

Author's summary of professional accomplishments

1. Name and surname: Jolanta Orzeł-Gryglewska

2. Diplomas and academic degrees:

12th February 1981 – Master's Degree in Biology obtained at the Faculty of Biology and Earth Sciences at the University of Gdansk; the title of the thesis: „*Transformation of activation time in the effect of EEG signal desynchronisation evoked by complex visual stimuli*”.

25th September 1998 – Doctoral Degree in Biology, the Faculty of Biology, Geography and Oceanology, University of Gdansk, on the basis of the thesis: "*Electroencephalographic changes after lesions of the lateral hypothalamus in the rat*"

3. Professional Development

1st February 1981 – 30th September 1982 – Biology Specialist at the Department of Animal Physiology, Faculty of Biology and Earth Sciences, University of Gdansk.

1st October 1982 – 15th October 1998 – Assistant, Department of Animal Physiology, Faculty of Biology and Earth Sciences (from 1985 the name changed into the Faculty of Biology, Geography and Oceanology), University of Gdansk (in the period from 6th November 1984 to 17th February 1990, and from 9th May 1991 to 11th September 1991 maternity and parental leave).

16th October 1998 – 30th September 2010 – Assistant professor, Department of Animal Physiology, Faculty of Biology, Geography and Oceanology, University of Gdansk (from 23rd August 1999 to 27th December 1999 - maternity leave);

From 1st October 2010 until now – Senior Specialist, Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk.

4. Scientific achievements as indicated in the Article 16, Act no 2 of 14th March 2003 regarding scientific degrees and titles (Dz. U. nr 65, poz. 595 ze zm.):**A) Title of the scientific project:**

A cycle of publications: "The role of the midbrain ventral tegmental area in the system of hippocampal theta rhythm induction".

B) Original papers constituting the basis of the scientific achievement:

- [1]. Orzeł-Gryglewska* J., Jurkowlaniec E., Trojnar W. Microinjection of procaine and electrolytic lesion in the ventral tegmental area suppressed hippocampal theta rhythm in urethane-anesthetized rats. *Brain Res. Bull.*, 2006, 68, 295-309. doi: 10.1016/j.brainresbull.2005.08.026
IF - 1,684; points awarded by the Ministry of Science and Higher Education: 20
- [2]. Orzeł-Gryglewska* J., Kuśmierczak M., Jurkowlaniec E. Involvement of GABAergic transmission in the midbrain ventral tegmental area in the regulation of hippocampal theta rhythm. *Brain Res. Bull.*, 2010, 83, 310-320. doi: 10.1016/j.brainresbull.2010.09.006
IF - 2,498; Ministry points: 20
- [3]. Orzeł-Gryglewska* J., Kuśmierczak M., Majkutewicz I., Jurkowlaniec E. Induction of hippocampal theta rhythm by electrical stimulation of the ventral tegmental area and its loss after septum inactivation. *Brain Res.*, 2012, 1436, 51-67. doi: 10.1016/j.brainres.2011.12.003
IF - 2,879; Ministry points: 25
- [4]. Orzeł-Gryglewska* J., Kuśmierczak M., Matulewicz P., Jurkowlaniec E. Dopaminergic transmission in the midbrain ventral tegmental area in the induction of hippocampal theta rhythm. *Brain Res.*, 2013, 1510, 63-77. doi: 10.1016/j.brainres.2013.03.021
IF - 2,828; Ministry points: 25
- [5]. Orzeł-Gryglewska* J., Matulewicz P., Jurkowlaniec E. Theta activity in local field potential of the ventral tegmental area in sleeping and waking rats. *Behav. Brain Res.*, 2014, 265, 84-92. doi: 10.1016/j.bbr.2014.02.023
IF - 3,391; Ministry points: 30
- [6]. Matulewicz P., Orzeł-Gryglewska* J., Kuśmierczak M., Jurkowlaniec E., NMDA-glutamatergic activation of the ventral tegmental area induced hippocampal theta rhythm in anesthetized rat. *Brain Res. Bull.*, 2014, 107, 43-53. doi: 10.1016/j.brainresbull.2014.06.001
IF - 2,974; Ministry points: 20

* - corresponding author

Total Impact Factor of the aforementioned publications: 16,254.

IF values were given according to the year of publishing.

Total number of the points awarded by the Ministry: 140.

Ministry points were given according to the current ministry list of journals (2014).

Individual contribution of the author to each of the articles written by more than one person is described in the Appendix 3 (the list of published papers).

Declarations of all the co-authors, stating their individual contribution, can be found in the Appendix 5.

C) description of the scientific objectives and results presented in the papers forming the basis of the scientific achievement

Introduction

The hippocampal theta rhythm is a regular, almost sinusoidal electrical signal of the brain, which can be recorded in certain behavioural states. A particularly stable form of the rhythm, with amplitude reaching 1 mV, can be recorded from deep electrodes implanted in rodents or cats. Frequency of the rhythm oscillates between 3 and 12 Hz (Vanderwolf et al., 1975) depending on the level of wakefulness and the species. In the case of the human electroencephalographic recording, the term „theta” is used for the band 4-7.5 Hz; therefore, the animal theta, encompassing a wider range of frequency, was also named the „rhythmic slow activity” (RSA). The latter name, however, has not successfully replaced the term “theta” in the literature. Whether theta rhythm appears in the signal, depends on the neurotransmitter basis (Bland, 1986; Gemma et al., 1999) and the type of behaviour. With regard to these criteria, two types of theta rhythm have been distinguished: type I, present during exploratory locomotor activity (serotonin is probably the chemical basis here), and type II (cholinergic), appearing during the periods of freezing with intensified attention and during episodes of paradoxical sleep (REM) (Kramis et al., 1975). In rats under light urethane anaesthesia, theta rhythm appears periodically on its own, and if the anaesthesia is deeper, it can be induced by sensory stimulation such as tail pinch or stroking the animal’s back. Theta rhythm is considered to play the key role in the formation of memory trace in the phenomenon of long-term synaptic potentiation (Lynch, 2004). It has also been reported that theta is related to the mechanisms of spatial orientation learning and spatial memory in animals and humans (Cornwell et al., 2008, 2012; Kaplan et al., 2012; Lega et al., 2012; Sammer et al., 2007; Watrous et al., 2011; White et al., 2012). Because theta rhythm is related to such cognitive processes in wakefulness and because it also appears in the state in which consciousness is abolished (i.e. REM sleep), it is considered to be one of the most interesting symptoms of electrical activity of the brain.

The structures of the brain stem that are engaged in the induction and regulation of theta rhythm form the „theta synchronisation system” (Bland and Oddie, 1998; Vertes, 1981, 1982; Vertes and Kocsis, 1997; Vertes et al., 2004). The beginning of the system is commonly considered to be situated in the pontine reticular nuclei such as the nucleus reticularis pontis oralis (Nunez et al., 1991; Vertes, 1981; Vertes et al., 1993) and the pedunclopontine tegmental nucleus (Kinney et al., 1998; Nowacka et al., 2002; Vertes et al., 1993). These areas activate certain parts of the hypothalamus such as: medial and lateral mammillary nuclei (Alonso and Llinas, 1992; Kocsis and Vertes, 1994; Llinas and Alonso, 1992), the supramammillary nucleus (Kirk and McNaughton, 1993; Kirk et al., 1996; Pan and McNaughton, 2002) and the posterior hypothalamus (Bland and Oddie, 1998; Kirk, 1998;

Kirk et al., 1996). Subsequent pathway of activation leads through the medial septum of the forebrain, which is the nodal point in the route of these projections to the hippocampus.

There are also other structures that can be considered to be elements of the theta regulation system: the area of the dorsomedial hypothalamus (Woodnorth et al., 2003), the raphe nuclei (Nitz and McNaughton, 1999; Vanderwolf and Baker, 1986), fasciculus retroflexus (Valjakka et al., 1998), the ventral tegmental nucleus of Gudden (Bassant and Poindessous-Jazat, 2001; Kocsis et al., 2001), nucleus incertus (Nunez et al., 2006), and the habenula (Aizawa et al., 2013). The growing number of reports suggested that the midbrain ventral tegmental area (VTA) could be involved in either regulation or modulation of theta rhythm. The VTA and its ascending mesolimbic projections are primarily known to be directly related to the mechanisms of emotion and motivation as well as motor activation, and these functions may be aimed at receiving reward. However, the VTA is also connected with the septum and hippocampus (Oades and Halliday, 1987), and is involved in such reactions that are accompanied with theta activity (e.g. the exploration of the environment). Importantly, these theta-related reactions are also easily induced by electrical stimulation of the VTA (Trojniar and Klejbor, 1999). Moreover, chemical lesions of dopaminergic neurons (DA) in the VTA (performed with the use of 6-OHDA) changed the parameters of theta rhythm in sleep and waking in freely moving rats (Jurkowlanec et al., 2003; Sei et al., 1999).

The aims of my research were following:

- to show the involvement of the VTA in the system of theta rhythm induction;
- investigating the pathways that stem from the VTA and are potentially engaged in activation of the hippocampus;
- investigating the main neurotransmitter systems in the VTA in the context of theta rhythm regulation.

I used the standard experimental model of theta induction, which consists in sensory stimulation (pinching the tail base) in rats remaining in urethane anaesthesia. In such conditions only the cholinergic, type II, theta appears (characteristic for REM sleep and freezing with heightened attention, and because of the anaesthesia the highest power of the rhythm occurs in the range of 3-6 Hz.

In the first stage of the experiments (publication No. 1) I aimed to find out whether the VTA can indeed participate in the induction of theta rhythm when it is evoked by tail pinch. The following method was employed in my experiments to achieve this goal: just after obtaining a stable response to tail pinch (i.e. theta rhythm during the periods of 1-min stimulations at 10-min intervals), I performed local microinjections of procaine into the VTA, which blocked neuronal transmission in this area. As a result of this blockade in the VTA, induction of theta rhythm through tail pinch became impossible: the effect lasted for about 20 minutes. In the second series of experiments, I damaged the VTA by the use of electrolytic lesions. During the first hour post-lesion, the ipsilateral signal was significantly desynchronised and had low power, especially in the 3-6 Hz band. The sensory stimulation could evoke theta only in the hippocampus contralateral to the lesion, but even here the power in the 3-6 Hz band was largely decreased. On the 5th day post-lesion, the ability of theta to

appear during tail pinch recovered only slightly, but the power in this band derived from many frequency components existing in this range, none of which dominated. These results indicate that the VTA is a significant component of the theta synchronisation system, and temporary blockade (by procaine injections) or damage of this area makes the possibility of evoking theta by sensory stimulation impossible or much more difficult.

The VTA contains projection dopaminergic neurons (ca. 70%) related to the mesolimbic and mesocortical systems, and they form the A10 group of dopaminergic cells according to the classification by Dahlstrom and Fuxe (1964). The second significant group of cells in the VTA are the GABAergic cells (ca. 25%), a part of which sends projections to the nucleus accumbens and the prefrontal cortex. However, the majority of GABAergic cells in the VTA are the interneurons, which act as the inner inhibition mechanism limiting the activity of the dopaminergic neurons (Oades and Halliday, 1987). Another source of GABA transmitter in the VTA are the projections from the nucleus accumbens and the ventral striatum, which terminate mainly on the dopaminergic neurons (Bayer and Pickel, 1991). Because of their ability of somatodendritic releasing of the dopamine (Adell and Artigas, 2004; Kalivas, 1993) and because of the D2 receptors present on their surface, the dopaminergic neurons also possess the mechanism of auto-inhibition, which allows to limit any overactivity.

In the second stage of experiments (papers 2 and 4) I investigated the inner neural circuits within the VTA in the context of the phenomenon of theta induction. Dopaminergic neurons are inhibited by descending pathways and by tonic activity of the GABAergic interneurons. I made an attempt at abolishing this inhibition by microinjections of antagonists of GABA receptors into the VTA. Bicuculline microinjection (GABA_A receptors blocker) resulted in immediate appearance of synchronous theta rhythm with high amplitude, lasting for 30-40 minutes without any breaks. After phaclofen microinjection (GABA_B blocker) theta rhythm appeared with about 18-min latency, and in this case the theta was interrupted with short episodes of less synchronous hippocampal activity. This effect remained for around 30 minutes. An opposite effect was observed after muscimol microinjections (GABA receptors agonist): theta power gradually decreased during consecutive sensory stimulations, until it was replaced by delta power 1 hour after the injection. These data show the role played by the GABAergic system of the VTA in the regulation of theta rhythm: the role is related particularly to GABA_A receptors.

Overactivity of dopaminergic neurons increases dopamine release not only at the terminals but also in the somatodendritic area. Such outcome leads to D2 receptors activation on the dopaminergic cells, which limits the activation of these cells. In the next series of experiments I used local injections of cis-flupenthixol (D2 receptors blocker). At a certain concentration of this pharmacological agent, episodes of theta rhythm appeared after about 7 minutes of latency; the episodes were interrupted with short episodes of less synchronous hippocampal signal. The effect lasted for about 50 minutes. Similar was the effect of local injections of amphetamine, which increases dopamine release in the target areas of the VTA (Sulzer et al., 2005): 10 minutes after the injection theta rhythm episodes appeared and they were interrupted with short episodes of less synchronous hippocampal activity. This outcome could be observed for about 35 minutes. Particular episodes of theta rhythm were shorter by a half on the average than theta episodes after flupenthixol, but so were the episodes of

desynchronisation of the EEG signal. In summary, the results obtained in the second stage of experiments allow to conclude that intensification of dopaminergic transmission in the VTA (achieved directly through influence over dopaminergic cells or indirectly through limiting GABAergic inhibition) results in induction of theta rhythm in the hippocampal signal.

In the next set of experiments (paper No. 3) I focused on describing the pathways beginning at the VTA level and participating in theta rhythm induction. Neuronal activation coming from the VTA can reach the hippocampus through two routes: direct (dorsally, through the fornix and the cingulate, or ventrally, through the entorhinal cortex) and indirect, encompassing the connections in the medial and lateral forebrain septum (Gasbarri et al., 1997; Oades and Halliday, 1987; Scatton et al., 1980; Verney et al., 1985). In order to obtain theta rhythm, electrical stimulation applied to the VTA was used in these experiments. The medial forebrain septum was blocked by procaine (a non-specific blocker of neuronal transmission). The assumption was that if the theta inducing pathway from the VTA reaches the hippocampus directly, the blockade in the septum should not influence the appearance of theta during the VTA stimulation. However, the septal blockade made induction of theta by the means of the VTA stimulation impossible for about 20 minutes, which indicates that the septum is the necessary element of the VTA-hippocampal connection in the context of theta. To investigate which structures are activated when theta activity is evoked at the level of the VTA, I employed immunohistochemical methods. Analysis of Fos protein expression (performed on brain slices of stimulated animals) showed some level of increase in c-fos activity in the structures typically involved in theta induction (hypothalamus, medial and lateral septum, diagonal band of Broca, dentate gyrus and layers CA1-CA3 of the hippocampus). However, these were not statistically significant changes, perhaps because of the generally lower activity in the nervous system caused by anaesthesia. It is also possible that Fos activity is not related to changes in the electrical signal: in the control group, after stimulation of the zona incerta, there was 200% increase in Fos activity in the hippocampus, but power spectrum of the hippocampal signal was not affected by the stimulation. This part of the results (described in paper 3) suggests that the VTA participates in the induction of theta and that its projections join the main theta-related ascending route, where the septum is the main node between the pons and the hippocampus.

The experiments described so far were performed in urethanised rats, and the subsequent study (paper No. 5) was carried out in freely moving animals, and theta rhythm was observed here as naturally appearing signal during active waking and REM sleep. The purpose of the study was to analyse the spectrum of field potential in the VTA during theta appearance in the hippocampus, as well as to find time correlation between maximal power values of the signal recorded in these two structures. From the periods of waking voluntary movements and paradoxical sleep, which are connected to theta rhythm, I collected signal samples from the VTA and hippocampus and I subjected them to spectral analysis. I found that the maximal power in signals from both structures was found in the theta band, and the frequency corresponding to this power was significantly correlated between these two centres; I also found that the signals were cross-correlated in the theta band. Signal from the VTA preceded the hippocampal signal by 10 ms. Particularly strong correlation was present in the 6-9 Hz band, the correlation was also higher for theta rhythm from paradoxical sleep than the one from exploration of the environment. The observed results indicate precise relationship

between the electrical signals in the theta band from both structures (the VTA and the hippocampus) during exploration and paradoxical sleep and they constitute another piece of evidence confirming that the VTA belongs to the structures regulating the theta rhythm.

In the light of the results, it became necessary to assess the influence of the glutamatergic system over the VTA in the context of changes in the hippocampal signal. The VTA receives projections from many structures related to the theta rhythm (the habenula, oral pontine reticular nucleus, pedunculo pontine tegmental nucleus, laterodorsal tegmental nucleus, raphe nuclei), which use glutamatergic transmission to a considerable extent (Geisler et al., 2007; Geisler and Wise, 2008; Kalivas, 1993). Therefore, glutamatergic neurons could be a potential source of activation transmitted to the hippocampus through the VTA. In the last stage of experiments (paper No. 6) I made an attempt to describe the role of the glutamatergic receptors in the VTA in the processes of theta induction. Local microinjections of N-Methyl-D-aspartic acid (NMDA, a specific agonist for glutamatergic receptors) performed in rats remaining under urethane anaesthesia induced long-lasting (about 30 minutes) episodes of theta rhythm after 12 minutes of latency. When a blocker of NMDA receptors (MK-801) was injected, it was not possible to induce theta rhythm with sensory stimulation for 30 minutes post-injection, and during the subsequent 20 minutes the stimulation could induce theta but with a latency of over a dozen seconds. Thus, the results show that the glutamatergic centres of the pons, which are engaged in the induction of theta, can impose their influence also through connections with the VTA. The VTA then dopaminergically activates the neurons in the septum of the forebrain, and the final result of the whole route is appearance of theta rhythm in the hippocampus.

In summary, the results allow to indicate the following:

- The VTA belongs to the structures regulating theta rhythm;
- The VTA modifies theta rhythm through dopaminergic projections to the septum of the forebrain, and not through direct projections with the hippocampus;
- The VTA, similarly to the supramammillary nucleus or posterior hypothalamus, can constitute a relay between the reticular pontine nuclei and the septum in the ascending route of hippocampal theta rhythm activation;
- It is possible that within the VTA, motivation-related behaviour is linked with theta-related activity – the signal important for central cognitive and memory processes.

The studies mentioned here permitted explanation of the functioning of the neural circuits within the ventral tegmental area – the “game” between the transmitter circuits (interaction of excitatory and inhibitory systems with dopaminergic VTA neurons) in relation to modifying the hippocampal signal has become clearer. The studies also extended the knowledge on the ascending system of theta rhythm regulation.

References:

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D) Overview of other scientific achievements

I commenced my work as a researcher when I was a fourth year student at the Faculty of Biology and Earth Sciences at the University of Gdansk. The scope of my thesis, supervised by Prof. Juliusz Tokarski at the Animal Physiology Department, related to the dynamics of reactions at the level of the central nervous system. I participated in the implementing new methods in the EEG Laboratory. One of my tasks was to perform experiments and analysis of relation between time of exposure of a complex visual stimulus (a colourful abstract picture) and the central arousal time expressed as alpha rhythm blockade in human EEG. The results were presented at the Congress of the Polish Physiological Society in 1981.

After I finished my studies (1981), I was employed at the the Animal Physiology Department at the University of Gdansk. In the beginning I continued experiments concerning inertia effects of a reaction at the level of the central nervous system. In 1984 I began cooperating with Dr Weronika Trojnar and Dr Edyta Jurkowlanec at the Department of Human Physiology at the The Medical University of Gdansk, and I participated in research devoted to changes in behaviour and EEG recording after lesions of the lateral hypothalamus

(LH) in rats. One of the most prominent symptoms observed after this lesion is reduced amount of sleep (hyposomnia), which may last longer than other behavioural disturbances (such as adipsia and aphagia). Hyposomnia in this case is also connected to akinesia, i.e. reduced motor activity in experimental animals. In the following years I engaged in analysis of neuroanatomical and neurotransmitter basis of the hyposomnia resulting from electrolytic lesions of the LH; I also analysed the changes in movements occurring in this syndrome. We came to a conclusion that despite the reduction in the general amount of locomotor activity, the rats showed increase in minor postural adjustments and perioral movements. I took part in creating a new actographic method allowing to record locomotor activity that is changed in the aforementioned way. At the same time we tried to find the answer to the question whether the lack of sleep in the light phase (of the 24 hrs period), seen after LH lesion, could be compensated by increase in sleep in the dark phase. Twenty-four recordings of EEG showed that there is no such compensation, and the intensity of hyposomnia was similar in 1-hour and 12-hour recordings during the light phase. The control group, which was subjected to damage of the zona incerta, i.e. one of the structures neighbouring with the LH, did not display any disturbances of sleep. These results were presented at meetings of scientific societies during the years 1990-1994.

Hyposomnia is observed after lesions performed at various antero-posterior levels of the LH and its occurrence does not depend on the size of the damage. The LH constitutes a part of the medial forebrain bundle (MFB), which is an important neural pathway connecting the structures within the forebrain and diencephalon. The LH also contains neurons that are potentially important for sleep-waking regulation. Because of these complex anatomic issues, one of the most significant points of my research was to investigate whether the hyposomnia is caused by the damage to the LH neurons or to the fibres passing through this area. To find the answer, I performed lesions of cytotoxic type localised in the LH and I did not observe changes in the amount of sleep and wakefulness, which proves that the hyposomnia is rather the result of damage to the MFB fibres, not cells. Locomotor activity in animals is connected to the mesolimbic and nigrostriatal dopaminergic systems, and the ascending pathways of these systems pass through the MFB. Suppressing these systems' activity by intraperitoneal administration of haloperidol (a dopaminergic antagonist, mainly of D2 receptors) does not reduce the hyposomnia after the LH lesion. This suggests that the dopaminergic system is not one of those systems that would participate in the sleep reduction occurring after lesions of the LH. In my subsequent experiments, I showed that it is the GABAergic system that plays a role in the development of hyposomnia. Muscimol injections (GABA_A receptor agonist) into the LH induce hyposomnia that has similar intensity as in the case of electrolytic damage of this structure. Muscimol effect is blocked by bicuculline (GABA_A receptor antagonist). A part of the results concerning electroencephalographic effects of the LH damages was analysed in detail and presented as the main part of my doctoral thesis titled *Electroencephalographic changes after lesions of the lateral hypothalamus in rat*, supervised by Prof. Weronika Trojnar at the University of Gdansk. I defended the thesis in September of 1998 at the Faculty of Biology, Geography and Oceanology at the University of Gdansk. In 2000 the thesis received Prof. Juliusz Narebski Prize, the award given by the Polish Sleep Research Society. The thesis was also published as articles in 2 parts: in *Acta Neurobiologia Experimentalis* and in the Journal of the Polish Sleep Research Society, *Sen*.

After I received my PhD degree, i.e. on 15th October 1998, I was employed at the Department of Animal Physiology at the University of Gdansk, and I continued scientific research under supervision of Prof. Weronika Trojniar. One of the projects I participated in pertained to plasticity of the central activating processes in conditions of stimulation and damages to the mesolimbic system. In the first cycle of experiments, I investigated how repeated (14-day) electrical stimulation of the VTA, which results in feeding or locomotor reaction in rats, would influence power spectrum of the local field potentials recorded from the same area. The effects of this repeated stimulation included an increased tendency toward desynchronisation in the VTA field activity during wakefulness, a tendency of slow wave sleep to become shallow, and disturbance in paradoxical sleep pattern. These changes persisted until the second day after the stimulation had been completed.

Using the abovementioned method of obtaining feeding or locomotor reaction through the VTA electrical stimulation, our team also investigated functional relationship between the VTA and the pedunculopontine tegmental nucleus (PPN), which is known to send projections to the VTA. When procaine was injected into the PPN, we could observe significant elevation of threshold for both behavioural reactions induced by the VTA stimulation; local injection of GABA_A receptor blocker gave a similar, albeit weaker effect. These results show that the PPN and VTA belong to the same circuits engaged in the regulation of psychomotor activation, and that the VTA is activated by the PPN.

I also participated in experiments investigating relationship between the dopaminergic system and interindividual differences in the intensity of exploratory locomotor reactions performed in new environment; these reactions are related to the level of dopamine and activation of the hypothalamic-pituitary-adrenal axis. One of our methods consisted in estimating the number and morphology of the dopaminergic cells in the hypothalamus of rats divided into high and low reactivity groups. After classifying the animals into the two locomotor groups, we identified hypothalamic cells containing tyrosine hydroxylase (TH+ cells). We found out that in the rats showing higher exploratory activity the number of TH expressing cells in the hypothalamus was higher and their size was bigger (measured on the basis of area, axes and diameter). Highly reactive individuals show higher propensity for addiction, and perhaps the histological features we observed can be the marker of such a tendency.

The other direction of experiments encompassed analysis of the hippocampal theta rhythm: we analysed neurotransmitters and electroencephalographic features related to the theta rhythm evoked by sensory stimulation in the conditions of urethane anaesthesia. We were particularly interested in the functional connections between the pontine structures engaged in generating theta rhythm. As mentioned before, the multi-level system of theta rhythm regulation begins in the pontine reticular nuclei, which are responsible for activating certain hypothalamic areas. The subsequent pathway of theta regulation passes through the forebrain medial septum, which is a node for ascending pathways reaching the hippocampus. Among the initial elements of theta regulation system, two reticular nuclei are the most significant: the oral pontine reticular nucleus (RPO) and the pedunculopontine tegmental nucleus. We investigated the role of each of the nuclei and functional relation between them, and we obtained the following results:

- stimulation of the RPO and PPN through local injections of carbachol leads to theta rhythm appearance in hippocampal recording; however, electrical stimulation is effective in this way only when applied to the RPO. In our experiments, performed in rats under urethane anaesthesia, the RPO was stimulated chemically and electrically, and at the same time we performed procaine inactivation of the PPN in the contralateral hemisphere. The PPN inactivation did not block theta induced by intra-RPO carbachol injection, but it completely blocked the theta evoked through electrical stimulation of the RPO. The results indicate that at the level of the pons, the PPN plays an important role in the generation of theta rhythm, although its effectiveness may depend on the method of theta induction (cholinergic versus electrical activation of the RPO).
- procaine injections into the RPO showed that sensory induction of theta rhythm is impossible when it is the posterior part of the nucleus that is chemically blocked (experiments in the conditions of urethane anaesthesia). When procaine was microinjected into the anterior part of the RPO, there was either no blockade of sensory-induced theta or an appearance of long lasting theta episodes without any need of sensory stimulation. These results show functional differences between the anterior and posterior parts of the RPO concerning the mechanisms of theta rhythm induction.
- the PPN activity may be modulated by the serotonergic system, particularly by the raphe nuclei, which can influence the PPN neurons through various classes of serotonergic receptors. We found that microinjections of serotonin agonists (8-OH-DPAT and 5-CT) into the PPN induced long lasting episodes of theta with a short latency (performed in urethane anaesthetised rats). The conclusion is that local inhibition which is achieved through serotonin and which probably concerns the cholinergic neurons in the PPN acts as a trigger for hippocampal theta.

Apart from the main direction of experiments, i.e. the one involving the morphological aspects of theta activity, I also participated in research into frequency-amplitude dynamics of the hippocampal signal in the conditions of theta induction. Analysis of the changes in frequency and power of the theta rhythm occurring in the course of time showed that their values increased rapidly at the beginning of the stimulation, and then gradually decreased with the time of stimulation. Changes in frequency of the theta rhythm were appearing before the changes in power. The observed dynamics seems to be characteristic of theta rhythm since similar changes are not observed in the case of other EEG signal components in the hippocampus after theta is pharmacologically blocked. Another investigated issue concerned involvement of gap junctions in the appearance of synchronous electrical activity in the hippocampus. Gap junctions (electrical synapses) provide fast communication among cells. Earlier reports on the role of gap junctions in theta rhythm induction were based on *in vitro* experiments performed in isolated hippocampal slices. Our team performed such experiments in urethanised rats and showed that intraperitoneal injection of carbenoxolone (blocker of electrical synapses) induced slow but complete suppression of hippocampal theta rhythm evoked with sensory stimulation. This effect consisted of gradual reduction of the power at the theta band and of increase in the delta power during consecutive sensory stimulations. Thus, gap junctions can be considered as an important element in the induction of synchronous hippocampal activity.

As a member of the Sleep Laboratory and an academic teacher, I have also become interested in the wide issue of sleep-waking cycle regulation: consequences of sleep deprivation and the involvement of dopamine in the regulation of the cycle have been the most prominent topics in this part of my research. As a result of investigating the literature within the field, I wrote two review papers. The first review paper (2010), based on many long-term studies, focused on the finding that sleep limitation leads to serious impairments in functioning. These impairments include worsened vision and perception, weakened concentration, slowed reactions, increased number of mistakes and decreased precision of performed activities. Sleep deprivation also leads to schematic thinking, making wrong decisions, emotional disturbances and worsened memory. The exhaustion occurring after 20-25 sleepless hours causes such disturbances in performing tasks as is observed after ethanol intoxication at the level of 0.10% in blood. In the second review paper (2014), I presented facts confirming the involvement of the mesostriatal and the mesocorticolimbic systems in the regulation of sleep and wakefulness. I discussed the influence of dopamine, its agonists and the dopamine transporter over the characteristics of sleep and waking, and sleep disturbances accompanying dysfunctions of the dopaminergic systems. I also elaborated on the issue of the involvement of dopamine in REM sleep and induction of dreams.

Currently, our team investigates the role of the VTA in the regulation of electrical activity of the hippocampus in rats subjected to aversive conditioning. This experimental model allows to achieve theta rhythm in the state of high activation in the central nervous system during wakefulness, and this possibility concerns both immobility (“freezing”) and voluntary locomotor activity (avoidance). This way both types of theta rhythm can be investigated: the cholinergic and the non-cholinergic kind. In subsequent experiments I intend to investigate what is the role of the PPN projections into the VTA in the mechanisms of theta rhythm induction.



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