Behavioural characteristics and neuronal mechanisms of amygdala kindling

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ABSTRACT

In the central nervous system changes occur in response to internal and external stimuli. These alterations are of great importance for the functioning of the individual in her/his environment, and in neurobiology fall under the term plasticity. In this thesis, the interaction between three aspects of plasticity are investigated, namely epilepsy, learning and memory and stress. A general introduction of these aspects is given in **chapter 1**, which results in two central questions: (*i*) How does stress interact with epilepsy?, and (*ii*) How does epilepsy interact with learning and memory?

Certain types of human epilepsy are characterized by attacks occurring suddenly. The arms and legs of patient shake and consciousness may be lost for several minutes. Clinical studies showed that stressful situations can provoke these attacks. An explanation of this observation is, however, not available. This thesis attempts to start to explain the observation. In the present research on male rats, a well-known animal model for epilepsy is used, namely kindling of the amygdala. The term kindling means "fan the fire", which points to the increase in the response of the brain to local electrical stimulation. The stimulation is of short duration and low intensity and is applied daily through electrodes implanted in the brain. At the start a response is visible only in the electro-encephalogram (EEG) as an afterdischarge, which is a synchronization in the activity of a number of neurons. Finally, repeated stimulation results in a tonic-clonic seizure, which bears great similarity to attacks in humans. This response will remain present for the rest of the rat's life.

Two types of psychosocial stressors are used in research on the relationship between epilepsy and stress. (*i*) The experimental rat is confronted by a naive, male intruder rat in his own territory. The subsequent fight results in a victory experience for the experimental rat. (*ii*) The experimental rat is placed into the territory of a dominant opponent rat. The fight results in a defeat experience for the experimental rat. Both social stimuli evoke strong stress-responses. Experiments described in **chapter 3** show that repeated defeats somewhat accelerate the development of kindling, while victories on the contrary cause a retardation. These differences may be explained in terms of a difference in controllability in each situation. During the confrontation with a naive intruder the experimental rat is able to actively control the situation, while there is an absence of control during the confrontation with the dominant opponent. A considerable degree of controllability is accompanied by an

activation of noradrenergic mechanisms that have anti-convulsive properties. **Chapter 4** demonstrates that exposure to a defeat shortly before application of the kindling stimulation results in a less severe epileptic seizure in kindled rats, while victory has no effect. This acute effect as a result of defeat is probably caused by an increased release of opiates, which have an anti-convulsive effect.

The second central question of this thesis is dealt with in **chapter 5 and 6**. Every other day one group of rats is trained in a spatial learning task in addition to the delay development of kindling. In comparison with this group, another group of control rats is exclusively trained in the learning task. The development of short-term and long-term memory in both groups is studied for several days in the holeboard. In this free-choice maze the rat can find food at several locations on the basis of environmental, spatial cues. After a few days of testing the control rats locate the food pellets without errors. However, generalized seizures in rats of the kindling group cause a deficit in long-term, but not short-term, memory. Beside these differences in behaviour alterations at the cellular level are observed in the brain. Spatial learning in control rats is accompanied by a gradual increase in immunoreactivity against protein kinase C and muscarinic acetylcholine receptor protein. The increased immunoreactivity of PKC appears only against the γ and not α or β isoform. Alterations occur in the pyramidal and granular neurons of the hippocampal formation, a brain structure implicated in learning and memory in both man and animal. These cellular alterations are also observed in kindled rats displaying partial seizures, but are absent in kindled rats with generalized seizures. Kindling disturbs the cellular and molecular processes that occur during spatial learning dependent of the extent of the development.

The development of kindling is further investigated in **chapter 2** using recently developed signal analytical methods. For this purpose the relationship between epileptiform EEG measured daily in the amygdala of both hemispheres is analyzed. The amount of association is expressed in the linear and non-linear regression coefficients, r^2 and h^2, respectively. The progression from partial into generalized seizures is accompanied by an increase in both coefficients, but mainly in h^2. Consequently, a coupling between both amygdalae is built up during kindling. Subsequent kindling stimulation, however, results in a reduction of this coupling. This suggests the development of an independent focus in the hemisphere contralateral to the stimulation side. This suggestion corresponds with results,

described in **chapter 7**, on long-term changes in the brain of rats after completion of kindling. An increase in immunoreactivity against PKC-γ and muscarinic acetylcholine receptor protein is observed in the piriform and entorhinal cortex of the contralateral temporal lobe of rats which had a three month seizure-free period after an average of ten clonic-tonic seizures. This increase in immunoreactivity indicates an increase in excitability.

Finally, the vested results on the relationship between epilepsy, stress, and learning and memory are interrelated in **chapter 8**. For this purpose the underlying molecular biology of the separate aspects are discussed and integrated into one concept. This last chapter finishes with a description of two lines of research for the future.