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DYNAMICS OF CORTICAL THETA ACTIVITY CORRELATES WITH STAGES OF AUDITORY AVOIDANCE STRATEGY FORMATION IN A SHUTTLE-BOX

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Abstract—By comparing behavioral performance and cortical theta activity (4–8 Hz) on a trial by trial basis we examined how the different behavioral stages of tone-induced avoidance learning in the shuttle-box may be distinguishable by theta power as a potential correlate of changing strategies of information processing. Electrocorticograms with pronounced theta content were recorded across the cortical surface of gerbils during avoidance learning and analyzed in each trial in conjunction with reaction times and unconditioned and conditioned responses. The focus of theta analysis in this paradigm with a 5-s delay between tone and foot-shock onsets was on the 14-s periods after hurdle crossing where feedback information from a trial is available. The strongest theta activity occurred in stage 1 of initial tone conditioning which was sharply reduced to a minimum during stage 2 of optimization of unconditioned escape responses from the foot shock. A few initial successful avoidance responses gave rise to a reversal of the decline of theta activity that later reached a second maximum. A systematic increase of theta activity during this stage 3 of avoidance conditioning was found for the occasional trials with unconditioned responses and not for the increasing number of conditioned responses suggesting that error processing is a major correlate of this new increase of theta power. After the second maximum the theta power slowly declined together with a further improvement of behavioral performance indicating that stage 4 of retrieval of the consolidated avoidance response was reached. The results suggest that behind a previously reported general trend of decreasing theta power with increasing performance in this paradigm there is a hidden microstructure of theta activity across trials which separates stages of avoidance conditioning and is partially mirrored by known changes of prefrontal dopamine release.

Key words: theta rhythm, avoidance learning, learning stages, gerbil, dopamine.

Avoidance conditioning, e.g. in a shuttle-box, is a complex learning paradigm with several stages during which an initial escape strategy from a foot shock is replaced by a tone stimulus–conditioned avoidance strategy. Subjects obviously use behavioral feedback information from a few initial, and presumably, spurious avoidance successes, but little is known how this is shaped into a consistent avoidance strategy. There is already extensive work on the auditory learning and memory aspects of the shuttle-box paradigm in Mongolian gerbils (*Meriones unguiculatus*) using frequency-modulation as stimuli in discrimination learning and categorization which all contain avoidance conditioning as an element. This work has shown that this learning involves physiological plasticity and protein synthesis in auditory cortex, whose functional and anatomical organization is largely understood (Scheich et al., 1997; Stark and Scheich, 1997; Wetzel et al., 1998; Ohl et al., 2001; Budinger et al., 2006; Schicknick and Tischmeyer, 2006).

In order to differentiate stages of learning and retrieval in gerbils during auditory avoidance conditioning in a shuttle-box we here investigated the relationship between changes of individual behavior and the power of theta rhythm. Performing brain microdialysis during avoidance conditioning we have previously shown that the activity of the medial prefrontal dopamine system was transiently increased during the initial stage of acquisition of the avoidance response. This increased extracellular prefrontal dopamine release became reduced during further improvement of performance and during retrieval of the learned behavior (Stark et al., 1999, 2001, 2004). This suggested that dopamine is involved in this associative learning covering especially the stage of what we called “fast improvement of performance,” i.e. the stage when the number of conditioned responses with shock avoidance steeply increased. Recent investigations revealed individual-specific, negative correlations between an overall decline of an initially strong theta activity and the learning progress during the course of this auditory avoidance learning (Stark et al., 2007). In the present trial by trial analysis, we focus on the question whether and how the behaviorally distinguishable stages of avoidance strategy formation are reflected by prominent “events” in the changing theta activity.

A prerequisite for a better understanding of the course of learning and of memory retrieval is a high time resolution of recorded physiological parameters. The interpretation of microdialysis data from extracellular dopamine release in the range of minutes is very limited in this respect (Stark et al., 2004) and also seems to reflect tonic rather than phasic components of a dopaminergic drive (Hernandez et al., 2006). As a candidate potentially reflecting similar processes with high resolution we recorded electrocorticograms in freely moving, learning animals. We analyzed temporal patterns of theta activity in each single trial with respect to the occurrence of conditioned or unconditioned responses during avoidance training. Theta activity ap-

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pears to be a correlate for the intensity of information processing specifically during associative learning (Balleine and Curthoys, 1991; Asada et al., 1999; Ishii et al., 1999). Accordingly, if the acquisition of the avoidance strategy consists of stages defined by physiological and behavioral observables the stepwise learning could be reflected by fast changes of theta activation.

Behavioral studies of auditory avoidance conditioning and parallel microdialysis from medial prefrontal cortex in the shuttle-box have shown that the learning of the conditioned avoidance response follows a hierarchy of the three main associations "signal detection," "signal evaluation," and "retrieval of avoidance strategy" (Stark et al., 2000). The stage "signal evaluation" consisting of components of escape and avoidance learning was accompanied by a strong increase and a subsequent attenuation of prefrontal dopamine release. A differentiation of the components was impossible due to low time resolution in dopamine measurements. The present work aims at high-time resolved electrophysiological and behavioral data to extend our knowledge about the formation of an avoidance strategy in several stages. Particular emphasis was placed on finding events that define the transition from merely escape learning to true avoidance learning. The methodical approach we used (Stark et al., 2007) for an objective demarcation and further differentiation of stages is now based on unique single trials defined by correlation of electrophysiological and behavioral data during avoidance conditioning. Analysis and understanding of the stages with specific information processing and their transitions allow presumably a more profound investigation of the neuronal mechanisms involved.

The basic hypothesis of the experiment is that the strength of theta activity may reflect the demand of actual information processing during the learning process. As a general trend the magnitude of theta power across trials may characterize the course of intensity of information processing from the start of learning up to the retrieval of the matured behavioral strategy. Recently, we already found an overall negative correlation of theta activity in the intertrial interval after compartment change with individual learning progress (Stark et al., 2007). Beyond this general trend, a detailed comparison of actual theta activity with behavioral events in a trial by trial analysis may disclose markers for the formation of relevant associations and for the separation of stages of the learning process. For a detailed analysis the theta activity in the intertrial epochs after compartment change until the start of a new trial was chosen. In these trial epochs an animal can update the previous cumulative behavioral feedback experience with the actual outcome of the preceding trial. The result of this processing may be used behaviorally in the next trials.

Generally, theta activity seems to be involved in brain states of arousal and consciousness (Ishii et al., 1999). Oscillations of brain potentials between 4 and 8 Hz are generated in subjects during decision making (Jacobs et al., 2006). Hippocampal theta activity is involved in communication between prefrontal cortex and hippocampus essential for some types of memory formation and recall (Sarnthein et al., 1998; Jones and Wilson, 2005; Jensen, 2005). The maintenance of hippocampal theta activity is essential in initial learning in rats (McNaughton et al., 2006). Physiological studies in rat and human have also indicated that mental tasks involving working memory are accompanied by synchronized neuronal activity in the theta-frequency range (Givens, 1996; Sarnthein et al., 1998). Together, such results suggest that theta activity may also reflect learning during the formation of behavioral strategies that require a continuous updating of information.

EXPERIMENTAL PROCEDURES

Animals

Four male gerbils (*Meriones unguiculatus*) weighing 80–100 g were kept in single cages (24×20×14 cm) with ambient illumination on a 12-h light/dark cycle with free access to pellets and water. Each animal was extensively handled daily 1 week before the start of experiments minimizing stress to prevent non-specific effects on learning. The animals were treated in accordance with National Institutes of Health procedures for care and use of laboratory animals, and experiments were approved by the ethics committee of the state of Sachsen-Anhalt (No. 42502/2-553IfN). All efforts were made to minimize the suffering and number of animals used.

Surgery

The animals were stereotaxically implanted with stainless steel screws (diameter 1.0 mm) in the scull. Electrocorticograms were recorded from a screw placed epidurally over the right medial prefrontal cortex through a hole in the right frontal bone (2.0 mm rostral and 0.7 mm lateral to bregma point) against an electrode placed epidurally over visual cortex through a hole in the right caudal parietal bone (5.0 mm caudal and 3.0 mm lateral). A third screw placed onto the dura through a hole in left caudal parietal bone (5.0 mm caudal and 3.0 mm lateral) served as ground electrode. This electrode configuration among several others was chosen because under the experimental conditions it produced the largest and most continuous theta activity, a prerequisite to study theta dynamics by its power in this first attempt. Anesthesia was induced by 400 mg pentobarbital (Sigma, Taufkirchen, Germany) per kg body weight intraperitoneally. The head mount with electrode contacts (Plastics One, Roanoke, VA, USA), and Teflon-coated stainless steel wires (SS-3T/HH, Science Products, Hofheim, Germany) connecting the socket contacts and screws were fixed with dental cement on the skull.

Behavioral test

Five days after surgery gerbils were habituated for 30 min to the shuttle-box. On the next day animals were subjected to two auditory avoidance training sessions in a shuttle-box (Coulborn Instruments, Allentown, PA, USA) separated by a pause of 6 h, followed by a third training session at the next morning. The training sessions consisted of 60 trials with 30-s intertrial intervals from trial onset to trial onset. Individuals not reaching the behavioral criterion of at least 100 accumulated conditioned responses were excluded from analysis. The conditioned tone stimuli were frequency modulated tone (FM) a1 (linear FM 1–2 kHz, duration 0.25 s, 60 dB SPL) and FM a2 (linear FM 2–1 kHz, duration 0.25 s, 60 dB SPL) delivered in pseudo-randomized order. The tones lasted maximally 15 s. The unconditioned electric foot shock (0.6 mA pulses for a maximum of 10 s, scrambled via a grid floor) followed 5 s after tone onset. Tone and shock were interrupted when the gerbil had moved into the other shuttle-box compart-