Postnatal Development of Rat Hippocampal Gamma Rhythm In Vivo

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INTRODUCTION

The current interest on gamma-band (20–100 Hz) (Bragin et al. 1995; Whittington et al. 1997) network oscillations stems from the suggested role of oscillatory synchronization in sensory encoding, cognitive functions and synaptic plasticity (Traub et al. 1998). Neocortical gamma oscillations occur, e.g., in response to sensory stimuli (Jefferys et al. 1996; Singer 1999), while in the rat hippocampus, theta-associated gamma oscillations are most prominent during exploratory activity, attentive immobility, and rapid-eye-movement (REM) sleep (Bragin et al. 1995; Buzsáki et al. 1983; Freund and Buzsáki 1996). Despite the apparent importance of cortical gamma oscillations, their ontogeny has remained unelucidated.

In altricial species (species born in an immature state), early postnatal sleep has been thought to be undifferentiated in terms of electroencephalography (EEG) (Gramsbergen 1976). More specifically, in rats, EEG-based classification of slow-wave (SWS) and REM sleep has been thought to be possible only after the second postnatal week (Frank and Heller 1997; Gramsbergen 1976). During the “pre-EEG” period, sleep classification is based only on behavioral criteria: Active sleep (AS) is characterized by irregular breathing, intermittent muscular twitches and rapid eye movements, whereas during quiet sleep (QS), the animal is lying still and shows a very regular pattern of respiration (Hilakivi and Hilakivi 1986). It has been suggested that AS and QS represent immature forms of REM sleep and SWS, respectively, yet this view has recently been challenged (Frank and Heller 1997). AS is the dominant form of sleep in rats immediately after birth and its amount declines sharply by the end of the second postnatal week (Jouvet 1980). This parallels with the timing of rapid synaptic development in the cortex, after which the rat pups open their eyes and reach a degree of motor maturity.

Spontaneous bursts of correlated neural activity are a typical feature of the developing CNS, and they are implicated in the formation of synaptic contacts (Crair 1999). It has also been long hypothesized that the intense cortical activity during AS may be essential for the development and maintenance of cortical circuitry—thus explaining the high amount of AS in the newborns of altricial species (see Jouvet 1980; McCormick 1999). In the newborn rat hippocampus in vitro, spontaneous electrical activity is characterized by periodical synchronous bursts (for reviews, see Ben-Ari 2001; O’Donovan 1999). In the area CA3, these developmentally regulated events are seen as brief oscillations at gamma frequencies (Palva et al. 2000). However, it is not known whether such activity exists in the developing hippocampus in vivo. Here we have addressed the developmental profile of rhythmic population behavior in the area CA3 in the newborn rat hippocampus in vivo as well as the relationship of the hippocampal EEG activity with the early sleep stages, QS and AS. Parts of the work have been presented in an abstract form (Lahtinen et al. 1999).

METHODS

The animals used were Wistar rat pups of the postnatal day (P) 4–6 at time of the electrode implantation (the day of birth is referred to as day P0). They were operated under deep hypothermia using a miniaturized hypothermic instrument attached to Kopf’s stereotaxic frame,

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a system developed especially for the surgery of neonatal rats (Cunningham and McKay 1993). A tungsten field potential electrode (30 \( \mu \)m) was implanted into the hippocampus, and the tip of the electrode was aimed at the pyramidal cell layer in the CA3 region through a hole in the skull (2.0–2.5 mm posterior, 2.0 mm lateral, and 2.0–3.0 mm ventral to the Bregma). Stainless steel screws attached into the bone above the cerebellum served as reference and ground electrodes. The electrodes were fixed with dental acrylate. The operated pups were always kept together with their mothers and the rest of the litters (5–10 pups) except at the time of surgery and recovery (~45 min) and the daily recording sessions (~1 h) starting from the day following surgery. The animals were housed with free access to food and water in a temperature (20 ± 2°C; mean ± SD)– and light (0700–2100)–controlled environment. During recording sessions, the EEG, static charge sensitive bed (SCSB), and respiration sensor (see following text) data were amplified and filtered (0.3–300 Hz) by preamplifiers (a purpose-built EEG-preamplifier, and Biorec BA-8R, Finland) and digitized with a National Instruments AD-board and LabView-based software for later off-line analysis. After the recordings, the rat was anesthetized, and the brain was dissected out. A horizontally cut block of the brain containing the dorsal hippocampus was fixed in 4% paraformaldehyde and embedded in paraffin. Electrode position verification was done under light microscopic evaluation from Nissl-stained coronal sections (10 \( \mu \)m).

Time-frequency analysis

For the time-frequency analysis, the EEG-signal was convolved with a Gabor wavelet: 
\[ h(t,f) = k \exp(-\pi^2/2 + imx), x = 2\pi it/m, \]
where time and frequency are denoted with \( t \) and \( f \), \( m = 9, i \) is the imaginary unit, and \( k \) is the normalization constant. Modulus of the complex valued outcome represents the amplitude of the signal at a narrow frequency band as a function of time (Sinkkonen et al. 1995). To cover the frequency band from 1 to 100 Hz, the center frequency \( f \) of the wavelet was varied in small steps of 1 Hz and the convolution was carried out 100 times. The time-frequency as well as amplitude-spectrum analyses were computed with LabView-based software.

Sleep-wake recordings

In the newborn (<10 days) rats the states of vigilance can be classified by behavioral criteria. Neonatal sleep-wake recordings at P (postnatal day) 5–10) were performed using the SCSB method (Hilakivi and Hilakivi 1986, Taira et al. 1990). In brief, movements of the animal induce a redistribution of static charge in the conducting layers inside the mattress, which are transduced into potential differences. A piezoceramic movement-sensitive breath sensor (BS) was attached to the animal’s abdominal skin to record respiratory movements. The sleep-wake behavior was classified into three different states: awake (W), QS, and AS. In W, the animal is moving actively, and both SCSB and respiratory recordings show irregular, very large amplitude activity. During the QS, the rat is mostly still although changes in body posture and startle responses occur intermittently. In AS, trunk muscle tone is lost, and the rat is in a recumbent position. Small amplitude twitches occur in the whiskers and extremities, and the respiration is highly irregular. SCSB recordings show repetitive, short-lasting activity peaks, and the respiration sensor reveals irregular, low, and medium amplitude activity. The rat pup was kept on the SCSB under a Plexiglas cylinder for 60 min at a time. The recorded data were visually scored in 20-s epochs. Transitions between states or otherwise ill-defined segments were put into “uncertain” (UC) category. All surgical, handling and animal housing procedures were approved by the Committee for the Welfare of Laboratory Animals of the University of Helsinki.

Results

An intrahippocampal EEG electrode was implanted into a total of eight rat pups of which seven survived to the day following surgery. In three of seven animals, we did not find evidence for rhythmic population activity at P5–P10, and a post mortem inspection of the electrode locations revealed that the electrode tips in these animals had been located in the hippocampal fissura, dentate gyrus, or stratum radiatum. The electrodes of the remaining four animals, hereafter referred to as animals A–D, were located in the pyramidal cell soma layer of the CA3 region. These four animals showed similar patterns of EEG activity during the course of development. Simultaneously with the hippocampal EEG, we monitored the behavioral state of the newborns (see Methods).

Time-frequency characteristics of spontaneous activity

First, we inspected all recordings visually with Gabor-wavelet based time-frequency representations (TFRs) of the EEG signal. Overall two trends were seen; the broadband signal amplitude increased dramatically during development (\( P < 0.0001 \) in 4/4 rats, 1-way ANOVA) and was greater during AS than during QS (\( P < 0.0001 \) in 4/4 rats, Fig. 1, see also Fig. 3). Also the interaction between sleep state and age was significant in all animals (\( P < 0.0001 \), 2/4 rats, \( P < 0.05 \), 4/4 rats, 2-way ANOVA). The TF analyses further revealed that, already at P5, brief (<500 ms) bursts of gamma oscillations occurred during AS and, to a lesser extent, also during QS (Fig. 1, top). At P6 and later, the bursts rapidly gained amplitude and were more and more confined to AS (Fig. 1, middle). The bursts also progressively occurred closer to each other forming longer periods of gamma activity so that, at P10, the gamma rhythmicity was close to being continuous (Fig. 1, bottom). Further, the onsets and offsets of longer periods (>5 s) of gamma activity reliably marked the switches from QS to AS and from AS to QS, respectively (Fig. 1, middle and bottom). These findings are well in line with the known defragmentation of AS during the early postnatal development in rat (c.f. Hilakivi and Hilakivi 1986) thus further supporting the idea of an association between gamma oscillations and AS.

Emergence of stable gamma oscillations during AS

To compare the frequency distributions of AS and QS, we divided the EEG signal in segments of 5 s into three categories (AS, QS, and W) according to the behavioral state of the animal (see Methods). We then computed amplitude spectra for the segments and averaged them within the categories. Initially, at P5, the spectra of both AS and QS were smooth and 1/f-like over 1 to 100 Hz, indicating the lack of macroscopically well-organized rhythmicty on any predominant frequency band (Fig. 2A). Already at P6, however, a small but discernible peak between 20 and 30 Hz appeared in AS but not in QS (Fig. 2A). In line with the TF analyses, the gamma peak grew larger and extended to higher frequencies during P6–P10, but remained confined to AS (Fig. 2A). At P8, an another peak ~3–5 Hz appeared and was enhanced during development. Similarly to gamma oscillations, these low-theta frequency oscillations appeared only in AS.

The developmental appearance of AS-linked theta-gamma oscillations is demonstrated in pooled data from all animals.
The difference between the amplitude spectra from QS and AS (black line, averaged data from 4 rats) illustrates the stability of the 30-Hz peak as well as demonstrates the emergence of theta rhythmicity at around P8. Narrowband gamma and theta oscillations thus increased in magnitude during development and gradually became distinct indicators of AS. Broad-band activity in the range of 1–100 Hz, on the other hand, behaved in the opposite way. At P5, the level of broadband activity was considerably greater in AS than in QS, but this difference diminished systematically toward P9–P10 (Figs. 2 and 3).

Intermittent small twitches and irregular breathing are behavioral hallmarks of AS and are not detected in QS (Frank and Heller 1997; Gramsbergen 1976). To confirm that the gamma oscillations found in AS were not artifacts generated by volume conduction of muscular activity, we compared AS with the W condition, in which considerable continuous muscle activity is always observed (this is one of the classification criteria for W). This muscle activity, however, did not give rise to a comparable gamma-frequency peak in the amplitude spectra, although the level of broadband activity was dramatically greater in W than in AS (Fig. 2B, gray line = W − QS). Finally, to confirm that the developmental enhancement of gamma-band activity was specific to AS, we estimated the 99.9% percentile (3 SD) of the amplitude distributions of broad- and gamma-band (here 24–40 Hz) filtered signals for AS and QS. Indeed, for AS, the ratio of gamma/broadband amplitude increased with age in all 3 animals from which we were able to make recordings up to P10 ($P < 0.05$, 1-way ANOVA, Fig. 3C). For QS, on the other hand, apart from the

(Fig. 2B).
initial increase in two animals recorded between P5 and P6, there were no consistent changes in the gamma/broadband ratio between P6 and P10 (Fig. 3D).

DISCUSSION

Postnatal organization of spontaneous broadband activity into gamma and theta rhythms

Our data indicate two trends in the developmental profile of the spontaneous mass activity in the neonatal rat hippocampus. First, narrowband rhythms at gamma and theta frequencies emerge progressively at P6 and P8, respectively. Especially after the first postnatal week, the gamma rhythmicity was a reliable indicator of behavioral AS, whereas QS was characterized by smooth amplitude spectra throughout P5–P10. Second, the magnitude of broadband activity increased during the course of development. Initially at P5, the level of broadband activity was considerably greater in AS than in QS, but this difference diminished toward P10. Large-amplitude broadband activity and narrowband gamma and theta oscillations are thus complementary characteristics of AS.

The increase in the overall broadband amplitude with age indicates that between P5 and P10, a rapid development of cooperative mass activity is taking place in the area CA3 of rat hippocampus. This finding is in line with a previous report on the developmental increase of broadband activity in the area CA1 in rat (Leblanc and Bland 1979). Intriguingly, some days after the emergence of gamma rhythmicity, theta oscillations appeared during AS. Hippocampal theta activity has been previously shown to develop slightly later in the CA1 and concomitantly with motor abilities (starting at P10) (Leblanc and Bland 1979). Thus the appearance of theta-band activity in CA3 seems to precede that of in CA1. Moreover, whereas joint theta and gamma oscillations characterize both exploratory and REM sleep states of adult rats, they seem to hallmark behavioral AS in the neonatal rat.

In the adult rat brain, the gamma frequency power has been reported to be highest in the dentate hilus (Bragin et al. 1995).

FIG. 2. A: at P6, a peak ~30 Hz emerged in averaged amplitude spectra of EEG epochs during AS but not during quiet sleep (QS). Thirty minutes of continuous recording from each day was divided in 5-s epochs into 3 categories [awake (W), QS, and AS]. Amplitude spectra were computed for the epochs and averaged within categories. Note also that the amplitude of broadband activity during AS (black line) was larger than during QS (gray line). The data are from one representative animal. B: hippocampal gamma and theta oscillations emerge concomitantly during AS in neonatal rat. Subtracting the amplitude spectra of QS from AS (black line) and averaging the results from 4 rats show the stability of the 30-Hz peak as well as demonstrate the emergence of theta rhythmicity at P8. There were no comparable peaks in the gamma frequency range in the W category (gray line) although the amplitude of broadband activity was larger in W than during AS.
However, when the hilar gamma activity was abolished by the removal of the entorhinal cortex, the dominant source of the gamma activity was confined to the CA3 region. Thus in the neonate hippocampus, long-range synaptic contacts may not yet be mature enough for the entorhinal region to overdrive the CA3-generated gamma rhythm. This idea is also supported by a number of anatomical studies (for a review, see Wyss and van Groen 1989). The granule cells of the dentate gyrus, to which the efferents from the entorhinal cortex make synapses, mature latest in the hippocampal formation. In rats, the outside-in gradient, i.e., the production of neurons from the superficial to the deeper layers of the granule cells, is still taking place until the third postnatal week (see Witter 1989). It should be noted, however, that in vitro hippocampal gamma oscillations can be seen even earlier than P5, although it is evident that these early gamma bursts are spatiotemporally rather restricted (see Palva et al. 2000), thus making them difficult to be detected in EEG recordings in vivo. Further, the synchronization mechanisms in the <P5 rat hippocampus are likely to be different from those in the adult (c.f. Lamsa et al. 2000) and thus there may also be a qualitative shift in gamma oscillations during the first postnatal week. Interestingly, a rapid conversion of depolarizing GABA<sub>A</sub> receptor-mediated responses to hyperpolarizing takes place around P5–P8 in the pyramidal neurons of rat hippocampus (Rivera et al. 1999). Hyperpolarizing GABA has been suggested to be a prerequisite for the synchronization of the hippocampal network to gamma frequencies (see Traub et al. 1997; but see also Lamsa and Taira 2001), thus possibly underlying the appearance of stable gamma oscillations also in EEG recordings in vivo by the end of the first postnatal week. Further, the slow kinetics of GABA<sub>A</sub> receptor-mediated inhibitory postsynaptic currents in developing hippocampus could explain the relatively low (≈30 Hz) gamma frequencies seen here (see Hollrigel and Soltesz 1997; Palva et al. 2000). Thus the present data do not exclude the possibility that the in vivo gamma activity is present in the CA3 even earlier than P5. This is obvious still after the notion that the gradual appearance of the gamma oscillations as well as their low frequency at P5 may be partially due to the recovery of hippocampal tissue from the electrode-implantation-induced damage (Soltesz and Mody 1995).

### Hippocampal gamma oscillations and active sleep

Interestingly, the CA3 gamma oscillations in the newborn hippocampus were confined to the periods of behavioral AS. According to previous reports, AS and QS remain undifferentiated in neocortical EEG recordings until the end of the second postnatal week. Thereafter desynchronized cortical EEG and myoclonia-coupled theta (4–7 Hz) during AS start to develop giving rise to adult-like REM sleep (Frank and Heller 1997). It has been recently suggested that AS bears only phenomenological similarity to the adult REM sleep and is more likely related to the spontaneous fetal activity (SFA) typical of the immature nervous system (Adrien et al. 1984; Robertson and Smotheran 1990). The idea is supported by the fact that neither AS or SFA are attenuated by brain stem lesions known to inhibit REM sleep (Adrien et al. 1984; Robertson and Smotheran 1990). Further, it was proposed that AS can be considered as an undifferentiated behavioral state from which both SWS and REM sleep develop (Frank and Heller 1997). The present results show that hippocampal gamma bursts dissociate AS from QS well before the emergence of cortical EEG patterns characterizing REM sleep and SWS. Thus there is also an electrophysiological basis for the early AS/QS differentiation. In the rat there is a parallel developmental decline in the occurrence of spontaneous hippocampal network bursts in vitro (Ben-Ari et al. 1989) (yet the bursts merge into longer oscil-
lations during development) (see Garaschuk et al. 1998) and of phasic muscular twitches and the related respiratory pattern (i.e., by definition AS) (Jouvet 1980; Lapointe and Nosal 1979). Intriguingly, hippocampal structures are known to mediate strong transient changes in drive to breathing (Harper et al. 1998). Thus behavioral AS could at least partially arise from the prominent spontaneous hippocampal activity during development, in particular during the first week of life when the descending inhibitory pathways in the dorsolateral funiculus at the thoracic level are not yet matured (Fitzgerald and Koltzenburg 1986).

Conclusions

Stable gamma oscillations can be detected in the rat hippocampal EEG as early as P5. The gamma rhythm was associated with AS, a developmentally regulated behavioral state probably reflecting the ongoing gross CNS activity. The initially brief gamma bursts at P5 grew longer, gained amplitude and shifted to higher frequencies toward P10; at around P8 the theta rhythm also became detectable. These changes may be linked to suggested increase in sensory processing in the rat during the second postnatal week.

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REFERENCES


