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Theta Activity in Neurons and Networks of the Amygdala Related to Long-Term Fear Memory

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ABSTRACT: With a combined in vitro/in vivo electrophysiological and behavioral approach, we have correlated conditioned fear behavior to electrophysiological activities in the lateral amygdala and the hippocampal formation in rodents. Data indicate that projection neurons in the lateral amygdala display a continuum of spike patterns including accommodating patterns, regular firing, and oscillatory activity at theta frequencies. The firing pattern is controlled to an important part by the intracellular cAMP system, in that an increase in intracellular cAMP concentration facilitates regular firing and theta oscillations. Oscillatory electrical activity, in turn, provides an important cellular element of synchronized theta activity at 4–8 Hz (indicating atropine-sensitive type 2 theta) occurring in amygdalo-hippocampal pathways during conditioned fear responses. This type of rhythmic network activity is associated with the retrieval of long-term fear memory following cued and contextual fear conditioning, but is not related to the expression of fear behavior per se or to short-term fear memory. Synchronization at theta frequencies is suggested to represent activity in amygdalo-hippocampal pathways associated with system consolidation of fear memory, which is supported by the cholinergic system. © **2005 Wiley-Liss, Inc.**

KEY WORDS: oscillation; theta rhythm; fear conditioning; hippocampus; amygdala

INTRODUCTION

Fearful experiences are rapidly learned and long remembered. Much research has therefore focused on aversive conditioning paradigms to elucidate mechanisms of learning and memory (McGaugh et al., 1996; LeDoux, 2000). An established experimental model is Pavlovian fear conditioning (LeDoux, 2000; Maren, 2001), in which a sensory stimulus (such as a tone, the conditioned stimulus, CS) or a context is fear-conditioned by pairing with an aversive stimulus (such as an electrical foot stimulus, the unconditioned stimulus, US). Evidence from such studies suggests a critical involvement of the amygdala and hippocampus in the process of memory formation. Within the amygdala, the lateral nucleus (LA) is the primary input station for sensory signals carrying CS information via a thalamic and a cortical route, and the functional convergence of CS and US information at this site is considered an important substrate of fear conditioning (LeDoux, 2000). Furthermore, the LA is a site of major reciprocal connections with temporolimbic areas, in particular the hippocampal formation (Pitkänen et al., 2000). A large body of evidence has indeed accumulated for synaptic plasticity in various amygdaloid subnuclei during cued and contextual fear conditioning, and for hippocampal plasticity during contextual conditioning (Clugnet and LeDoux, 1990; Maren and Fanselow, 1995; Rogan et al., 1997; Blair et al., 2001; Rodrigues et al., 2004). Amygdalo-hippocampal interactions may be more generally involved in the conversion of short-term fear memory into long-term fear memory related to emotional arousal, including cued fear memory (McGaugh et al., 1996; McGaugh, 2004; Richter-Levin, 2004). Theta rhythms (Vinogradova, 1993; Buzsaki, 2002) may provide a means for such an interaction, as is indicated by the following line of evidence: projection neurons in the LA possess a high propensity to generate rhythmic oscillations of the membrane potential at theta frequencies (Paré et al., 1995; Pape et al., 1998; Pape and Driesang, 1998), which in turn seems to constitute an important cellular basis for the synchronization of amygdalo-hippocampal activity at theta frequencies during conditioned fear responses (Seidenbecher et al., 2003). What remained unclear from these previous studies was how the theta-generating neurons fit into the accepted classification scheme of amygdaloid neurons and how theta activity can be regulated on the cellular level. Another open question was whether synchronized theta oscillation relates to the expression of conditioned fear, the formation of the memory, or a combination thereof. In the present article, we review some of the previous evidence and present new data on the regulation of theta oscillations in amygdaloid neurons, through the intracellular adenylyl cyclase/cAMP system, which may link the cellular oscillators to cholinergic input systems fostering theta activity at the network level. Novel data also indicate that theta synchronization at the amygdalo-hipppcampal network level occurs during long-term stages of fear memory but not during short-term stages of fear memory.

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MATERIALS AND METHODS

All experiments were carried out in the accordance with the European Communities Council Directive (86/609/EEC) and approved by the Regierungspräsidium Dessau (NR 42502/2–411 UniMD).

In Vitro Experiments

Coronal slices (400 μm thick) of the amygdala were prepared from rat brains (male Wistar, 3–8 weeks old) under halothane anesthesia, as described previously (Pape et al., 1998; Szinyei et al., 2000). The slices were maintained in an interface type recording chamber at (35 ± 1)°C during continuous perfusion (1–1.5 ml/min) in an ACSF containing 126 mM NaCl, 2.5 mM KCl, 2 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, and 10 mM dextrose, buffered to a final pH 7.4 through continuous perfusion of 95% O_2–5% CO_2. Intracellular recordings were made with glass micro electrodes pulled from thin-walled capillaries (TW-100F, World Precision Instruments, Sarasota) and filled with 2 M potassium acetate (resistance 40–80 MΩ). In some experiments, biocytin (1%) was added to the electrode solution. Recorded signals were amplified by an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA) with head stage gain at 0.1. Recordings were performed in bridge mode, with the bridge balance being continuously monitored. Only data obtained from cells with membrane resting potential more negative than −50 mV, overshooting action potentials and input resistance higher than 40 MΩ (determined from electrotonic responses to hyperpolarizing current steps of 500 ms duration) were collected for analysis. Spike firing patterns and oscillatory properties were assessed through responses to depolarizing currents steps of 500 ms duration at different amplitudes and slow depolarizing current ramps (90 pA/s; maximal depolarization to −20 mV) injected at different holding potentials. The time course of the hyperpolarizing after potential (AHP) was best approximated by exponential fits according to the equation

$$Y(t) = A_0 + A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$$

with $Y(t)$ being the amplitude of the AHP at time t, and A and t being amplitude coefficients and time constants, respectively. Pharmacologically active substances were dissolved in ACSF and applied by local superfusion to distinct areas on the exposed surface of the slice through pipettes with a larger tip diameter (10–20 μM), by pulses of low pressure (Picospritzer II, General Valve, Fairfield, NJ). 8-br-cAMP and rp-cAMPs were obtained from Sigma. Slices containing biocytin-labeled cells were fixed (2% paraformaldehyde), transferred to 20% sucrose in 0.2 M phosphate-buffered saline (pH 7.4), frozen, and resectioned at 80 μm. Sections of the amygdaloid complex were incubated with avidine-biotine-horseradish peroxidase complex solution (ABC kit; Vector Labs, Burlingame, CA) and processed to reveal the intracellular biocytin staining. Values are given as mean ± SD. Statistical significance was proven with the t-test or with the Mann-Whitney U-test, as applicable, with $P < 0.05$ considered significant.

In Vivo Experiments

Under deep pentobarbital anesthesia (50 mg/kg intraperitoneal), steel electrodes were implanted into the left hemisphere of adult male C57B/6 mice, aiming at the pyramidal layer of the hippocampal area Cornu Ammonis 1 (CA1) (AP-1.94 mm, L-1 mm from bregma, and DV-1.25 mm from the brain surface level) and the lateral amygdala (AP-2.06 mm, L-3.25 mm from bregma, and DV-3.2 mm from the brain surface), as described previously (Seidenbecher et al., 2003). Silver electrodes were implanted close to the midline over the nasal and cerebellar region for reference and ground, respectively. Electrode location was always verified histologically. For auditory cued conditioning, animals were adapted twice to the training context combined with a set of six neutral acoustic stimuli (CS−; 2.5 kHz, 85 dB, for 10 s, inter stimulus interval 20 s). On the next day, animals were exposed to three conditional stimuli (CS+; 10 kHz, 85 dB, 10 s) that each coterminated with an aversive US (scrambled foot shock, 0.4 mA, 1 s). Training was repeated once. Behavioral and electrophysiological responses were recorded for 2 min during the training ("immediate response"), and 2 h later to a CS+ presented in a neutral context ("short-term memory"). Retrieval and specificity of "long-term memory" were tested with a set of each four CS− and CS+ in the neutral context, 24 h after training. For foreground context conditioning, animals were trained in the same context, but without pre-exposure or acoustic signaling. Mice underwent three training sessions with each six unsignaled US at interstimulus intervals of 20 s, which were identical to those used during cued training. Freezing responses were recorded for 2 min during training ("immediate response") and 30 min later in the training context ("short-term memory"). On the second day, responses were recorded in a single 8 min exposure to the training context ("long-term memory"). For detailed off-line behavioral evaluation, a time line version of the public domain program Wintrack was used; freezing (complete immobilization except respiratory movements) and risk assessment behavior (alert observing and stretched attending) were measured as behavioral parameters of fear memory. Recorded field potentials were fed through a differential amplifier, filtered, and stored on magnetic tape for off-line analysis of behaviorally associated activities. Field potential waveforms were analyzed using the Spike2-software package. By keeping the second maximal positive peak as a fix point, 3 s periods were taken for the analysis of freezing-related correlation analysis and 10 s periods of CS− and CS+ were taken for stimulus-related correlation analysis. Statistical analyses of electrophysiological and behavioral data were done with Student's unpaired t-test, keeping the criterion for significance at $P < 0.05$.

RESULTS AND DISCUSSION

Control of Theta Oscillatory Activity in Amygdaloid Projection Neurons Through cAMP

The basic synaptic circuitry in the LA processes afferent sensory signals carrying CS and US information. Within the LA,

there are two major types of neurons (which can be further grouped): principal cells, whose axons leave the local synaptic environment (hence termed projection neurons), and GABAergic local circuit neurons (termed inhibitory interneurons), which mediate inhibition of principal cells through both feed forward and feedback pathways (Washburn and Moises, 1992; Weisskopf and LeDoux, 1999; Szinyei et al., 2000). In earlier studies, we had found that LA projection neurons in various mammalian species (mouse, rat, guinea pig, and cat) can produce rhythmic-oscillatory electrical activity and resonance behavior, both in vivo (Paré et al., 1995) and in the slice preparation in vitro (Pape et al., 1998). Two types of oscillations, termed low-threshold and high-threshold, were discerned, which prevail at levels of the membrane potential subthreshold and suprathreshold to spike generation, respectively. The oscillations are intrinsic in nature in that they are generated through the periodic interaction of functionally opposing sets of membrane currents: a persistent Na^+ current and an M-type K^+ current mediate low-threshold oscillatory activity, whereas high threshold oscillations result from activation of Ca^{2+} channels and Ca^{2+}-dependent (as well as voltage-gated) K^+ channels (Pape and Driesang, 1998). The oscillations occur at the theta frequency range (4–(1)2 Hz) and functionally interact, thereby shaping the action potential output of the projection neurons toward activity at the theta frequency. At a first glance, these results seemed to be at odds with the more traditional classification of amygdaloid projection neurons into accommodating and regular firing types, depending on the individual spike pattern generated upon depolarizing current injection (Washburn and Moises, 1992; Rainnie et al., 1993). In addition, more recent studies indicated that there is a continuum of cells displaying the various spike patterns rather than two distinct classes (Faber et al., 2001), and that these patterns are controlled through cAMP-regulated Ca^{2+}-dependent K^+ currents (Faber and Sah, 2002).

In view of these results, we investigated the effects of the adenylyl cyclase/cAMP system on oscillatory properties in LA projection neurons, using standard intracellular recording techniques in rat LA slices in vitro (Pape et al., 1998). Projection neurons were identified through morphological criteria, in particular pyramidal-like cell bodies and spiny dendrites (Fig. 1A), and electrophysiological properties as described previously (Rainnie et al., 1993; Faber et al., 2001). In the population of recorded cells, a majority ($n = 61$) showed spike frequency accommodation (thence termed A-type cells) upon injection of depolarizing current steps. The remainder ($n = 23$) were regular firing cells (R-type) generating tonic series of action potentials upon maintained depolarizing current stimuli (Fig. 1B). In essence, these properties matched with those described previously of the two classes of amygdaloid projection neurons (Rainnie et al., 1993; Szinyei et al., 2000; Faber et al., 2001). Membrane resting potential, input resistance, and single spike properties were not significantly different between the groups of cells. Major differences were observed between the hyperpolarizing after potential (AHP) following termination of the depolarizing current step. The AHP was described by a two exponential function, with the two components making a significantly different contribution in the two groups of neurons. In R-type neurons, the fast ($t_1 = 0.17 \pm 0.75$ s) and slow component ($t_2 = 1.70 \pm 0.76$ s) contributed 59 and 41% to the overall AHP, whereas in A-type neurons a very slow component ($t_2 = 5.11 \pm 0.76$ s) was dominating (65%) the fast component ($t_1 = 0.18 \pm 0.60$ s). Pharmacological manipulation of the intracellular cAMP level resulted in a significant alteration of the slow component of the AHP and an associated change in firing pattern. Local application of 8-br-cAMP (1 mM), a membrane permeable analogue of cAMP, in A-type neurons ($n = 5$) resulted in a significant *reduction* of the very slow AHP component (Fig. 1C). Under these conditions, the slow component contributed only 31% to the AHP, and its time constant ($t_2 = 1.60 \pm 0.75$ s) was similar to those in R-type neurons under control conditions, whereas the time course of the fast component was unaltered (0.14 ± 0.75 s). Moreover, regenerative activity was shifted toward regular firing patterns (not shown). In conclusion, an increase in intracellular cAMP concentration seemed to change the properties of A-type neurons toward those of R-type neurons. Vice versa in R-type neurons, a decrease in the effects of ambient cAMP through blockade of protein kinase A activity through rp-cAMPs evoked A-type properties. More specifically, local application of rp-cAMPs (50 μM) in R-type neurons ($n = 5$) resulted in a significant *enhancement* of the slow AHP (Fig. 1C) in that its relative contribution to the overall AHP was increased to 58%, and its time constant was increased toward that of the very slow component found in A-type neurons under control conditions (2.97 ± 1.22 s). The time course of the fast component remained unaltered (0.15 ± 0.73 s), and the cells displayed spike frequency accommodation upon maintained depolarizing stimuli (not shown). Most importantly, these properties were tightly linked to oscillatory membrane behavior. In none of the A-type neurons ($n = 61$) did we observe high threshold theta oscillations under control conditions, whereas all cells tested during action of 8-br-cAMP displayed stable membrane potential oscillations ($n = 5$; Fig. 1D). Likewise, high threshold oscillations typically occurring in R-type cells ($n = 23$) were reversibly blocked upon inhibition of protein kinase activity by rp-cAMPs ($n = 5$; Fig. 1D).

These results corroborate the previous notion that the firing pattern in LA projection neurons is regulated through the intracellular cAMP system acting on a slow AHP current (Faber et al., 2001). They make the important addition that also oscillatory membrane properties are controlled through the cAMP system (most likely acting via the slow AHP current, which functionally counteracts the membrane currents mediating the oscillation). They further let us conclude that theta-type oscillations are fostered during states associated with increased levels of intracellular cAMP, such as those that occur upon increased stimulation of muscarinic or monoaminergic receptors (Faber and Sah, 2002) associated with increased activity of ascending brainstem transmitter systems (Steriade and McCarley, 1990).

Synchronized Theta Activity in Amygdalo-Hippocampal Networks During Retrieval of Long-Term Fear Memory

The functional importance of these oscillatory cellular properties on an extended network level is indicated by findings of synchronized-oscillatory in temporolimbic circuits during various behavioral states. Synchronized oscillatory activities are considered mechanisms of network communication in the brain, and rhythms in the theta frequency range have been shown to be of particular relevance to different states of information processing and behavior (Klimesch, 1996; Klimesch et al., 1997; Buzsaki, 2002; Buzsaki et al., 2003). In fact, an earlier report on fear conditioning-induced neural plasticity detected increased synchronization of rat lateral amygdala neurons after fear conditioning (Quirk et al., 1995). This was later confirmed in amygdala-perirhinal networks (Paré et al., 2002) as well as in the LA of freely behaving cats during periods of US anticipation (Collins and Paré, 2000). Recently, we reported on a synchronization of neural activity in the LA with rhythmic theta oscillations in the CA1 subfield of the hippocampus during the retrieval of Pavlovian fear memory in mice, and the specific association of such synchronized activity patterns with the expression of conditional freezing behavior (Seidenbecher et al., 2003). Mice were used as experimental subjects, opening exciting perspectives for the consideration of molecular and genetic mechanisms. The experimental paradigms were chosen after having carefully determined behavioral parameters of conditioned fear in mice and training parameters that account for fear memory generalization and sensitization (Laxmi et al., 2003). In brief, mice were fear conditioned through explicitly paired presentation of conditioned (CS+) and unconditioned (US) stimuli and their responses during fear memory retrieval were compared with those of control animals undergoing explicitly unpaired training. Conditioned freezing behavior was monitored in the retrieval session 24 h after training so as to assess fear memory and the emotional relevance of the CS+ and an indifferent control stimulus (CS−). By comparison, risk-assessment behavior (i.e., overt orienting and stretched attending) was examined as a control measure of species-specific defensive behavior with minimum locomotor activity. Simultaneous with the behavioral assessment, electrophysiological activity was determined through recording of field potentials in both the LA and the CA1 of the dorsal hippocampus. In control animals, activity in CA1 was distributed around the theta frequency, but no prominent pattern of activity was observed

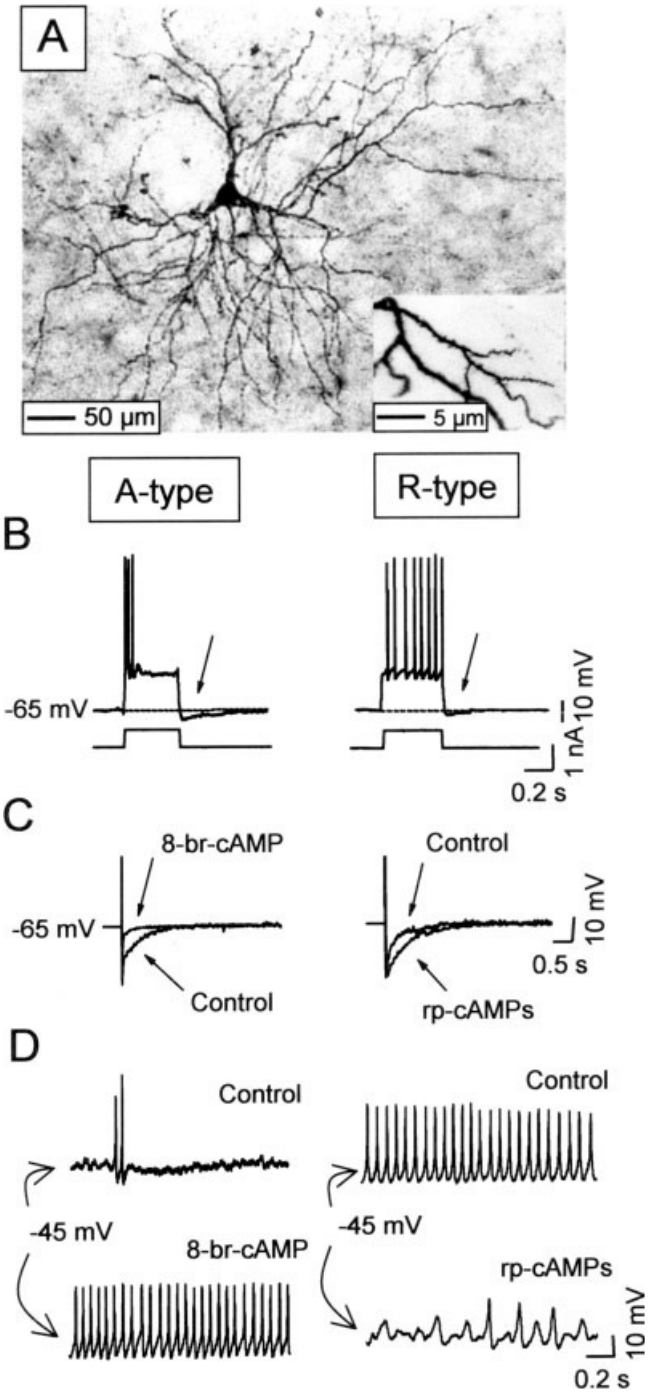

FIGURE 1. Properties of projection neurons in the rat LA. A: Morphological characteristics, after intracellular labeling with biocytin. Note pyramidal-like cell body and spiny dendrites (inset). B–D: Projection neurons can be grouped into accommodating (A-type; left column) and regular firing (R-type; right column), based upon electrophysiological properties. B: Responses to depolarizing current steps (bottom traces) injected at resting potential (as indicated near traces) with spike frequency accommodation (A-type) and tonic series of spikes (R-type). Note slow hyperpolarizing after potential (AHP) in A-type, but not in R-type neuron (indicated by arrow). C: Slow AHP observed under control conditions in A-type neuron is blocked through local application of 8-br-cAMP (1 mM). In R-type neuron, application of rp-cAMPs (100 µM) promotes slow AHP. D. Maintained depolarization (membrane potential as indicated) is associated with single spikes in A-type and oscillatory activity in R-type neuron. Application of 8-br-cAMP (1 mM) uncovers oscillatory activity in A-type, and rp-cAMPs blocks oscillatory activity in R-type neuron. Intracellular recordings are from the same A- and R-type neuron, respectively.

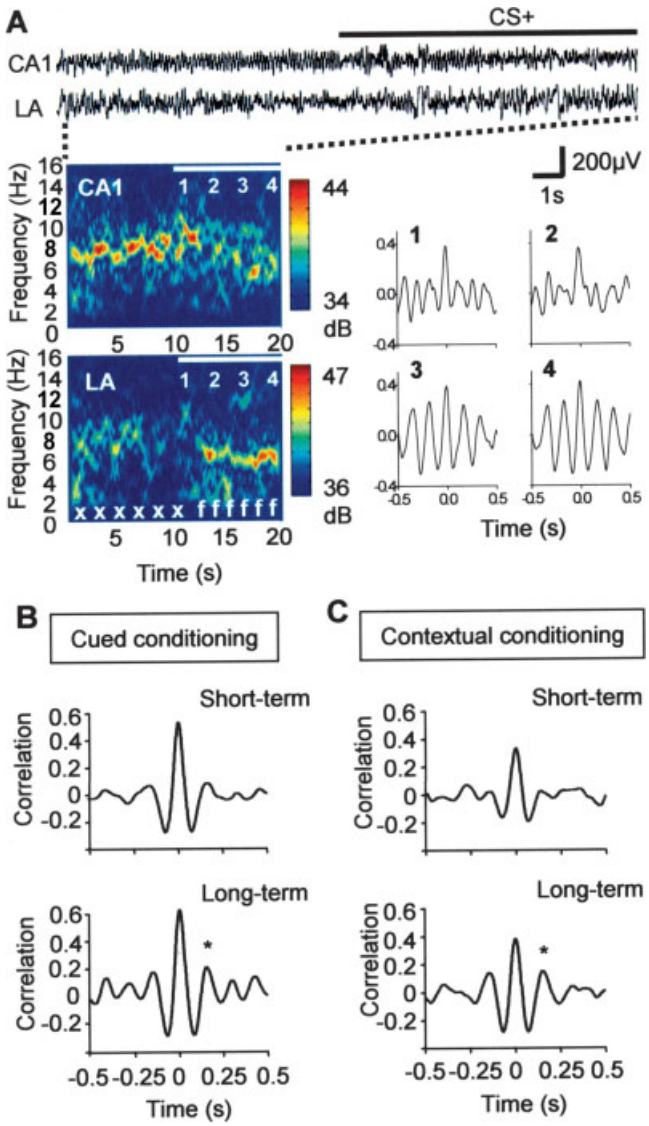

FIGURE 2. Neural activity in the CA1 and the LA during retrieval of fear memory in mice. A: Original traces of field potential recordings obtained in the CA1 and LA, as indicated, before and during presentation of the fear conditioned stimulus (CS+; presentation time marked by horizontal line) in a retrieval session (24 h after conditioning). Color-coded power spectra of the field potential recordings demonstrate theta activity at around 8 Hz in the CA1 during exploratory behavior (x) before CS+ presentation, and no dominant pattern of activity in the LA. CS+ presentation is associated with theta activity at 5–(6) Hz in both areas and the expression of freezing behavior (f). Cross correlograms of activities in CA1 and LA calculated at four successive 3-s intervals during CS+ presentation (start/end at 1s before/after the stimulus) reveal a progressive increase of correlated theta activity. B: Averaged cross correlograms of neural activity in CA1 and LA of auditory cued conditioned animals (n = 6), calculated during CS+−induced freezing during short-term and long-term memory retrieval. Note theta synchronization at around 5 Hz associated with long but not short term fear memory. C: Averaged cross correlograms of neural activity in CA1 and LA calculated during freezing behavior in retention tests after foreground contextual conditioning (n = 5). Similar to the cued conditioning, a significant increase in theta synchronization occurs during long-term as compared with short-term memory retrieval. ∗ denotes significant differences in the degree of synchronization (measured at the second positive peak in the cross correlograms) between long-term and short-term states. Part A modified from Seidenbecher et al., 2003.

in the LA (not shown; Seidenbecher et al., 2003). In fear-conditioned animals, theta activity prevailed in the CA1, and activity in the LA before and during CS− presentation resembled that in control animals. However, upon presentation of the CS+, activity in the LA shifted into a highly rhythmic pattern centered at the theta frequency band (Fig. 2A). Cross-correlation analyses revealed a progressive increase in synchronized activity at a frequency of 4–8 Hz (representing atropine-sensitive type 2 theta; Bland, 1986; Vanderwolf, 1988; Vinogradova, 1993), which progressively developed during presentation of the CS+ (Fig. 2A). By taking the second positive peak of the cross correlogram as a quantitative measure, a significant increase in theta synchronization could be demonstrated in fear-conditioned animals (n = 8) compared with control animals that had received explicitly unpaired training (n = 6). A partial, nonsignificant increase in theta synchronization was observed in fear conditioned mice during presentation of the CS−. Furthermore, freezing of fear-conditioned mice during the CS+ was associated with significantly stronger theta synchronization than the risk-assessment response of controls (data not shown; Seidenbecher et al., 2003). Theta activity at 8–14 Hz (theta 1) was observed during exploratory behavior in CA1, but not in LA, which argues against volume transmission as a major element of theta synchronization between these two areas during conditioned fear responses (data not shown; Seidenbecher et al., 2003).

What remained unclear from our previous study was whether this pattern of neuronal activity relates to the expression of conditioned fear, the formation of the memory, or a combination thereof. Therefore, we investigated the development of rhythmically synchronized activities in the amygdalo-hippocampal circuitry at different stages of fear memory formation. Following adaptation to a neutral auditory stimulus (CS−), mice (n = 6) were fear conditioned through the explicitly paired presentation of CS+ and US. Their behavioral responses to the CS+ were compared 2 min ("immediate"), 2 h ("short-term"), and 24 h ("long-term") after conditioning, and electrophysiological activity (field potential recordings) in CA1 and LA was simultaneously assessed. Presentation of the CS+ evoked pronounced freezing responses at all stages (immediate, short-term and long-term; not shown). The duration of CS+−evoked freezing was not significantly different at the various response stages, indicating a similar level of fear. Despite similar freezing responses, significant differences were observed in neurophysiological activity in the CA1-LA pathways. During short-term fear memory retrieval, no prominent change in activity patterns in the LA and the CA1 was observed before and during presentation of the CS+, and cross-correlation-analyses did not show any significant synchronization of activity between the two brain areas (Fig. 2B). By comparison, freezing during long-term fear memory retrieval was associated with a shift in activity in both the LA and the CA1 toward a highly rhythmic pattern centered at around the theta frequency band in the same animals, and cross-correlation analysis revealed a strong increase in synchronized activity at a frequency of 4–8 Hz during freezing

(Fig. 2B). On average, a significant increase in theta synchronization could be demonstrated during long-term fear memory retrieval, compared to immediate responses and short-term fear memory retrieval. Results similar to those from cued conditioning experiments were obtained upon foreground contextual conditioning ($n = 5$), in that freezing behavior upon US application or upon short-term fear memory retrieval in the shock context was not associated with synchronized activity in the LA / CA1, whereas freezing during long-term fear memory retrieval was associated with a significant increase in theta rhythm synchronization in these pathways (Fig. 2C). It is important to add that (i) the level of freezing was similar at the various stages, (ii) fear behavior or rhythmic activities in LA /CA1 were not observed in the neutral context, and (iii) risk assessment behavior was not associated with significant theta synchronization, neither in the shock nor in the neutral context (data not shown).

These data indicate that theta rhythm synchronization in CA1/LA circuits is specifically associated with long- but not short-term fear memory retrieval nor expression of fear behavior per se.

CONCLUSIONS

In conclusion, projection neurons in the LA display a continuum of basic spike patterns in response to maintained depolarizing stimuli, including accommodating patterns, regular firing and oscillatory activity at theta frequencies. The firing pattern is controlled to an important part by the intracellular cAMP system, in that an increase in intracellular cAMP concentration facilitates regular firing and theta oscillations, presumably through a reduction of a functionally counter acting slowAHP current. The intrinsic oscillatory properties of amygdaloid projection neurons, in turn, seem to provide adequate recurring time windows for the facilitated integration of synaptic inputs at theta frequencies. Synchronization of theta rhythms (type 2) occurs in amygdalo-hippocampal pathways during retrieval of conditioned fear, may support synaptic signal transfer between functionally connected neuronal populations during these periods, and thereby reflect a neurophysiological mechanism related to long term fear memory in these pathways. This conclusion is supported by the findings that muscarinic receptors are involved both in the generation of type 2 theta rhythms (Bland, 1986; Vanderwolf, 1988; Vinogradova, 1993; Buzsaki, 2002) and in the control of the slow AHP current (Faber and Sah, 2002) facilitating theta oscillations in LA projection neurons. As a corollary of this, the cells and systems in the amygdalo-hippocampal pathways seem to be prone to oscillate at theta frequencies during increased activity of the cholinergic system, as occurs during periods of increased arousal and attentiveness (Steriade and McCarley, 1990) and is important in long-term memory consolidation (Power et al., 2003).

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