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The posterior hypothalamic area as an independent generator of rhythmic theta oscillatory activity

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Abstract

Theta rhythm is one of the most prominent examples of rhythmic oscillatory activity in mammalian brain and it is generated mainly in structures of the limbic cortex, including the hippocampal formation. In the 1970s it was shown that theta rhythm may be also recorded in diencephalic region including the posterior hypothalamic nuclei and supramammillary nucleus, together considered as the posterior hypothalamic area (PHa). For decades it was stated that local posterior hypothalamic oscillatory activity is controlled by the descending inputs going to the PHa from the septohippocampal system. However, the latest studies indicated that theta rhythm can be recorded in deafferented PHa in vitro preparations which indicates that the posterior hypothalamic area should be considered as an independent of the other brain structures theta generator. In subsequent research the neurochemical and cellular basis of PHa theta were examined in both *in vivo* and *in vitro* conditions. In the light of multiple evidence obtained in these studies, it is the author’s intent to summarize the data concerning the role of the posterior hypothalamic area in hippocampal theta rhythm generation as well as the ability of that brain structure to independently generate theta rhythmicity.

Keywords: theta rhythm, hippocampal formation, hypothalamus, posterior hypothalamic nuclei, supramammillary nucleus

Introduction

The theta rhythm, being one of the model examples of oscillation and synchronization of neuronal activity occurring in the central nervous system (CNS), has been the subject of research since the early 1950s (Buzsáki, 2002). Today, this rhythm is one of the most studied patterns of rhythmic electroencephalographic (EEG) activity recorded in mammalian brain. This type of EEG activity is generated in many regions of the central nervous system, but the main sites of its generation are the structures of the limbic system, including

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the hippocampal formation (HPC; Bland, 1986). Theta rhythmic activity in humans observed during extracranial EEG examination is recorded from medial regions of the frontal lobe and temporal regions of the cortex. This EEG phenomenon is characterized by a frequency in the range from 4 to 7 Hz and amplitude reaching 80 μV (Mitchell *et al*., 2008). Human theta oscillatory activity is observed during many physiological processes, such as spatial navigation (Caplan *et al*., 2003; Chrastil *et al*., 2022), paradoxical sleep (Cantero *et al*., 2003; Girardeau and Lopes-Dos-Santos, 2021), language processes (Cross *et al*., 2022; Mitchell *et al*., 2008), or performing arithmetic operations and tasks based on working memory (Raghavachari *et al*., 2001).

Interestingly, rhythmic oscillatory activity in the theta band is also observed in human EEG recordings in pathological conditions, thus it may be considered a non-specific indicator of CNS disorders (Kowalczyk *et al*., 2013). One of the most prominent examples of such theta co-occurrence is the inhibition of theta rhythm in patients with temporal lobe epilepsy (TLE). Between seizures, significant additional neuronal firings, called interictal spikes, are observed in EEG recordings taken from patients with the TLE. Interestingly, immediately after those spikes, the power of theta rhythm recorded from anterior hippocampus and the entorhinal cortex (EC) is reduced, and during the prolonged between-spikes periods the inhibitory effect on theta rhythm might sustain (Clemens *et al*., 2021; Fu *et al*., 2018). These observations suggest that the theta inhibitory effect of interictal spikes may be a significant biomarker of the TLE. Another interesting example of changes in theta band oscillations can be observed in EEG recordings taken from patients with Alzheimer's disease (AD). It was shown that theta rhythm increases in its power in AD patients and seems to be more regular compared to the EEG recording of healthy subjects. Furthermore, AD patients show theta frequency-dependent abnormalities in EEG synchrony (Baik *et al*., 2022; Gallego-Jutglà *et al*., 2015). Interestingly, abnormalities in theta rhythm also occur in EEG recordings of patients with posttraumatic stress disorder (PTSD). During the performance of more attentionally-demanding tasks, patients with the PTSD exhibited 'late-stage' theta hyperconnectivity observed in right parietal cortex which contributed to their diminished mental flexibility (Dunkley *et al*., 2015; Toll *et al*., 2020).

Theta rhythm in rodents

The results of human studies presented above, highlight the importance of the theta rhythm in both physiological and pathological states of the CNS, and justify the ongoing research on that oscillatory activity (Kowalczyk *et al*., 2013). Most of those theta rhythm investigations, carried out since the early 1950s, were conducted with the use of rodent models, specifically rats. Rhythmic oscillations in the theta band generated in the HPC of these mammals exhibit a larger frequency band than in humans, ranging from 3 to 12 Hz. Moreover, theta rhythm in rodents is characterized by an almost sinusoidal pattern of regular waves, with a high amplitude of 1 to 2 mV in deep brain recordings (Bland, 1986). In this group of animals, theta activity is associated with physiological processes such as spatial navigation (Hasselmo *et al*., 2002), performance of voluntary movements (Li *et al*., 2021), long-term synaptic potentiation (LTP; Huerta and Lisman, 1995), and sensorimotor integration (Bland and Oddie, 2001). Similar to humans, the hippocampal formation is the main limbic structure in which this EEG activity is recorded and studied in rodents (Kowalczyk *et al.*, 2013).

Considering the different neurochemical basis and the correlation with the animal’s behavior, the hippocampal theta rhythm in rodents was divided by Vanderwolf into two types: type I and type II (Vanderwolf, 1969). Type I theta rhythm is generated when an animal is performing voluntary movements, such as walking, running, standing on hind legs, swimming or adjusting posture (Bland, 1986; Vanderwolf, 1969). During the above-mentioned types of behavior, the local hippocampal field potentials occur in the form of waves with a frequency from 6 to 12 Hz. The described type of theta rhythm is resistant to the application of even high doses of atropine sulfate (a cholinergic, muscarinic receptor antagonist). Therefore, that movement-dependent type of theta rhythm has a noncholinergic nature. There are studies suggesting that the generation of type I theta rhythm is related to serotonergic transmission (Bland and Colom 1993).

In contrast, type II theta oscillatory activity, is observed during the absence of the animal’s voluntary movements or occasionally, while performing automatic behaviors, such as licking, chewing or cleaning the fur. Rhythmic theta oscillations recorded during an animal's immobility occur in the frequency range from 4 to 9 Hz. It was documented that injection of atropine sulfate results in the abolition of type II theta rhythm, indicating its cholinergic basis (Bland, 1986). This type of theta rhythm can also be recorded in animals under urethane anesthesia, as it is not suppressed by the administration of most anesthetics. In addition, it was also shown that deafferented hippocampal formation preparations maintained *in vitro* are capable of generating type II theta activity after administration of cholinergic receptor agonists such as carbachol or acetylcholine (Kowalczyk *et al.*, 2013).

Both types of theta rhythm are recorded from hippocampal formation as a result of characteristic features of its neurons. Those neurons are called theta-related, because they discharge in a specific relationship with the theta rhythm observed extracellularly in the HPC (Fig.1). Widely accepted theta-cell classification was introduced by Colom and Bland in the 1980s. These authors found that the neurons associated with the theta rhythm in the hippocampal formation could be divided into two distinct populations, referred to as theta-on and theta-off cells. Theta-on cells increase their activity when HPC theta rhythm is present, whereas theta-off cells decrease their activity during ongoing hippocampal theta synchronization. Each cell within these two subtypes, can discharge in one of two characteristic patterns. The first pattern, referred to as phasic, is characterized by spike discharges that occur in constant phase correlation to each theta wave cycle. The second pattern, referred to as tonic, is characterized by irregular or regular cell discharges with no consistent relationship to each theta cycle. Both theta-on and theta-off cells include phasic and tonic subtypes (Fig.1; Colom and Bland, 1987; Bland and Colom, 1993). Furthermore, it was shown that theta-on phasic neurons are characterized by the ability to generate rhythmic membrane potential oscillations (MPOs; Artemenko, 1972). The extracellularly recorded theta rhythm is closely correlated with MPOs, which is why they are also referred to as the intracellular theta rhythm (Bland *et al.*, 2002). It was accepted, that the spatial summation of fluctuations in membrane potentials of hippocampal theta-on phasic cells is observed as a local field theta oscillation (Bland and Colom, 1993).

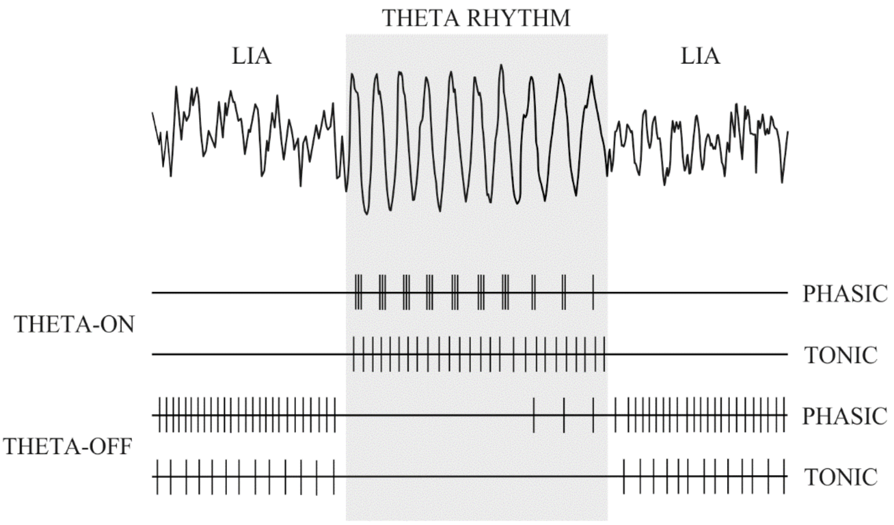

Figure 1. A diagrammatic representation theta-related cells classification. The upper row represents an example of theta rhythm (marked with grey rectangle) and large irregular activity (LIA) recording. The next four rows represent firing patterns of different subtypes of theta cells. Details in the text (Colom and Bland, 1987; Bland and Colom, 1993; modified).

Ascending brainstem-hippocampal synchronizing pathway

The theta field potentials described in the previous section are generated in the hippocampal formation and other structures of the limbic system as a result of extrinsic inputs received from the so-called the ascending brainstem-hippocampal synchronizing pathway or ascending synchronizing system (Fig.2). This pathway comprises numerous structures, located within the pontine region, diencephalon, and basal parts of the forebrain. The process of ascending pathway activation begins with the arousal of specific brainstem nuclei, which send continuous excitatory information through the posterior hypothalamic area to the medial septal area, from where the impulsation is transmitted to the limbic system structures, including the HPC (Kowalczyk *et al.*, 2013).

Pontine nuclei

As it was stated previously, the process of activating the ascending brainstem-hippocampal system begins within the specific brainstem nuclei (Fig. 2): the nucleus reticularis pontis oralis (RPO) and the pedunculopontine tegmental nucleus (PPT; Woodnorth *et al.*, 2003). Early studies investigated afferents of the PPT by using the examination of retrograde transport combined with the immunohistochemical examinations. These investigations revealed that the PPT provides a widespread innervation to the caudal diencephalon and basal parts of the forebrain (Hallanger *et al.*, 1987). Subsequent mapping studies showed that the PPT sends prominent projections to the RPO (Semba *et al.*, 1990) which, in turn sends its afferents to the caudal diencephalic region, mainly the supramammillary nucleus (SuM; Vertes *et al.*, 1986). In addition to extensive connections with other structures of the

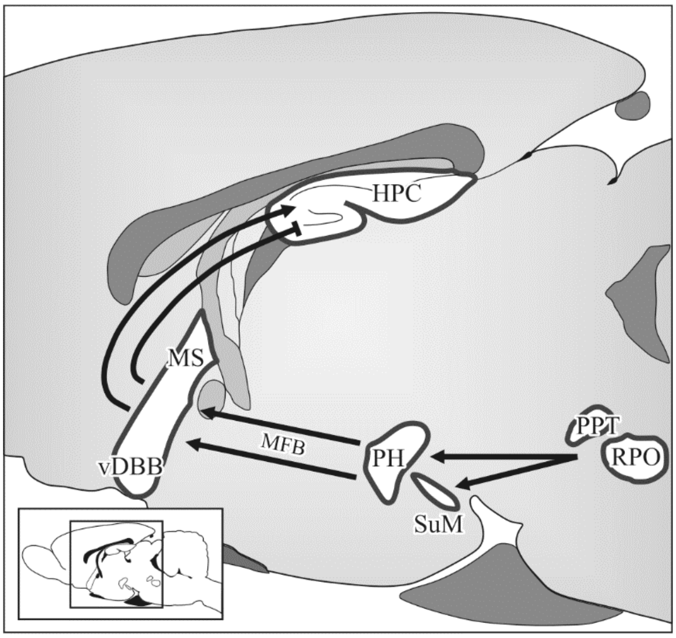

Figure 2. Diagram showing connections between the structures forming the ascending brainstem-hippocampal synchronizing system. RPO – nucleus reticularis pontis oralis, PPT – pedunculopontine tegmental nucleus, SuM – supramammillary nucleus, PH – posterior hypothalamic nuclei, MFB - medial forebrain bundle, MS – medial septum, vDBB – vertical limb of the diagonal band of Broca, HPC – hippocampal formation (Bland and Oddie, 1998; Kowalczyk *et al*., 2021; modified).

ascending system, the significant role of the RPO and PPT in theta rhythm generation in the HPC was indicated by the fact that a prominent population of theta-related cells is present in both of these nuclei. The presence of theta-neurons was confirmed by Kirk *et al*. (1996), who investigated cellular activity recorded from caudal areas of the midbrain including the SuM, posterior hypothalamic nuclei (PH) and medial mammillary nucleus (MM). Recordings were made during a continuous hippocampal theta rhythm induced by an electrical RPO stimulation. The results of this study showed that theta-related neurons can be found in multiple caudal diencephalic sites, including the RPO. Interestingly, all cells recorded from SuM and MM were classified as phasic theta-on, while the theta-on PH and RPO neurons discharged in a tonic manner (Bland *et al*., 1995). Moreover, studies with inhibition of brainstem nuclei confirmed their important contribution to the generation of hippocampal theta rhythm. It was shown that the injection of procaine (local anesthetic) into caudal part of the RPO resulted in inhibition of sensory-elicited theta rhythm in the HPC (Kroplewski *et al*., 2010). Similar results were obtained in the studies on the role of the PPT in hippocampal rhythm generation. The unilateral microinjections of procaine into the PPT successfully blocked tail pinch-elicited hippocampal theta rhythm, thus emphasizing the significant role of the PPT in ascending brainstem-hippocampal synchronizing pathway (Nowacka *et al*., 2002).

Posterior hypothalamic area

The posterior hypothalamic area (PHa) is another important part of the ascending synchronizing pathway (Fig. 2), which is constituted of the posterior hypothalamic nuclei and the supramammillary nucleus. Both these nuclei receive projections from the RPO and PPT (Kirk, 1998) and transmit excitatory impulses to the medial septum (MS; Bland *et al*., 2007) region.

There are multiple experimental evidences indicating the crucial role of the PHa in the generation of hippocampal theta oscillations. Electrolytic lesions of the posterior hypothalamus (including the SuM) in curarized cats resulted in inhibition of HPC theta rhythm even after the application of intense RPO electrical stimulation. Under such conditions only desynchronization and very slow oscillations were present in the hippocampal EEG recordings (Kawamura *et al*., 1961). Another study showing the significant effect of PHa inactivation on rhythmic activity of the hippocampal formation was performed with the use of chemical lesions (procaine administration). When procaine was injected directly into the SuM or to its afferents, a significant reduction in the amplitude and frequency of the theta rhythm in the HPC was observed despite high-frequency electrical stimulation of the RPO (Kirk and McNaughton, 1993).

What is more interesting, the theta-dependent neurons are also present at the level of the posterior hypothalamic area. Kirk and McNaughton were the first to describe these cells in the PHa. In that study, simultaneous EEG recordings were performed in the SuM and hippocampal formation, in urethane-anesthetized rats. It was revealed, that SuM cells discharged rhythmically when the hippocampal theta rhythm occurred spontaneously or when it was evoked by a tail pinch. The firing pattern of SuM neurons was strongly correlated with the frequency and phase of ongoing HPC theta oscillations. Moreover, during periods of EEG desynchronization observed in the HPC the discharge patterns of SuM neurons were irregular (Kirk and McNaughton, 1991; Kowalczyk *et al*., 2021). Interestingly, in subsequent cellular investigations it was documented that neurons recorded from the SuM may be classified as phasic theta-on, whereas PH neurons represent tonic subtype of theta-on neurons (Bland *et al*., 1995; Kirk *et al*., 1996). The results of these studies confirmed the presence of different populations of hippocampal theta-related cells at caudal diencephalic level of the ascending synchronizing system (Kowalczyk *et al*., 2013, 2021).

Medial septum

Another component of the ascending brainstem-hippocampal synchronizing pathway is the medial septum and the vertical limb of the diagonal band of Broca (vDBB). The MSvDBB plays a significant role in generating hippocampal theta rhythm by combining multiple inputs going from different brainstem and caudal diencephalic sites and distributing rhythmic impulsation into the HPC and other limbic structures (Fig. 2; Bland and Oddie, 1998). It was experimentally proven, that deafferentation of the MSvDBB inhibits the ability of the HPC to theta rhythm generation (Bland, 1986). Moreover, the electrical stimulation of the MSvDBB leads to the production of hippocampal theta and resulting HPC synchronous oscillations follows the frequency of septal stimulation (Kramis and Vanderwolf, 1980). It was also indicated, that MSvDBB neurons discharge pattern is clearly related to the occurrence and time course of HPC theta rhythmic field potentials recorded in urethane-anesthetized rats. The majority of septal theta-related cells were classified as theta-on phasic neurons (Ford *et al*., 1989).

Multiple studies emphasized the role of the MSvDBB in modulation and peacemaking of hippocampal theta rhythm based on its cholinergic, GABAergic, and glutaminergic inputs to the HPC (Dannenberg *et al*., 2015; Vandecasteele *et al*., 2014). The significance of cholinergic projections was shown in the study, in which the injection of 192 IgG-saporin (the selective eliminator of cholinergic neurons) into the MSvDBB resulted in the absence of hippocampal theta oscillations (Yoder and Pang, 2005). Other studies have shown that MSvDBB GABAergic neurons discharge rhythmically to pace the hippocampal theta oscillations (Ford *et al*., 1989). Based on the firing correlation of these neurons to either the trough (178 degrees) or the peak (330 degrees) of simultaneously recorded hippocampal theta waves, GABAergic medial septal cells were classified into two different populations (Borhegyi *et al*., 2004). It was concluded that the firing pattern of those neurons constitute the basis of the MSvDBB as the main external pacemaker of hippocampal theta oscillations (Hangya *et al*., 2009). Additionally, the results of the latest studies revealed, that the glutamatergic MSvDBB neurons showed a significant increase in firing rate during hippocampal theta oscillations, but without clear relationship to HPC theta phase. This may suggest that these glutamatergic cells contribute to MSvDBB theta pacemaker properties by providing additional tonic excitation necessary to induce HPC theta synchronization (Kocsis *et al*., 2022).

Local theta field activity in the posterior hypothalamic area

The previously described studies emphasized the relevance of the PHa as a part of ascending brainstem-hippocampal synchronizing pathway. Taking into consideration the presence of theta-related cells in both the SuM and PH, especially the subpopulation of theta-on phasic neurons which are highly involved in theta rhythm appearance in a given brain structure, the question arises: is the PHa capable of generating the local theta rhythm by itself? Interestingly, the results obtained from studies conducted since the 1970s suggested that PH and SuM are not just simple modulators of the hippocampal theta rhythm but may also be capable of producing rhythmic oscillatory activity locally.

The first studies addressing the ability of caudal diencephalic region to generate local theta field potentials were performed on freely moving rats by Komisaruk (1970) and concerned the theta oscillatory activity recorded in the mammillary bodies (MB) and hippocampal formation. In both of these structures, the movement-related subtype of theta rhythm was recorded. Theta oscillations in the MB were appearing with constant lag (about 20ms) compared to simultaneously recorded HPC rhythmic theta activity. The same time delay in the appearance of rhythmic oscillations between EEG recordings from the HPC and MB was observed in rats immobilized by a muscle relaxant, gallamine (Komisaruk, 1970). Another interesting results were obtained during simultaneous EEG recordings taken from the dorsomedial-posterior hypothalamus (DMPH) and the hippocampal formation during the animal’s spontaneous behavior (Bland and Vanderwolf, 1972). Data obtained in these studies revealed a clear correlation between the animal behavior and synchronous rhythmic EEG activity recorded from the DMPH, and this correlation appeared to be very similar as in the case of HPC theta field potentials. In the same studies, electrical stimulation of hippocampal dentate area led to the suppression of regular rhythmic activity in both the HPC and DMPH which was associated with the arrest of animal behavior. Only after retrieval of rhythmic oscillatory activity in both structures to its normal amplitude, spontaneous locomotor activity recommenced which indicated the potential involvement of DMPH theta activity in the control of behavior. However, the described results were obtained only from two rats and the detailed characteristic of DMPH rhythmic oscillatory activity was not performed (Bland and Vanderwolf, 1972).

A more detailed analysis of posterior hypothalamic theta activity was compiled during the research conducted by Sławińska and Kasicki (1995). The results were obtained from nine rats, while simultaneous depth EEG signals were recorded from the posterior hypothalamic nuclei and the CA1 pyramidal layer of the HPC in various experimental situations (spontaneous motor activity in a home cage and spontaneous locomotion along a runway). Additionally to EEG recordings, the electrical stimulation of subthalamic and hypothalamic areas was performed in these studies. The authors found that, EEG activity obtained from both structures (i.e. the posterior hypothalamus and hippocampal formation) was related to the animals' spontaneous behavior. What is more interesting, the EEG signals recorded from the posterior hypothalamic nuclei during spontaneous locomotor activity or that evoked by electrical stimulation were characterized by pronounced synchronization and it never occurred when the animal was motionless (Sławińska and Kasicki, 1995).

In subsequent studies, performed on urethane-anesthetized rats, the similarities between hippocampal and posterior hypothalamic theta rhythmic activity were analyzed (Kocis and Vertes, 1997). The authors conducted EEG examination taken from structures in the caudal diencephalon including the posterior hypothalamic nuclei, the supramammillary nucleus and the mammillary bodies, along with recordings in the hippocampal formation (specifically the CA1 and dentate gyrus). The obtained results indicated different populations of SuM and MB neurons, which rhythmical firing pattern was strongly correlated with hippocampal theta oscillations at different preferred phases. Furthermore, the posterior hypothalamic area theta field oscillations exhibited a consistent time delay of about one quarter of theta cycle in comparison with simultaneous HPC theta. The authors developed the hypothesis that the theta rhythm observed in the PHa could be controlled by the descending inputs going to the posterior hypothalamic area from the septohippocampal system (Kocis and Vertes, 1997). It was assumed, that these descending inputs, eliciting locally generated PHa theta could be subicular to MB connections (Allen and Hopkins, 1989), or medial septal to posterior hypothalamus projections (Gonzalo-Ruiz *et al.*, 1999; Kirk and McNaughton, 1991; Kirk *et al.* 1996). Interestingly, the results of further studies also emphasized an important role of the HPC in generating theta rhythm locally at the level of the posterior hypothalamus. Specifically, during water maze tests, the similarities between theta rhythm recorded from the HPC and SuM were studied along with assessing the level of coherence of theta oscillations recorded simultaneously in both structures. The main finding of that study was that the coherence of HPC and SuM theta rhythm is s not particularly high. Furthermore, it was shown that the SuM was driven by HPC descending activity rather than the opposite (Ruan *et al.*, 2011). Subsequent studies by these authors addressed the characteristics of local theta field potentials recorded in the supramammillary/mammillary areas as a result of bi-directional connections to the HPC through the septal complex (Ruan *et al*., 2017). It was documented that, the inactivation of the MS in freely moving rats did not attenuate MS theta field potentials, but it increased posterior hypothalamic theta frequency simultaneously diminishing its power. The authors stated that the SuM is preferentially involved in coding HPC theta at higher frequencies whereas the MS-HPC neuronal circuit modulates the frequency over the SuM/MM area by its limitation. Furthermore, all of the circuits connecting the SuM through the MS with the HPC, and the HPC descending inputs which innervates the MM, exhibit the bi-directional control of their activity (Ruan *et al*., 2017).

The data presented above strongly suggested that the posterior hypothalamic area is capable of generating theta rhythm locally. However, no systematic analysis of theta rhythmic oscillatory activity observed locally in the PHa was conducted until the 2010s (Bocian *et al*., 2016a, 2016b; Caban *et al*., 2018; Kowalczyk *et al*., 2014). The first studies aimed at providing a more detailed insight into PHa theta rhythmicity, focused on the pharmacological and cellular basis of posterior hypothalamic theta, was conducted by Kowalczyk *et al*. in 2014 (Fig. 3A). The authors have used in their studies both *in vivo* and *in vitro* experimental models. In the urethane-anesthetized rats the simultaneous EEG recordings were performed in both the HPC and the PHa. It was shown that the posterior hypothalamic theta oscillations were 7-8 times lower in amplitude compared to hippocampal theta i.e. spontaneous PHa theta rhythm was characterized by mean amplitude values of approximately 150 μV. The authors concluded, that it was due to the different cellular organization of nuclei forming the posterior hypothalamic area in comparison to the HPC. Specifically, the hippocampal formation is characterized by strict laminar organization which promotes the emergence of synchronous oscillatory phenomena. It is consistent with a statement that a given brain structure, capable of generating local field theta oscillations, should possess a laminar cytoarchitectonic organization of neurons necessary to create electrical dipoles (O’Keefe, 2007). Spontaneous PHa theta activity recorded in anesthetized animals was also examined according to its neurochemical basis. The authors documented cholinergic nature of this EEG phenomenon observed in the PHa since it was blocked after the administration of atropine sulphate (Fig. 3B; Kowalczyk *et al*., 2014). Another important observation was that the time duration of both theta and irregular activity episodes in the posterior hypothalamic area, was different compared to EEG recording taken from the HPC. Interestingly, four different variants of coappearance were observed in rats under urethane anesthesia: the presence of PHa theta oscillations characterized by the same frequency and time course as the hippocampal theta oscillations, the presence of PHa theta with the simultaneous absence of hippocampal theta, the presence of HPC theta with the simultaneous absence of PHa theta, and the simultaneous appearance of theta rhythm in both the HPC and PHa but characterized by different frequencies and time course. That finding was in contrast with previous studies indicating that the theta rhythm generated locally in the PHa is the result of descending information going from the septohippocampal system (Kocis and Vertes, 1997; Ruan *et al*., 2011; 2017). Moreover, in the same studies the ability of posterior hypothalamic in vitro preparations to generate theta oscillations was examined. It was shown that the bath

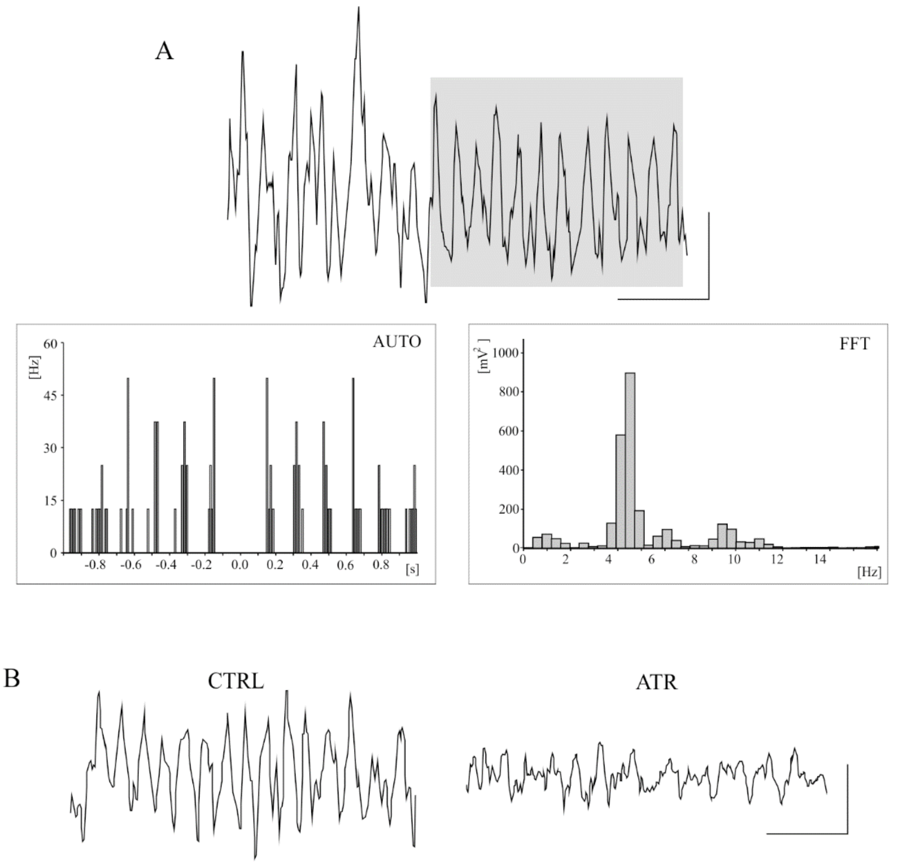

Figure 3. Theta rhythm recorded in the posterior hypothalamic area of a urethane-anesthetized rat. (A) An example of theta field potential recorded in the PHa (upper row). The bottom left row shows an auto-correlation histogram of theta sample, indicated in the upper row with grey rectangle; regularly repeating peaks in the histogram indicate the pronounced rhythmicity of the analyzed signal. The bottom right row shows the power-frequency spectrograph of the theta sample, indicated in the upper row with grey rectangle. (B) An example of PHa field potential recorded before (CTRL; left) and after (ATR; right) the administration of atropine sulphate (10 mg/kg i.v.). Calibration: 1 s, 100 µV (Kowalczyk *et al*., 2014; modified).

perfusion of completely deafferented PHa slices with cholinergic agonist carbachol (CCH) led to the generation of well-synchronized episodes of theta field activity, which again indicated that afferent inputs to the PHa are not essential for theta production in this diencephalic area (Kowalczyk *et al*., 2014).

In the subsequent studies PHa local theta field potentials were analyzed with regard to ongoing local neuronal firing repertoire (Bocian *et al*., 2016b). The PHa theta field potentials were evoked in urethane anesthetized animals by a local injection of carbachol in these studies. According to previously stated criteria of theta-related cells classification (Colom and Bland, 1987), neurons localized in the posterior hypothalamic nuclei and supramammillary nucleus were characterized on the basis of their discharge pattern examined during simultaneously recorded theta rhythm. More than half (specifically 57.7%) of the total number of theta-related PHa neurons identified in that study were classified as theta-on tonic cells. Smaller number (specifically 33.1%) of theta-related PHa neurons were classified as theta-off tonic cells. Both types of theta-related neurons were present in the SuM as well as in the PH. Interestingly, a new type of firing pattern of posterior hypothalamic neurons was distinguished. Since this discharge pattern was not correlated with ongoing local theta activity, thus according to the authors, these neurons could not be considered as theta-related. It was suggested that these neurons, named "timing cells", and characterized by a constant, long-lasting pattern of discharges, are part of the ascending synchronizing system and provide a regular rhythmic signal that facilitates the transduction of tonic discharges of brainstem cells into theta-frequency rhythmic firing pattern (Bocian *et al*, 2016b). In the same study, additionally to phasic subtype of PHa theta-cells the presence of posterior hypothalamic theta-on phasic cells was also revealed. These neurons constituted most (specifically 81.1%) of theta-related neurons isolated and recorded in PHa in vitro preparations perfused with carbachol (Bocian *et al*., 2016b). The results of the described studies, indicating the presence of local theta-related cells in both the SuM and PH endorsed the results of previous investigations (Kowalczyk *et al*., 2014) showing the ability of PHa to generate theta oscillations independently of other brain structures and thus ruled out the previous hypothesis considering local PHa theta activity as an effect of descending hippocampal projections.

Further studies aimed at estimating the ability of the PHa to the production of oscillatory theta field potentials at different stages of postnatal development (Caban *et al*., 2018). In these studies, the CCH-induced theta rhythm was examined in PHa slices taken from 8 to 24 days old rat pups. The recorded PHa theta rhythm was increasing its probability of occurrence, amplitude values and synchrony in accordance with the preparations taken from pups at higher age. The plateau phase of PHa theta rhythm was reached at age of 22-24 days. Furthermore, the number of theta-related neurons was also increasing in PHa slices correspondingly to the increasing pups' age thus providing evidence for gradually developing the capability of generating theta rhythm (Caban *et al*., 2018).

In subsequent research, conducted in both *in vivo* and *in vitro* conditions it was shown that theta rhythm observed locally in the PHa is not dependent on gap junction communication between neurons as it was previously demonstrated for hippocampal theta (Bocian *et al*., 2016a). Specifically, the effect of broad-spectrum gap junctions (GJs) blocker – carbenoxolone (CBX) was analyzed, and unlike in the HPC, blockage of GJs did not suppress the theta rhythm recorded from the PHa in urethane anesthetized rats indicating that the posterior hypothalamic area theta rhythm's generation does not involve electrical coupling via GJs. Moreover, the administration of trimethylamine (GJs opener), which enhanced hippocampal theta, did not affect posterior hypothalamic oscillations. What is more interesting, the application of CBX resulted in an increase in PHa theta rhythm amplitude, and the observed effect was attenuated by the application of spironolactone (mineralocorticoid receptors antagonist) suggesting that enhancement of posterior hypothalamic theta activity by CBX was mediated by mineralocorticoid receptors (Bocian *et al*., 2016a).

The studies described above concerned the theta rhythm generated in the PHa as a result of cholinergic activation. The next research aimed at examining the electrophysiological basis and potential involvement of glutamatergic receptors in the production of posterior hypothalamic oscillatory activity. It was shown that glutamatergic stimulation of the PHa with kainic acid induces well-synchronized local theta field potentials in both the supramammillary nucleus and posterior hypothalamic nuclei. The administration of non-NMDA ionotropic glutamate receptor antagonist (DNQX) successfully blocked the ability of the PHa to generate local theta oscillations in response to kainic acid in both in vivo and in vitro conditions (Kowalczyk *et al.*, 2023). The results obtained in these studies were consistent with previous experiments indicating the involvement of glutamatergic receptors in the generation of theta oscillations in the limbic cortex studies (Bonansco and Buño, 2003). In the same studies, the firing pattern of glutamatergically-activated PHa theta-related neurons was examined. In *in vivo* conditions, the discharge pattern of PHa neurons activated by kainic acid administration allowed to classify them as theta-on phasic, theta-on tonic, and theta-off subclasses of theta-related neurons. In the same studies the presence of KA-activated theta-on phasic neurons was also revealed in *in vitro* maintained PHa slices. Additionally, as it was shown that as it was in the case of cholinergically-induced theta rhythm (Bocian *et al.*, 2016b), also glutamatergic stimulation leads to the emergence of timing cells activity in both *in vivo* and *in vitro* conditions. What is particularly interesting, the results obtained in this study indicated, for the first time in the posterior hypothalamic area, that a subpopulation of theta-related neurons which activity was glutamatergically elicited exhibit clear subthreshold membrane potential oscillations in the theta frequency range (Kowalczyk *et al.*, 2023).

Summary

The experimental evidences presented in this review clearly show, that the posterior hypothalamic area including the supramammillary nucleus and posterior hypothalamic nuclei plays significant functions in the ascending synchronizing pathway. The role of the PHa as modulator and transducer of excitatory impulsation running from the brainstem regions to the limbic structures was widely discussed and demonstrated (Bland and Vanderwolf, 1972; Kowalczyk *et al.*, 2021). However, the results obtained over the last decade have shown that the impact of the posterior hypothalamic area on theta rhythm production appeared to be much more complex than it was previously thought.

Multiple studies have shown the ability of the PHa to the generation of theta oscillations and the association between these local theta oscillations with animals’ movement and behavior was indicated (Bland and Vanderwolf, 1972; Sławińska and Kasicki 1995). While the presence of local posterior hypothalamic theta activity was recorded in numerous studies, the basis of this EEG phenomenon was suggested to be an effect of descending inputs from the HPC and MSvDBB (Kocis and Vertes, 1997; Ruan *et al.*, 2011; Ruan *et al.*, 2017). However, the latest studies have clearly shown, that the local theta field potentials recorded in the PHa could be generated independently of the HPC in both anesthetized animals and the deafferented PHa slice preparations. What is more interesting, the analysis of both SuM and PH neuronal firing pattern revealed the presence of theta neurons, related to local, posterior hypothalamic oscillatory activity (Bocian *et al.*, 2016a; 2016b; Kowalczyk *et al.*, 2014), which exhibit clear membrane potential oscillations (Kowalczyk *et al.*, 2023). This finding is specifically important since MPOs, referred to an intracellular theta rhythm, seem to be a crucial electrophysiological phenomenon for theta rhythm to appear in a given brain structure (Bland *et al*., 2002). In subsequent studies it was also revealed that unlike in the hippocampal formation, PHa local theta oscillations are not dependent on electrical coupling via GJs, this activity may be induced by glutamatergic stimulation, and it is modulated by corticosteroids (Bocian *et al*., 2016a; Kowalczyk *et al*., 2023).

In the light of ability of the posterior hypothalamic area to generate local theta field oscillations, it seems to be particularly important to consider this EEG pattern not only in the context of animal's locomotor activity (Bland and Vanderwolf, 1972; Sławińska and Kasicki 1995), but in the wider context of CNS functions. It was shown that, supramammillary involvement in modulation of HPC excitability indirectly influences the place-learning ability and associative memory processing (Pan and McNaughton, 1997). In addition, according to its projection to the association cortex, the PHa significantly contributes to motivated behaviors, emotion, and arousal (Pan and McNaughton, 2004). Furthermore, presented above the possibility of modulating posterior hypothalamic theta rhythm by activation of mineralocorticoid receptors may indicate the importance of this EEG pattern in the organism's response to stress and adaptation to stressful stimuli, both at the behavioral and metabolic levels (Hunter *et al*., 2009). However, the possible contribution of the PHa to the animal's stress response along with others mentioned in this review OUN functions has not been yet sufficiently examined and thus it should state a goal of future posterior hypothalamic research.

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