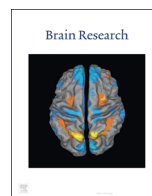




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Research report

## Cell discharge correlates of posterior hypothalamic theta rhythm. Recipe for success in recording stable field potential



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## ABSTRACT

The theta rhythm discovered in the posterior hypothalamus area (PHA) differs from theta observed in the hippocampal formation. In comparison to hippocampal spontaneous theta, the theta recorded in the PHA is rarely registered, has lower amplitude, often disappears, and sometimes returns after a few minutes. These features indicate that spontaneous theta recorded in the PHA is not an appropriate experimental model to search for the correlation between PHA cell discharges and local field potential. In this paper we present standard experimental conditions necessary to record theta-related cells in the PHA in anesthetized rats. Three pharmacological agents were used in the experiments to induce PHA theta rhythm in urethanized rats: carbachol (CCH), carbenoxolone and kainic acid, which are potent enough to induce well-synchronized PHA theta. However, CCH was found to be the best pharmacological tool to induce PHA theta oscillations, due to its longest duration of action and lack of preliminary epileptogenic effects. It seems that CCH-induced theta can be the most suitable pharmacological model for experiments with the use of protocol of long-lasting recordings of PHA theta-related cell discharges.

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## 1. Introduction

Electrical recordings from deep brain structures using wire electrodes are one of the relatively old methods in neuroscience. The combination of local field potential measurements with recordings of neuronal discharges is the best experimental tool available to study the behavior of small populations of neurons. Since the membrane current generated by single neurons passes electronically through extracellular space it can be measured by electrodes placed outside neurons. Field potentials recorded at any given site reflect the linear sum of fields generated by current sources and sinks distributed along multiple cells. If an electrode is placed close to a neuron, recorded extracellular currents will provide information about the discharges of this given cell and neighboring neurons (Nádasdy et al., 1998).

One of the field potentials which has been intensively studied since the early 1950s is theta rhythm (Green and Arduini, 1954; Liberson and Cadilhac, 1954; MacLean et al., 1952). It is one of the most synchronized electroencephalographic (EEG) activities that can be recorded in several brain regions, for example: the cingulate cortex (Landfield and McLaugh, 1972), the entorhinal cortex (Alonso and García-Austt, 1987a, 1987b) or the hippocampal

formation (HPC), which is considered to be the main structure involved in the generation of theta (Bland, 1986).

Previous studies reported that the generation of theta field potential recorded in different brain structures is accompanied by a characteristic pattern of cell discharges (Bland and Colom, 1993; Colom and Bland, 1987; Ford et al., 1989; Kowalczyk et al., 2013a, 2013b). Taking into consideration the spike train dynamics of a given cell in relation to changes in simultaneously occurring theta and large irregular activity (LIA), hippocampal neurons were classified as theta-related and theta non-related. Theta-related cells were classified as “theta-on” and “theta-off” (Colom and Bland, 1987). As the names implied, “theta-on” cells increased their activity during theta field activity, whereas “theta-off” cells decreased their activity during theta. Both “theta-on” and “theta-off” cells were subclassified as phasic and tonic (Colom and Bland, 1987). Some theta-related neurons can also be classified as gating cells (Konopacki et al., 2006; Kowalczyk et al., 2013b). All types of theta-related cells were described in the HPC, but in subsequent experiments they were also observed in the medial septum (Colom and Bland, 1991; Ford et al., 1989), the supramammillary (SuM) nucleus (Bland et al., 1995; Kirk et al., 1996; Kocsis and Vertes, 1994), posterior hypothalamus nucleus (PH; Bland et al., 1995; Kirk et al., 1996), and entorhinal cortex (Dickson et al., 1994, 1995) during simultaneously recorded hippocampal theta field activity.

Just recently Kowalczyk et al. (2014) demonstrated for the first time that theta activity could be also observed in the posterior

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hypothalamus area (PHa) in anesthetized rats. Furthermore, they showed that *in vivo* theta recorded in the PHa was produced independently of simultaneously occurring HPC theta. Experimental evidence indicates that the PHa, primarily the posterior hypothalamic and supramammillary nuclei of the posterior hypothalamic area (Vertes, 1982), comprise a critical part of the ascending brainstem synchronizing pathway (Oddie et al., 1994; Thinschmidt et al., 1995; Vertes and Kocis, 1997; Kirk, 1998; Bocian and Konopacki, 2001, 2004; Pan and McNaughton, 2004; Bocian and Konopacki, 2007).

Typically, in rodents hippocampal theta rhythm occurs spontaneously in short epochs divided by large irregular activity. Kowalczyk et al. (2014), demonstrated that theta rhythm observed in PHa differs significantly from theta recorded in the HPC. Firstly, the PHa theta field potential is much lower in amplitude (approximately 7–8 times) than the HPC signal. Secondly, when the appropriate level of anesthesia is applied a spontaneous transition from theta into irregular activity and vice versa can be observed. This effect is well pronounced in the HPC, whereas in PHa theta oscillations are unstable, i.e. theta activity can occur in the PHa for several seconds and then may disappear for a few minutes. These findings indicate that spontaneous theta activity recorded from the posterior hypothalamic area is not an appropriate experimental model to search for the correlation between PHa cell discharges and local theta field potential. Indeed, the *in vivo* extracellular recording requires a specific experimental conditions including carefully controlled level of anesthesia in which rats typically cycle spontaneously between theta and LIA field activity, each lasting for periods of 5–10 s or longer.

In this paper we present the standard experimental conditions necessary for recordings of theta-related local cell discharges localized in the posterior hypothalamic and supramammillary nuclei in anesthetized rats.

## 2. Results

Histological analysis revealed that in all rats the electrodes for recordings of posterior hypothalamic field activity were located either in PH or SuM and microinjection cannula tracks were localized in PHa in the frontal plane between the range of 3.6–3.8 mm (Paxinos and Watson, 2014). Moreover, histological procedures verified that all recorded neurons were localized in the PH or SuM nuclei of the posterior hypothalamic area.

### 2.1. The effect of CCH injections

Four doses of CCH were tested: 0.5 µg/0.5 µl, 1.0 µg/0.5 µl, 1.5 µg/0.5 µl and 2.0 µg/0.5 µl, respectively. Intrahypothalamic injection of the two highest doses (1.5 µg/0.5 µl and 2.0 µg/0.5 µl) induced only epileptiform discharges (data not shown). CCH applied in a concentration of 1.0 µg/0.5 µl was recognized as effective in producing repeatable epochs of PHa theta rhythm (Fig. 1). In the first stage of postinjection recordings (15 min) continuous and well-synchronized epochs of theta rhythm were observed. In the second stage (30 min), typically a few seconds epochs of theta were separated by large irregular activity (Fig. 1, left panel). Amplitude of theta rhythm measured 30 min postinjection was higher than amplitude observed in control conditions ( $251.9 \pm 8.1$  µV vs.  $169.3 \pm 18.7$  µV; Fig. 1, left panel). CCH-induced theta rhythm was still very well-developed 90 min postinjection. The effect of CCH injection was reversible after 120 min, i.e. theta rhythm observed in this postinjection time resembled theta activity recorded in the control conditions (Fig. 1, left panel). It is worth noting that intrahypothalamic injection of CCH not only facilitated theta rhythm in anesthetized rats that produced spontaneous theta, but also

induced theta rhythm when spontaneous activity in theta band was not observed in control conditions (Fig. 1, right panel). The lowest concentration of CCH (0.5 µg/0.5 µl) was found to be a subthreshold dosage: it did not induce any apparent changes in PHa field potential (data not shown).

### 2.2. The effect of CBX injections

In separate experiments two doses of CBX were tested: 25 µg/0.5 µl and 50 µg/0.5 µl, respectively. Intrahypothalamic administration of CBX in a concentration of 25 µg/0.5 µl never produced PHa theta rhythm (data not shown). A twice higher concentration of CBX (50 µg/0.5 µl) was found to be effective and in the first postinjection period (15 min) induced long-lasting and well-synchronized epochs of theta rhythm in the PHa (Fig. 2, left panel). In the second postinjection period (30 min) typically, a few seconds of theta epochs were separated by large irregular activity (Fig. 2, left panel). Amplitude of CBX-induced theta, measured 30 min postinjection, was higher than the amplitude observed in control conditions ( $234.5 \pm 9.4$  µV vs.  $148.5 \pm 11.2$  µV). The effect of CBX injection was reversed after 60 min, i.e. theta rhythm observed in this postinjection time resembled theta activity recorded in control conditions before drug injection (Fig. 2, left panel). It is worth noting that intrahypothalamic injection of CBX not only facilitated theta rhythm when spontaneous theta was observed in control conditions, but also evoked theta rhythm which was not observed in control conditions (Fig. 2, right panel).

### 2.3. The effect of KA injections

In separate experiments the effect of two concentrations of KA on the posterior hypothalamic EEG activity was evaluated: 0.1 µg/0.5 µl and 0.25 µg/0.5 µl. The concentration of 0.1 µg/0.5 µl was found to be a subthreshold one. It has never produced PHa theta rhythm (data not shown). Following intrahypothalamic injection of KA in concentration of 0.25 µg/0.5 µl in locally recorded EEG, two different patterns of field potentials were observed: epileptiform discharges and theta activity (Fig. 3). Epileptiform discharges were recorded immediately after compound administration and maintained at least for 15 min (Fig. 3, left panel). After this time well-synchronized epochs of theta rhythms were observed. Recorded 30 min postinjection, theta was characterized by increased amplitude in comparison to the control conditions ( $213.8 \pm 7.2$  µV vs.  $157.8 \pm 14.7$  µV; Fig. 3, left panel). KA-induced theta rhythm was still very well-developed 60 min postinjection. The effect of KA injection in concentration 0.25 µg/0.5 µl was reversible after about 90 min, i.e. theta rhythm observed in this postinjection time resembled theta activity recorded in control condition (Fig. 3, left panel). Intrahypothalamic injection of KA not only facilitated theta rhythm in anesthetized rats that produced spontaneous theta but also evoked theta rhythm when spontaneous activity in theta band was not observed in control conditions (Fig. 3, right panel).

### 2.4. Animal's age and probability of recording spontaneous PHa theta rhythm

Interestingly, the analysis of the rat age revealed that the age per se could be also the essential factor that determines success in recording hypothalamic spontaneous theta activity. We noted that the probability of recording posterior hypothalamic spontaneous theta rhythm gradually increased with age. Specifically, among the animals aging 40–45 days only 3 out of 20 rats (15.0%) generated spontaneous PHa theta rhythm (Fig. 4). When the rat's age reached 50–65 days the probability amounted to 73.9% (17 out of 23 rats generated spontaneous PHa theta rhythm, Fig. 4). Surprisingly, in the third group of rats aging 70–75 days, the probability of

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