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Electrical hippocampal activity during danger and safety signals in classical conditioning in the rat

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The effect of stimuli predicting danger (DS) and safety (SS) in Pavlovian aversive conditioning on hippocampal local field potentials (LFP) was studied in 25 partially restrained adult male rats (Long-Evans). DS lasting 5 s preceded tail-shock, while SS overlapping DS during DS last 3 s predicted omission of shock. The power spectra of LFPs during trials were analyzed in theta and delta frequency bands. In DS, theta frequency during the last 3 s was lower that in first 2 s. In danger and safety situation theta peak frequency was different for dorsal CA1 activity (5.99 Hz vs. 6.86 Hz, respectively), while delta peak frequency was different for ventral CA1 (1.56 Hz vs. 1.07 Hz) for the last 3 s of trial. Differences in theta frequency in danger and safety situation may reflect differences in sensory processing during induced emotional states and/or related differences in motor behavior.

Key words: hippocampus, theta rhythm, fear conditioning, safety/danger differentiation

INTRODUCTION

The hippocampal formation generates field potentials known as rhythmic slow activity (RSA) or theta rhythm (Teitelbaum et al. 1977). In rats, this sinusoidal like signal occurs at frequencies within the 4–12 Hz band. Despite many attempts there is no one commonly accepted theory explaining the function of the theta rhythm. In general, theta is present during voluntary movements (Vanderwolf 1969). It is suggested that theta rhythm is involved in various functions, like integration of motor programming (Vanderwolf 1969, Bland 1986), learning, memory, attention and motivation (Grastyan et al. 1959). It was also shown that the hippocampal theta has a substantial role in spatial localization and navigation (O'Keefe 1993).

Another group of studies has investigated the possibility that hippocampal theta plays an important role in the neural plasticity underlying behavioral learning (Berry and Seager 2001). Gray and McNaughton (Gray 1982, Gray and McNaughton 2000) have proposed that the hippocampus is involved in the resolution of conflict between competing approach and avoidance tendencies. The frequency of reticular-elicited theta may be decreased by application of anxiolytic drugs (McNaughton et al. 2007). It was also argued that theta rhythm is indicative of inhibition of sensory systems and is not directly involved in active motor programming process (Sainsbury 1998).

It is generally accepted that two types of theta exist. The type 1 theta rhythm usually has a higher frequency (7–12 Hz in rats) and occurs during voluntary movement performed by the animal, including head movements, changes in posture, manipulatory and locomotor behaviors, etc. Type 2 theta (usually 4–7 Hz) is not related to concurrent movement and can occur during immobility in a waking animal. The frequency of theta during locomotion is related to the animal's speed (Slawinska and Kasicki 1998). Both types of theta occur not only during spontaneous behavior, but can be elicited in awake animals by electrical stimulation of some brain structures, inducing locomotion or tonic motor reaction (Slawinska and Kasicki 1995) or even when animals remain immobile (Woodnorth et al. 2003).

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Type 2 theta may play an important role in acquisition and performance in classical conditioning. This theta has an impact on eye blink and jaw movement conditioning. In eye blink conditioning the higher rate of acquisition was correlated with the dominance of low frequency theta (2–8 Hz) in the hippocampal activity during the pretraining period, prior to the first conditioning session (Berry et al. 1978). The whole series of experiments performed by Thompson's group is reviewed in Berry's and Seager's paper (2001). When the conditioned stimuli were presented during a period of predominance of this theta (3–8 Hz), the animals learned in half as many trials as animals trained during non-theta hippocampal activity (Berry and Swain 1989). These experiments showed that the occurrence of low frequency theta is in favor of faster learning.

The amount of type 2 hippocampal theta and learning rate during classical nictitating membrane conditioning in the rabbit could be increased by water deprivation, which may be associated with arousal and changes in emotional state of the animal (Berry and Swain 1989). Similar result has been obtained in acute water-deprived rats (Maren et al. 1994). In experiments on anesthetized animals this deprivation elevated the power of theta rhythm (4–7.9 Hz band) in the hippocampus. In awake animals the water deprivation facilitated the rate of contextual fear conditioning, although in another paper water deprivation produced no effects on conditioned freezing to the contextual cues (Pouzet et al. 2001). In freely behaving fear-conditioned mice it was shown that conditioned freezing behavior is associated with type 2 theta activity (5.0 ± 1.5 Hz) in hippocampus (Seidenbecher et al. 2003). The amount of type 2 theta and learning rate may thus be positively influenced by arousal or negative emotional state.

In the experimental chamber fear as an anticipation of threat or pain can be induced using fear conditioning paradigm. The inhibitory conditioning procedure we used in the present experiment was designed to model the schedule of events that normally accompany successful coping behavior. Two signals are used in this procedure: the danger signal ending with shock delivery, and the safety signal predicting cancellation of shock that would otherwise occur (than the absence of it). Controlling the application moment of the conditioned stimulus enables analysis of related local field potentials (LFP) during stimuli presentation. Such aversive conditioning procedure can be used to induce not only the state of fear, but by introduction of a conditioned inhibitor stimulus it is possible to induce also relief from fear (Dess and Soltysik 1989).

The aim of the study was to analyze hippocampal activity occurring during presentation of danger and safety signals, inducing opposite (as we assume) emotional states of the animal: conditioned fear and relief from fear.

METHODS

Animals and Surgery

The experiments were performed on 25 male Long-Evans rats (300–350 g). The animals were housed individually in standard laboratory conditions with food and water *ad libitum*. Two weeks before the onset of the aversive conditioning training, the animals were implanted with electrodes for chronic monopolar recordings. The surgery was performed in aseptic condition with Butomidor i.m. injected before isoflurane anesthesia. For recording the hippocampal LFP activity the tungsten electrodes (wire of 0.2 mm diameter, insulated except the tip, the impedance around 30 kOhm/1 kHz) were stereotactically implanted into the dorsal CA1 pyramidal layer (dCA1), the dentate gyrus (DG) and the ventral CA1 layer (vCA1) (Paxinos and Watson 2005). A stainless steel screw, implanted anterior to the bregma, was used as a ground/reference electrode. Additional holes were drilled in the skull and small hooks were placed in them to strengthen the fixation of the socket to the skull. All electrodes were soldered to a socket, which was finally fixed with acrylic cement. After the experiment all animals were killed and the brains have been removed. The location of electrode tips (electrolytic lesion) was determined on 40 μm Cresyl violet stained sections. All experiments were approved by a local Ethics Committee.

Experimental set-up

The experiments were performed in a dimly lit experimental chamber (wooden box with walls covered with foam). The animals were placed in a treadmill/stand apparatus placed on the floor of chamber, for details see (Soltysik et al. 1996). The apparatus prevented rats from turning around, secured their sta-

bile position in relation to sources of stimuli applied during training, and enabled the animals to ambulate on a non-driven treadmill. The replaceable strip of cloth was changed after each experimental session. The treadmill enabled recording of possible locomotor activity of rats whose stepping movements could move the strip and supporting cylinders. Rotation of cylinders activated photocells and was recorded. During the experiment two types of conditioned stimuli were used: acoustic (700 Hz tone delivered by loudspeaker above the animal) and visual (light emitted by an array of diodes placed behind the animal). The unconditioned stimulus (1 mA shock, 0.2 s) with a stimulus isolation unit was applied with bipolar electrodes mounted to the tail. Behavior of the animal was monitored by a LCD camera located above the animal and recorded simultaneously with the LFP activity. The connector on the head of animal was connected with a flexible cable to an amplifier. LFPs were digitized using a micro1401 converter (CED). The delivery of stimuli was controlled by a Spike2 script, and the data were stored for further processing on a PC (Spike2 enabled synchronized recording of data and behavior).

Experimental sessions

Before the experiments, each animal was accustomed for 3 days (around 20 min per day) to the treadmill/stand apparatus and experimental chamber. The adaptation procedure was used to attenuate initial restlessness induced by a new situation and restraint of the subject.

The experiment consisted of 17 sessions (0–16), each composed of 10 computer-controlled trials. Each trial lasted 5 s each. Intertrial interval varied randomly between 90–150 s. On the first day of the experiment (session 0) the two new stimuli (5 s tone, 5 s light), used later as the signals of danger (DS) or safety (SS), were presented 5 times each in random order to the rats (in all experimental sessions the sequence of stimuli was set according to Gellermann's tables (Gellermann 1933). Sessions 1 and 2 consisted of 10 excitatory trials, in which the DS was immediately followed by the tail-shock (1 mA, 0.2 s) used as unconditioned stimulus (US). In consecutive sessions (3–16) five excitatory and five inhibitory trials (see Fig. 1) were randomly scheduled. During the inhibitory trials safety signal overlapped the danger signal by the last 3 s. Analysis was performed on two data segments. The first segment lasted 2 s, starting at the moment of DS stimulus onset, and was labeled as d1 segment. The other 3 s interval was labeled as d2 segment. The SS was a predictor of an omission of the otherwise expected aversive US. This procedure was used to elicit in inhibitory trials the phasic change or switch of the animals' emotional state – from fear to relief from fear.

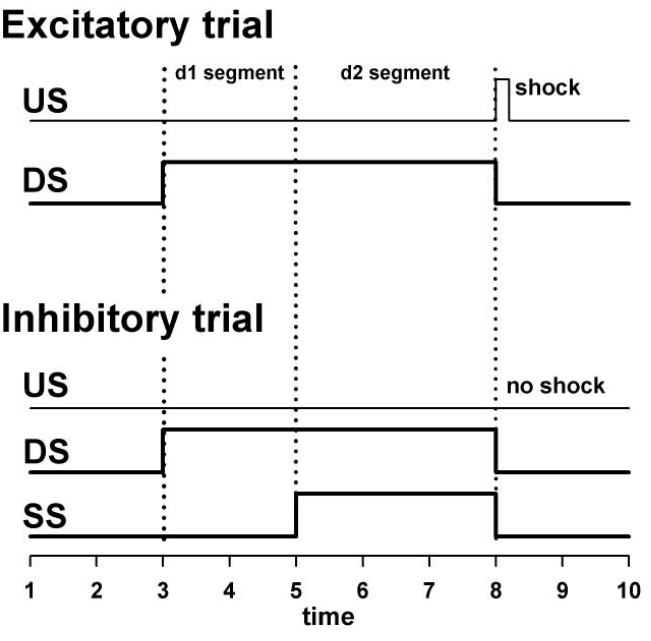

Fig. 1. The scheme of organization of stimuli in the excitatory and inhibitory trials. The LFPs from the intervals labeled as the d1 and d2 segments were analyzed. Denotations: (US), (DS) and (SS) – unconditioned, danger and safety stimuli, respectively.

Data acquisition and analysis

During conditioning sessions the hippocampal LFPs were amplified, digitized (sampling frequency 1 kHz) and recorded on a hard disk. Analysis of the data was performed off line. The recorded signals were "cleaned" manually by removing segments with artifacts. The power spectra (FTP, Hanning window) of the LFP signal in 0–50 Hz frequency band were calculated for two periods (2 s DS and 3 s DS/SS) for each trial of each rat. Using homemade scripts (Spike2) the mean spectra for these two data segments were calculated for every rat in every session and the dominating frequency in delta (0.1–4 Hz) and theta (4–12 Hz) frequency bands was determined in each session for each data segment. The significance of differ-

ences between the dominating frequencies during DS and SS trials was determined using a *t*-test. Additionally the total-mean spectra for every rat for all differentiation periods (sessions 3–16) were calculated.

After each session the number of feces on the belt and marks of urination were noted for statistical analysis. Then the mean values of this parameter were calculated for each rat in every session.

RESULTS

Location of all electrodes has been verified histologically and is shown in Fig. 2.

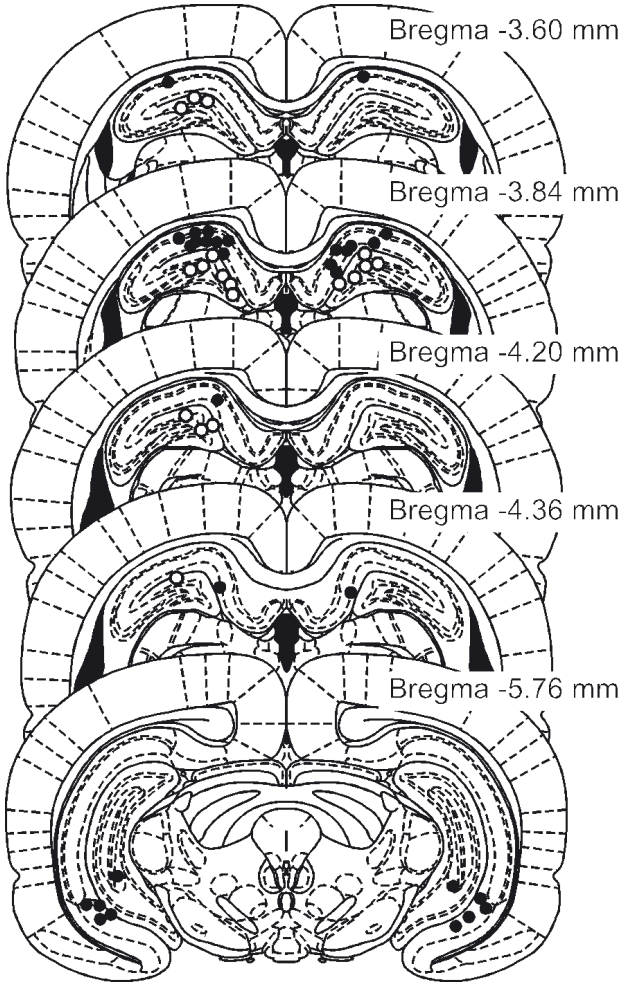

Fig. 2. Position of electrodes located in the dorsal CA1 field, dentate gyrus and ventral CA1 field. Placement of electrodes in the dentate gyrus marked by unfilled circles. (Modified from Paxinos and Watson 2005*).

* Modified from Paxinos G, Watson C, The Rat Brain in Stereotaxic Coordinates (5th edition), Figures 63, 65, 68, 69 and 81, Copyright (c) 2005, with permission from Elsevier

Behavior of animals

During the conditioning trials the rats quickly developed a typical freezing reaction to whole experimental situation. In a time course of differentiation they began to perform movements of the head and fore limbs during intertrial periods, but at the moment of DS onset (both for danger and safety trials) they became motionless. During the last few differentiation sessions the animals sometimes raised their head up at the moment of the SS onset and stayed immobile until the end of stimulus. Analysis of the number of feces showed that their number per session decreased with time. The correlation coefficient between the average number of feces and number of session was high and significant (r=0.9, P<0.001).

Analysis of dominating frequency in theta band

Analysis of power spectra of LFPs showed that in the dorsal CA1 activity the dominating theta frequency was significantly different in excitatory and inhibitory trials during the last 3 s (d2 segment). Results for all analyzed electrodes are presented in Table I. In 17 of 21 cases the difference between the peak theta frequency was statistically significant. Of note, for four not significant cases the P values were close to 0.05, what was set as the minimal significance level. The mean peak theta frequency during d2 segment in excitatory trials was significantly smaller than in inhibitory trials (respectively 5.99 ± 0.41 Hz vs. 6.86 ± 0.41 Hz, P<0.001). The same analysis performed for d1 data segment showed no significant differences between excitatory and inhibitory trials (6.37 ± 0.38 Hz vs. 6.44 ± 0.35 Hz). This shows clear differentiation of hippocampal theta to DS and DS/SS stimuli. An example of power spectra for excitatory and inhibitory trials is shown in Fig. 3.

Similar analysis was done for dominating theta frequency in the dentate gyrus activity during excitatory and inhibitory trials (Table II). The mean peak theta frequency during d2 segment in excitatory trials was significantly smaller than in inhibitory trials for the whole group of animals (5.77 ± 0.65 Hz vs. 6.10 ± 0.72 Hz, P<0.001). However, analysis performed for individual recording sites showed significant differences between peak theta frequency for these two situations only in 4 of 18 cases.

A correlation analysis was used to evaluate whether the magnitude of change in CA1 theta peak frequency during d2 period depended on the peak theta frequen-

Table I

Differences in dominating frequency in theta frequency band for dorsal CA1 between the excitatory and inhibitory trials (d2 segment)

No.	Structure	Θ_{DS}	Θ_{SS}	Θ_{SS}- Θ_{DS}	*P*	Significant
1.	dlCA1	6.38	7.36	0.98	0.000914	Y
2.	dlCA1	5.42	6.45	1.03	0.004068	Y
3.	drCA1	5.62	6.82	1.20	0.000151	Y
4.	dlCA1	5.80	6.69	0.89	0.022137	Y
5.	drCA1	6.25	7.03	0.78	0.017457	Y
6.	drCA1	5.44	6.09	0.65	0.069628	N
7.	dlCA1	5.33	6.64	1.31	0.001032	Y
8.	dlCA1	5.75	6.51	0.76	0.008897	Y
9.	drCA1	5.98	6.79	0.81	0.006183	Y
10.	dlCA1	5.83	6.80	0.97	0.003629	Y
11.	drCA1	6.45	7.16	0.71	0.054563	N
12.	drCA1	6.43	6. 87	0.44	0.042969	Y
13.	dlCA1	6.92	7.51	0.59	0.007813	Y
14.	drCA1	5.97	7.44	1.47	0.001870	Y
15.	dlCA1	5.73	6.37	0.64	0.072877	N
16.	dlCA1	6.13	6.67	0.54	0.071268	N
17.	drCA1	6.24	7.17	0.93	0.010188	Y
18.	dlCA1	5.96	6.68	0.72	0.016907	Y
19.	drCA1	5.65	6.68	1.03	0.004736	Y
20.	dlCA1	5.96	6.55	0.59	0.002175	Y
21.	dlCA1	6.45	7.73	1.28	0.002140	Y

Denotations: (Θ_{DS}) frequency of theta maximum in Hz for DS; (Θ_{SS}) frequency of theta maximum in Hz for SS; (Y) and (N) – significant and not significant difference; (d) dorsal; (l) left side; (r) right side

cy during the first 2 s of DS presentation (d1 segment). We calculated the correlation coefficients between the theta frequency during d1 interval and difference between dominating theta frequency during d2 and d1 segment independently for excitatory and inhibitory trials. For excitatory trials the correlation coefficient was low but statistically significant (r=0.38, P=0.046), for inhibitory trials the correlation coefficient was insignificant, although close to the accepted significance level (r=0.34, P=0.067). The results are presented in Fig. 4. In excitatory trials higher theta frequency during d1 segment was followed by a greater decrease in frequency during d2 segment. Although in inhibitory trials the correlation was insignificant there was a trend that lower frequency during the d1 segment was associated with a greater increase of frequency during d2 segment.

Analysis of power spectra for the LFP recorded in the ventral CA1 field of the hippocampus did not show significant activity in theta band.

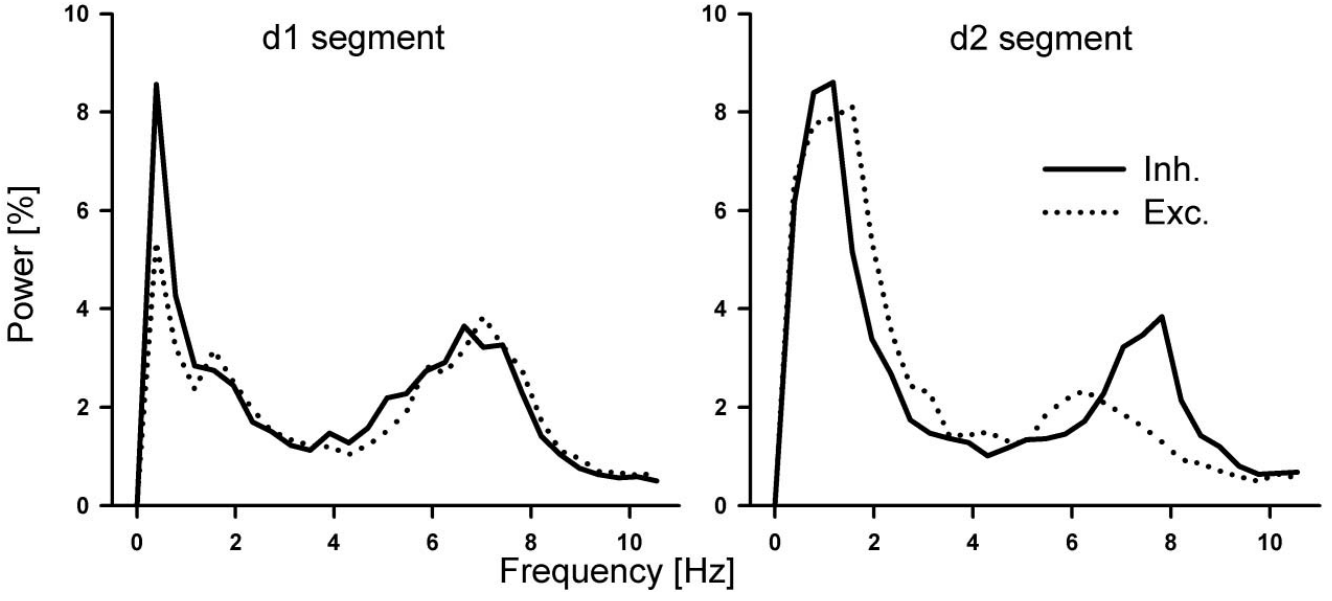

Fig. 3. The mean power spectra for dorsal CA1 activity during excitatory (Exc.) and inhibitory (Inh.) trials for the d1 and d2 data segment. Note the difference of peak location around 6–8 Hz between the excitatory and inhibitory trials for the inhibitory trials. The means calculated for all differentiation sessions.

Analysis of dominating frequency in delta band

Dominating frequency in delta frequency band for the ventral hippocampus during excitatory and inhibitory trials was significantly different for the d2 segment (1.56 ± 0.02 Hz vs. 1.07 ± 0.20 Hz, P=0.003). This analysis was done on frequencies significantly different in individual cases. The results for each recorded site are shown in Table III.

DISCUSSION

Across experimental sessions we observed a progressive decrease of anxiety level or emotional arousal. This conclusion is justified by the decreasing number of feces with time and changes in animal behavior during intertrial intervals. Across the session numbers the initially long lasting freezing in intertrial intervals was replaced by some movements of head, forelimbs or changes in posture. The decrease of anxiety level is probably related to the fact that during long lasting intertrial intervals no aversive, unconditioned stimulation occurred.

In the conditioning paradigm applied in the present experiment the danger signal predicted occurrence of the tail shock, while the safety signal predicted rather the cancellation of shock, that would otherwise follow, than simply the absence of shock. This enabled induction in animals two opposite emotional states – a fear and relief from fear (state of safety) (Dess and Soltysik 1989). In another paper using a similar experimental paradigm it was confirmed that the safety signal may reverse the behavioral effect of the danger signal, i.e.

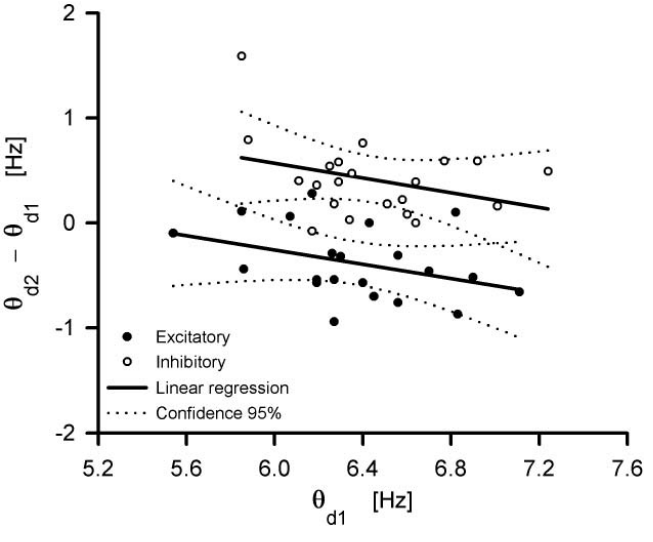

Fig. 4. The relation between the peak theta frequency in the d1 segment (X axis) and the magnitude of difference between theta frequency in the d2 and d1 segments (Y axis). Note that for excitatory trials the higher dominating theta frequency in the d1 segment, the bigger decrease in the d2 segment. For the inhibitory trials the correlation coefficient was insignificant. Note that X axis is placed at y = −2.

Table II

Differences in dominating frequency in theta frequency band for dentate gyrus between the excitatory and inhibitory trials (d2 segment)

No.	Structure	Θ_{DS}	Θ_{SS}	Θ_{SS}- Θ_{DS}	*P*	Significance
1.	rDG/rCA1	4.73	5.59	0.86	0.068936	N
2.	lDG	4.86	4.89	0.03	0.481811	N
3.	rDG	4.86	4.68	−0.18	0.355905	N
4.	lDG	5.7	5.88	0.18	0.306938	N
5.	rDG	5.2	5.57	0.37	0.316855	N
6.	lDG/lCA1	5.8	6.58	0.78	0.087627	N
7.	lDG/lCA1	5.26	6.17	0.91	0.103211	N
8.	rDG	5.78	6.67	0.89	0.000801	Y
9.	lDG	5.7	5.96	0.26	0.353415	N
10.	lDG	5.92	5.94	0.02	0.476213	N
11.	rDG	6.14	5.48	−0.66	0.121167	N
12.	lDG	6.4	6.5	0.1	0.38013	N
13.	rDG	6.24	6.38	0.14	0.374961	N
14.	lDG	6.09	6.26	0.17	0.321489	N
15.	rDG	6.6	6.21	−0.39	0.136452	N
16.	lDG/lCA1	5.72	6.73	1.01	0.022069	Y
17.	rDG/rCA1	7.29	7.78	0.49	0.000839	Y
18.	lDG/lCA1	5.49	6.6	1.11	0.024331	Y

Denotations: (Θ_{DS}) frequency of theta maximum in Hz for DS; (Θ_{SS}) frequency of theta maximum in Hz for SS; (Y) and (N) – significant and not significant difference

causing reappearance of ultrasonic vocalization. For a more detailed discussion of emotional states during this conditioning procedure see Jelen and colleagues (2003).

It is known that more intense or salient conditioned stimuli enhance conditioning. The auditory stimuli provoke faster avoidance acquisition, while visual are less effective (see e.g. Werka 1998). The visual stimulus used in our experiment as inhibitory might slow down the differentiation process. On the other hand, using it may prevent manifesting locomotor movements by animal. In experiments using a similar design some locomotion was observed during the d2 segment (according to terminology used in this paper) when a tone was used as SS (Jelen and Zagrodzka 2001). Thus, to avoid locomotor movements during presentation of stimuli we used light as safety signal.

The dominating theta frequencies during the d1 segment in both excitatory and inhibitory trials did not differ. This result confirms that animals could not predict which type of trial will occur on the basis of first 2 s of DS presentation.

The main result of present study is that frequency of the theta rhythm accompanying two different experimental conditions inducing opposite emotional states (fear and relief from fear) is significantly different. Dominating theta frequency during the d2 segment of the excitatory trial was significantly smaller than during

Table III

Differences in dominating frequency (Hz) in delta frequency band for the ventral hippocampus between the excitatory and inhibitory trials (d2 segment)

No.	Structure	Δ_{DS}	Δ_{SS}	$\Delta_{SS}-\Delta_{DS}$	*P*	Significant
1.	vrCA1	1.56	1.19	−0.37	0.042226	Y
2.	vrCA1	1.35	1.27	−0.08	0.297549	N
3.	vlCA1	1.56	1.13	−0.43	0.024036	Y
4.	vrCA1	1.61	0.98	−0.63	0.017081	Y
5.	vlCA1	1.53	0.75	−0.78	0.003724	Y
6.	vrCA1	0.96	0.78	−0.18	0.205594	N
7.	vlCA1	1.56	1.28	−0.28	0.046884	Y
8.	vrCA3	1.84	2.10	0.26	0.091384	N
9.	vlCA1	1.87	1.90	0.03	0.47	N
10.	vlCA3	2.08	1.58	−0.5	0.37402	N

Denotations: (Δ_{DS}) frequency of delta maximum in Hz for DS; (ΔS_S) frequency of delta maximum in Hz for SS; (Y) and (N) significant and not significant difference

the corresponding d2 segment of inhibitory trial (during SS) for the records in dorsal CA1 field.

It is tempting to relate observed effects on theta frequency with differences in emotional state or arousal level of the animals. It is known that exposing animals to various high arousal situations may induce type 2 theta. In immobile guinea pigs type 2 theta was present when the animals were exposed to owl sounds, snakes or female conspecifics (Sainsbury and Montoya 1984). In unrestrained immobile rats it was possible to elicit type 2 theta by exposing to them a cat or a ferret (Sainsbury et al. 1987). However, simple coincidence of theta presence and increased arousal or fear does not mean the causality. The cited authors hypothesized that type 2 theta occurred during sensory processing but only when the animal was in a high state of "arousal". In particular, in our experiment we cannot demonstrate a direct connection of different frequencies of theta rhythm in immobile rats specifically with fear and relief from fear. It is possible that changes in emotional state influence cognitive processes or sensory processing of conditioned stimuli in hippocampus in situation of passive defense reaction.

In our experiment the animals independently of the session number at the moment of onset of DS became motionless (or even froze). The immobility of the animal during the d1 segment may allow us to consider the theta occurring during this situation as theta 2. Reaction to this part of the stimulus should be independent on the type of trial in a given session. Thus, basing on these reasons we could expect a lack of differences between frequencies of theta in trials of both types. Our results are in accordance with this expectation. During the d2 segment in excitatory and inhibitory trials the animal may be in different emotional states. Transition to relief from fear depends on the onset of the safety signal. Fear and relief from fear were accompanied by theta which we hypothesize, on the basis of the rats behavior, was also of type 2. In future studies we believe that more precise discrimination of periods the animal presents freezing behavior could be obtained with dorsal neck EMG, as shown in a recent paper (Steenland and Zhuo 2009).

We showed that in excitatory trials (in which the animals were immobile during both d1 and d2 segments) the magnitude of changes in theta frequency during the d2 segment was related to the frequency during the d1 segment (Fig. 4). The higher theta frequency during d1 segment, the bigger decrease in theta frequency during d2 segment. The strength of reaction to the lack of safety signal depends both on the state of the animal during the d1 segment of the excitatory trial and individual emotionality. For this reason we cannot expect the same level

of reaction in all animals during the experiment. The magnitude of changes may reflect different levels of fear during the d1 segment. These results are in line with data shown in experiments done on mice in Pavlovian fear conditioning, where presentation of conditioned stimulus during retrieval session was accompanied by a progressive decrease of theta frequency to 5–6 Hz (Fig. 2E,F in Seidenbecher et al. 2003).

In inhibitory trials the correlation between the magnitude of difference in theta frequency between d2 and d1 segments and the frequency during d1 segment was insignificant. The differentiation process may change the emotional value of the first two seconds of DS presentation. It might mean that during the d1 segment the animals were alert (with lower level of fear) thus the effect of safety stimulus presentation was limited. In contrast, the lack of SS presentation resulted in significant emotional changes.

In the jump avoidance test it was shown that in rats during "immobile movement processing" theta frequency increased in a short time interval preceding the jump (Bland et al. 2006). In our case in inhibitory trials the SS inhibits fear and related freezing behavior. However, the animals did not perform vigorous movements, but remained mostly immobile, although they raised the head and changed posture. Thus, we cannot explain the higher theta frequency as related to sensorimotor preparation to trained motor reaction. However, we cannot reject the hypothesis, that such change may reflect also inhibition of passive defense reaction. On the other hand, it is possible that during SS presentation the animals' attention was focused on the stimulus inducing relief from fear. Thus, greater frequency of theta in safety trials in comparison to danger trials may be related to the sensory processing (including attention) of SS.

CONCLUSIONS

On the basis of immobility of animals during aversive differentiation conditioning we assume that the theta activity occurring during presentation of stimuli can be classified as type 2 theta. The exposition of animals to danger and safety signals, inducing opposing emotional states (fear and relief from fear), was associated with different peak frequencies of theta activity. The differences in theta frequency in excitatory and inhibitory trials may reflect differences in sensory processing during induced emotional states and/or differences in motor behavior.

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