

Topic: Midbrain dopaminergic and GABAergic neurons' responses to the aversive stimulus across alternating brain states of urethane anaesthetized rat - electrophysiological *in vivo* studies.

Supervisor: dr hab. Tomasz Błasiak (Department of Neurophysiology and Chronobiology)
tomasz.blasiak@uj.edu.pl

Background information:

Ventral tegmental area (VTA) is one of the main sources of dopamine (DA) in the mammalian brain. This structure is the centre of dopaminergic pathways and plays a key role in regulation of motivation (Wise, 2005), goal-directed behaviours, reinforcement learning or reacting to reward-related cues (Schultz, 1997; Morita, 2013). Better understanding of how DA circuits work and are modulated can contribute to explanation of mechanisms of dysfunctions laying at the root of some nervous system disorders such as addiction, anxiety, PTSD or some of depression symptoms (anhedonia or lack of motivation).

It has been shown that, occurrence of unexpected reward, reward-related cue or novelty causes phasic release of DA in VTA target structures (Goto et al, 2007). Those phasic changes in DA neurons activity lie at the root of forming reward prediction error (RPE) signal which encodes difference between expected and delivered reward - updating reward expectations and forming the basis for learning (Schultz, 2016). DA neurons also code response to the aversive or noxious stimuli by development of quick, short latency pause in firing. For a long time, it has been assumed that DA neurons code both reward and aversive stimuli homogeneously across the entire dopaminergic neurons population forming positive and negative prediction error signals respectively (Schultz, 1998; Ungless et al., 2004). However, study conducted by Brischoux et al. (2009) revealed subpopulation of VTA DA neurons that exhibit excitatory response to the footshock (electrical shock delivered to the animal's hind paw; aversive stimulus). Those neurons also appear to form distinct subpopulation located mostly in the ventromedial parts of VTA suggesting functionally different group of DA cells within the structure. Based on this results Brischoux and colleagues proposed existence of unique population of DA neurons that is excited by significant stimuli (positive or negative) and in this way encodes motivational salience of the stimuli rather than value. Recent study had shown that both level and pattern of VTA dopaminergic neurons activity is modulated by ongoing brain states under urethane anaesthesia. DA neurons' firing rate is elevated during SWA and their pattern of electrical activity is dominated by irregular firing and/or bursting whereas during cortical activation VTA DA neurons fire more regularly and level of their electrical activity decreases (Walczak and Błasiak, 2017).

Considering these findings, as well as our preliminary results, it is hypothesized that responses of VTA dopaminergic neurons to noxious stimuli are modulated by alternating states of the brain. Most interestingly, our preliminary findings indicate the existence of, so far not described, subpopulation of VTA DA neurons which can be differentiated based on their dynamically changing direction of response to an aversive stimulus.

The main question to be addressed in the project:

The main objective of this PhD project will be to investigate brain state dependent changes in the spontaneous and aversive stimuli evoked activity within the neuronal network of the ventral tegmental area (VTA) and the rostral-medial tegmental nucleus (RMTg). First part of

the project will be aimed to describe types and parameters of responses of VTA dopaminergic (DA) and GABAergic neurons' to the aversive stimulus (electrical footshock) observed across two alternating brain states (activation and slow wave activity) in urethane anaesthetized rats. Histological verification and reconstruction of the recordings sites will allow to produce spatial map along with biochemical identification of recorded VTA neurons. Lastly, the project will describe brain state dependence of spontaneous and aversive stimuli evoked electrical activity of the neurons in one of the major inhibitory inputs to VTA - rostral-medial tegmental nucleus (RMTg). Characterization of RMTg neurons' responses to the footshock will allow to further establish its involvement in encoding aversive event - related information by VTA DA neurons. Literature suggests the existence of two subpopulations of midbrain DA neurons, that can be differentiated on the basis of their stable responses to aversive stimuli (excitation or inhibition). However, our preliminary results indicate further heterogeneity of DA neurons within VTA in urethane anaesthetized rat. Our hypothesis is that heterogeneity of the midbrain dopaminergic neurons' reactions to aversive stimuli results, at least in part, from brain state dependent changes in strength of GABAergic input to VTA.

Information on the methods/description of work:

In order to verify stated hypothesis, electrophysiological and histological studies will be performed on urethane anaesthetized Sprague-Dawley male rats. Electrophysiological experiments will involve juxtacellular recordings of VTA dopaminergic and GABAergic neurons' spontaneous activity and responses to the aversive stimuli (electrical footshock) across cyclically alternating brain states. Juxtacellular labelling combined with immunohistochemical staining will allow to precisely localize and biochemically identify recorded neurons. This will result in creating spatial map of dopaminergic neurons' responses to the aversive stimuli. Spontaneous and footshock evoked responses of large representation of RMTg neurons will be recorded with the use of array of 32 electrodes. During the experiments the ongoing brain state (activation, slow wave activity) will be identified on the basis of spectral content of intrahippocampal local field potential recorded from the stratum lacunosum moleculare of the CA1 region. Literature and our preliminary results support the notion of existence of distinguishable dopaminergic neurons subpopulations within VTA responding in different manner to aversive stimuli and level of electrical activity that is modulated by ongoing brain state. Types and parameters of responses of recorded cells as well as their spontaneous activity will be further analysed with the use of custom-made scripts designed for proposed project.

Special requirements from the student:

Candidates with prior experience in at least one of research techniques listed below are encouraged to apply:

- extracellular recordings *in vivo* (rodents) using juxtacellular technique or multichannel microelectrodes arrays,
- neuronal tract-tracing and neuroanatomical techniques.

References:

Wise RA. (2005) Forebrain substrates of reward and motivation. *J. Comp. Neurol.*, 493(1), 115–121; Schultz W. (1997) Dopamine neurons and their role in reward mechanisms. *Curr. Opin. Neurobiol.* 7(2), 191- 197 (1997); Morita K, Morishima M, Sakai K, Kawaguchi Y. (2013) Dopaminergic Control of Motivation and Reinforcement Learning: A Closed-Circuit Account for Reward-Oriented Behavior. *J. Neurosci.*, 33(20), 8866-8890; Goto Y, Otani S, Grace AA. (2007) The Yin and Yang of dopamine release: a new perspective. *Neuropharmacology*, 53(5), 583–587; Schultz W. (2016) Dopamine reward prediction error coding. *Basic Research. Clin. Oral Implants Res.* 26, 42-42; Schultz W. (1998) Predictive reward signal of dopamine neurons. *J Neurophysiol.* Jul; 80(1):1-27; Ungless, MA, Magill, PJ, Bolam JP. (2004) Uniform Inhibition of Dopamine Neurons in the Ventral Tegmental Area by Aversive Stimuli. *Science.* 303, 2040-2042.; Brischoux F, Chakraborty S, Brierley DI, Ungless MA. (2009) Phasic excitation of dopamine neurons in ventral VTA. *Proc. Natl. Acad. Sci. U.S.A.* 106(12) 4894-4899; Walczak M, Błasiak T. (2017). Midbrain dopaminergic neuron activity across alternating brain states of urethane anaesthetized rat. *Eur. J Neurosci.*, 45: 1068-1077.