hz. Power spectra and spectrograms were constructed using the multi-taper method in MATLAB with the Chronux toolbox (www.chronux.org). Band-limited (2 Hz) functional connectivity analysis of each experiment was performed in MATLAB using three coupling measures (Pearson correlation, phase-locking value and phase-lag index) independently. Specifically, each coupling measure was computed across all PFC-HPC contact pairs over non-overlapping time windows (1s) [10]. In essence, Pearson correlation is a linear coupling measure that captures instantaneous (zero-lag) amplitude fluctuations of both areas. In contrast, phase-locking values quantify the average phase coupling between pairs of signals, thus including zero-phase (zero-lag) and non-zero phase contributions. Finally, the phase-lag index can be regarded as refinement of the phase-locking measure, where zero-phase contributions are discarded for the average computation. The frequency bands considered include delta (3-5 Hz), theta (9-11 Hz), beta (15-25 Hz), low gamma (30-50 Hz), and high gamma (50-80 Hz). We used Wilcoxon ranked test and Cohen's D [11] to test for statistical significance and corresponding effect sizes between baseline and drug-period samples (power and connectivity).

3 Results

Selective activation of 5-HT_{1A}R with 8-OH-DPAT at 1 mg/kg (*n = 7* mice) exerted complex spectral changes in the neural signals of the PFC and HPC that were not observed after a previous injection of saline. Overall changes were larger in HPC compared to PFC ($p<0.05$). In both areas, 8-OH-DPAT produced sharp decreases of theta waves accompanied by reductions in beta and gamma oscillations ($p<0.001$). The gamma decrease was particularly pronounced at high frequencies (>60 Hz) (Fig. 1). The 5-HT_{1A}R antagonist WAY-100635 at 0.5 mg/kg completely reversed 8-OH-DPAT′s effects in PFC ($p<0.001$). In HPC, it restored theta and beta power but only partially reversed the decrease of high gamma oscillations ($p<0.001$). Interestingly, it also produced a rebound increase in delta and beta oscillations in both areas ($p<0.001$). By contrast, the effects of WAY-100635 when administered alone were smaller than those after 8-OH-DPAT ($p<0.001$). Theta and high gamma oscillations were also reduced ($p<0.001$), especially in HPC, but less than after 8-OH-DPAT. WAY-100635 alone also increased delta and beta oscillations in both areas ($p<0.001$) (Fig. 1). These effects were not observed in the control group where only saline was injected (*n = 5*) (data not shown).

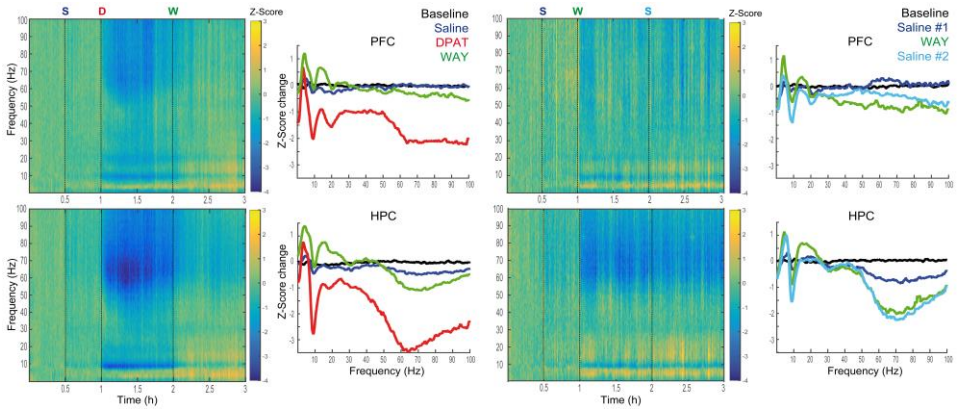

Fig. 1. Spectrograms showing the effects of 8-OH-DPAT 1 mg/kg (*n = 7 mice, n = 18 electrodes*) in the PFC (*upper left*) and HPC (*lower left*) and WAY-100635 0.5 mg/kg (*n = 4 mice, n = 9 electrodes*) in the PFC (*upper right*) and HPC (*lower right*). Spectrograms were normalized by the baseline (Z-scored) and averaged across animals. Lateral panels show the z-score change of power during the post-drug period with respect to baseline averaged across 15 min of recording during baseline (black), saline (dark and light blue), 8-OH-DPAT (red) and WAY-100635 (green).

We further investigated whether selective activation or inhibition of 5-HT_{1A}R influenced PFC-HPC functional connectivity. We analyzed three complementary connectivity measures that reflect different aspects of circuit dynamics related to phase synchronization: global, zero lag effects of the drugs that occur simultaneously in both areas (Pearson correlation); non-zero lag effects that quantify constant lags

between areas and likely reflect direct PFC-HPC connectivity (PLI); and a mix of zero lag and non-zero lag effects (PLV). Both, 8-OH-DPAT and WAY-100635 decreased functional connectivity at theta and beta frequencies, although this decrease was more pronounced after the former. Interestingly, 8-OH-DPAT increased, whereas WAY-100635 decreased, connectivity at low gamma ($p<0.001$). Importantly, these alterations occur for connectivity measures that include non-zero lag effects (PLV and PLI), suggesting that 5-HT_{1A}R play a role in the neural communication between the PFC and HPC independent from global effects (Fig. 2).

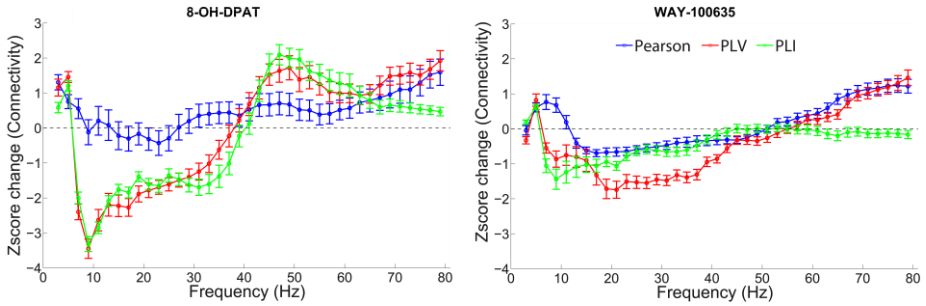

Fig. 2. Shown are the effects of 8-OH-DPAT 1 mg/kg (*left*) and WAY-100635 0.5 mg/kg (*right*) on prefronto-hippocampal functional connectivity. Pearson correlation, phase-locking value (PLV) and phase-lag index (PLI) were normalized by the baseline (Z-scored) and averaged across animals.

4 Discussion

We report that selective pharmacological activation or inhibition of 5-HT_{1A}R exert strong and complex influences on network dynamics in the PFC and HPC and prefronto-hippocampal functional connectivity. Several oscillatory bands (i.e. delta, theta, beta, and gamma) are disrupted during 5-HT_{1A}R abnormal neurotransmission suggesting a complex microcircuit effect that likely reflects the sophisticated pattern of expression of 5-HT_{1A}R in both brain structures. Interestingly, both 8-OH-DPAT and WAY-100635 exert greater changes in HPC compared to PFC, revealing that HPC is more sensitive to 5-HT_{1A}R manipulation. This work also unravels a specific role of 5-HT_{1A}R in shaping PFC-HPC functional connectivity independent from global effects. Overall, either excessive or insufficient 5-HT_{1A}R activation disrupts PFC-HPC network activity both locally and as a circuit. We conclude that psychiatric treatments targeting 5-HT_{1A}R may exert strong influences on PFC-HPC neurodynamics having an impact on cognitive processing shown to depend on this circuit.

Acknowledgements. We thank J. Chanovas and P. Nebot for excellent technical assistance. This work was supported by a NARSAD Young Investigator Award and a SAF2016-80726-R (AEI/FEDER, UE) to M.V. Puig.

References

1. Pompeiano, M., Palacios, J.M., Mengod, G.: Distribution and Cellular Localization of mRNA Coding for 5-HT1A Receptor in the Rat Brain: Correlation with Receptor Binding. J. Neurosci. vol. 12, no. 2 (1992) 440–53
2. Puig, M.V., Gener, T.: Serotonin Modulation of Prefronto-Hippocampal Rhythms in Health and Disease. ACS Chem. Neurosci. vol. 6, no. 7 (2015) 1017–1025
3. Leiser, S. C., Li, Y., Pehrson, A.L., Dale, E., Smagin, G., Sanchez, C.: Serotonergic Regulation of Prefrontal Cortical Circuitries Involved in Cognitive Processing: A Review of Individual 5-HT Receptor Mechanisms and Concerted Effects of 5-HT Receptors Exemplified by the Multimodal Antidepressant Vortioxetine. ACS Chem. Neurosci. vol. 6, no. 7 (2015) 970–86
4. Robbins, T.W., Arnsten, A.F.T.: The Neuropsychopharmacology of Fronto-Executive Function: Monoaminergic Modulation. Annu. Rev. Neurosci. vol. 32, no. 1 (2009) 267–287
5. Robbins, T.W.: Shifting and Stopping: Fronto-Striatal Substrates, Neurochemical Modulation and Clinical Implications. Philos. Trans. R. Soc. Lond. B. Biol. Sci. vol. 362, no. 1481 (2007) 917–932
6. Meneses, A., Perez-Garcia, G.: 5-HT1A Receptors and Memory. Neurosci. Biobehav. Rev. vol. 31, no. 5 (2007) 705–727
7. Ögren, S.O., Eriksson, T.M., Elvander-Tottie, E., D'Addario, C., Ekström, J.C., Svenningsson, P., Meister, B., Kehr, J., Stiedl, O.: The Role of 5-HT1A Receptors in Learning and Memory. Behav. Brain Res. vol. 195, no. 1 (2008) 54–77
8. Meltzer, H., Massey, B.: The Role of Serotonin Receptors in the Action of Atypical Antipsychotic Drugs. Curr. Opin. Pharmacol. vol. 11, no. 1 (2011) 59–67
9. Carr, G.V., Lucki, I.: The Role of Serotonin Receptor Subtypes in Treating Depression: A Review of Animal Studies. Psychopharmacology (Berl). vol. 213, no. 2–3 (2011) 265–287
10. Tauste Campo, A., Martinez-Garcia, M., Nácher, V., Luna, R., Romo, R., Deco, G.: Task-Driven Intra- and Interarea Communications in Primate Cerebral Cortex. Proc. Natl. Acad. Sci. U. S. A. vol. 112, no. 15 (2015) 4761–4766
11. Cohen, J.: A Power Primer. Psychol. Bull. vol. 112, no. 1 (1992) 155–9