Abolition of the Neocortically Monitored Theta Rhythm after Ibotenic Acid Lesion of the Parafascicular Nucleus in Behaving Rats

Gabriella Marini¹, Giovanni Tredici² and Mauro Mancia³

Istituto di Neuroscienze e Bioimmagini, CNR, Milan 20090, Italy, ²Istituto di Anatomia Umana and ³Istituto di Fisiologia Umana II, Università degli Studi, Milan 20133, Italy

Seven adult Sprague-Dawley rats, chronically implanted with standard electrodes to monitor frontoparietal electroencephalographic (EEG) and nuchal electromyographic (EMG) activity, received, under deep anesthesia, unilateral or bilateral microinjections of ibotenic acid into the lateral portion of the parafascicular nucleus of the thalamus. Four days after the injections (corresponding to the period of neuronal destruction), obliteration of the oscillatory activity in the theta range was found on the side ipsilateral to the injection, while on the intact hemisphere the rhythm was well developed. The asymmetry between the two hemispheres was particularly evident during REM sleep but was also seen during attentive but immobile alertness. In bilaterally injected rats, the neocortical theta rhythm was abolished on both hemispheres. These results suggest that in freely-moving rats the lateral parafascicular neurons are part of the network on which the emergence of the theta rhythm relies.

CURRENT CLAIM: In freely moving rats, the lateral parafascicular neurons of the intralaminar thalamus play a role in the neocortically monitored synchronized theta waves.

Interest in brain rhythms is increasing. The renaissance of concern about the temporal aspect of nervous activity may reflect the development of the fascinating idea that rhythmicity of neuronal cortical populations is an important process that organizes the spatio-temporal coherence patterns necessary for coordinating the activity of remote groups of cells (Singer, 1993).

The rhythmic theta activity was first investigated in detail in the hippocampus by Green and Arduini (1954) who interpreted the "undulating series of large slow waves" as an "arousal rhythm". Many behavioral connotations of this rhythm (learning, memory, ongoing motor behavior, attention, orienting reflex) have been proposed, but despite intensive investigation, its functional and behavioral significance is still not fully clear.

There are various forms of rhythmic slow activity, and their frequency characteristics and behavioral correlates indicate different pharmacological responses and species-differences (Robinson, 1980). In rats, Vanderwolf and Robinson (1981) classified distinct types of theta rhythm based on their occurrence in temporal association with Type I (active movement, dominated by 6-9 Hz activity) and Type II (repetitive behavior and immobility, dominated by 4-7 Hz activity) behavior. In this report, we shall refer to the spontaneously occurring oscillatory activity between 4 and 8 Hz clearly seen in the cortical EEG during brain-activated behavioral states of attentive (but immobile) arousal and REM sleep of freely moving rats.

In an experimental series carried out in our laboratory on chronically implanted, behaving rats, the effects of injections of the excitotoxic ibotenic acid into various thalamic nuclei on the electrographic correlates of sleep have been studied. Here we report on the effects on the neocortical monitored EEG theta rhythm when the target was the lateral portion of the intralaminar parafascicular nucleus (PF). In the rat, the PF is the only component of the caudal group of the intralaminar complex since there is no centre médian (CM) (Jones, 1985). The PF is divided by the fasciculus retroflexus into a medial and a lateral part, which is considered to be the equivalent of the CM in larger mammals (Kuhlenbeck, 1954).

METHODS

Adult Sprague-Dawley rats (200-300 g) were chronically implanted with standard electrodes to monitor frontoparietal EEG and nuchal electromyographic activity under general anesthesia with chloralhydrate (300mg/kg, i.p.). All surgical procedures were made with full asepsis and conformed to the Guide for the Care and Use of Laboratory Animals (1996). EEG activity was also studied using spectral analysis; it was digitally sampled at 128 Hz (BAS 161, Basis, Verona, Italy) and the power spectra in EEG activity were computed on-line for 34 consecutive artifact-free 2-s epochs using a Fast Fourier transform routine. Recordings were made 6 hours per day, every day at the same time, in the freely-moving animal in a sound-attenuated and dimly lit cage whose temperature was

Correspondence: Gabriella Marini, D.Sc., Istituto di Fisiologia Umana II, Universita degli Studi, via Mangiagalli, 32, 20133 Milan, Italy, Tel: 39-2-7060-1087, Fax: 39-2-7063-8565, E-mail: marinig@imiucca.csi.unimi.it.
kept constant. The investigations lasted two weeks before and after the ibotenic acid administration. Ibotenic acid is a glutamate analogue which immediately excites and then kills the cell bodies, sparing the passing fibers. In ketamine-sedated rats, we injected ibotenic acid (Sigma, 1-2 µl of a 20 µg/µl solution in 1M phosphate buffer at pH 7.4) into the lateral portion of the PF (A-4.2; L1.3; H5.2 with respect to bregma according to standard coordinates from the rat brain atlas of Paxinos and Watson, 1986) through a needle (Hamilton microsyringe) guided stereotaxically. The drug was slowly delivered over 2 minutes and the needle was kept in the targeted region for 4 minutes. Successful lesions have been obtained in 7 rats. The injections were done either unilaterally (5 animals) or bilaterally (2 animals). The unilateral approach was preferred because a lesion on one side of the brain provides the opportunity to compare electrographic effects within the same animal. Moreover, animals with bilateral lesions have a higher postoperative risk of lethality.

On completion of the recording sessions, the animals were euthanized under deep anesthesia with 10 percent paraformaldehyde in 0.12M PBS. The location of the injection site was histologically verified in serial sections through the thalamus stained with neutral red or cresyl violet by assessing neuron loss and microglial proliferation. Maps of the lesioned region were reconstructed with a computer-aided system (Immagini e Computer).

RESULTS

In all the rats, 72 hours after unilateral injection of ibotenic acid (corresponding to the period of neuronal destruction) into the lateral part of the PF, the EEG recordings showed that the spontaneous neocortical theta rhythm was abolished on the side ipsilateral to the injection. The oscillations on the lesioned hemisphere were replaced by desynchrony, while the waves were well developed on the contralesioned hemisphere (Fig. 1A). The asymmetry between the two hemispheres due to the rhythm suppression was particularly evident during REM sleep but was also seen during attentive but immobile alertness. The spectral analysis further documented the obliteration of the oscillatory activity in the theta range. The power activity indicated the synchronized rhythm in the low frequency range on the intact hemisphere and its absence in the lesioned one (Fig. 1C).

In bilaterally injected rats, disruption of the neocortically monitored theta rhythm and desynchrony were observed on both hemispheres during REM sleep as well as immobile wakefulness. The effect peaked at 4 days after the injection.
which corresponds to the period of the maximum degenerative changes. Gradually the rhythm recovered and was normal after 10 days (not illustrated).

Histological analysis showed that neuronal loss was confined within the lateral PF and that the reduced density of the neurons was accompanied by glial reactions (Fig. 1B).

**DISCUSSION**

The main finding of this study was that ibotenic acid lesions in the lateral part of the intralaminar PF nucleus abolish the neocortically monitored theta activity spontaneously occurring during REM sleep and alert, but immobile wake in freely-moving rats. Synchronized oscillations imply the coherent activity of several neuronal aggregates. The present evidence suggests that the lateral PF neurons are part of the network of distinct anatomical areas on which the emergence of this rhythm relies.

Although Green and Arduini (1954, see their Fig. 5) had long ago observed that intralaminar stimulation induced the appearance of theta oscillations in the hippocampal recording, this finding was rather surprising and the explanation of such a thalamic influence is not readily apparent. Theta waves recorded from the neocortex may result either from hippocampal volume conduction (Gerbrandt et al., 1978) or they may be generated within the neocortex as occurs in the cingulate cortex (Holsheimer, 1982; Leung and Borst, 1987). If the latter proposition is true, the thalamic effect on this rhythm might have been mediated via the PF-cingulate areas links, which are strictly ipsilateral (Marini et al., 1996) and this would fit with the fact that the effect was unilateral. If the cortically recorded theta waves are caused by volume conduction from the hippocampus, the thalamic effect on the theta rhythm may have been mediated either directly or indirectly. The first possibility can be ruled out on the basis of anatomical work: injecting highly sensitive anterograde tracers in the lateral PF of rats, the septal nuclei and hippocampus were found to be completely devoid of labeling (Tredici et al., 1995). Instead, intense anterograde labeling has been seen in the ventral region of the reticularis pontis oralis nucleus (Marini et al., 1997), as well as in the raphe system (Marini and Tredici, 1995). The location of the labeled terminals in the pons corresponds to the cholinocceptive group of neurons whose activation elicits the hippocampal theta rhythm (Nunez et al., 1991; Vertes et al., 1993) while the serotonergic raphe system is involved in the control of hippocampal EEG desynchronization (Kinney et al., 1994). Since the septohippocampal cholinergic system generates theta waves by transforming the sustained brainstem input into rhythmic discharges (Petsche et al., 1965) that are cholinergically mediated (cfr. Bland, 1986), the effect may have been mediated via the PF-reticular pathway. However, since ultrastructural observations suggest an inhibitory function of the PF-raphe synapses (Marini and Tredici, 1995), it may be that the loss of PF neurons might have led to removal of the inhibition (dis-inhibition) of the serotonin-containing raphe cells. It will be necessary to investigate more fully the effect of the PF lesions also recording from the hippocampus to resolve the question.

The present experiments indicate an influence of the posterior intralaminar nuclei on the cortically monitored synchronized theta waves.

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