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The effect of deep brain stimulation of the amygdala in the pilocarpine model of temporal lobe epilepsy

(Abstract)

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-MARCH 2021 -

Epilepsy is one of the most common neurological disorders, affecting more than 70 million people worldwide. The most common form of epilepsy is temporal lobe epilepsy (TLE), mainly affecting the hippocampus, which is resistant to drug therapy in about 20-40% of cases. Deep brain stimulation (DBS) is a novel therapeutic method that consists of the implantation of electrodes in the central nervous system followed by application of electrical stimulation. In the case of pharmacologically refractory epilepsy DBS seems a promising alternative for reducing symptoms and slowing epileptogenesis. In temporal lobe epilepsy stimulation of the hippocampus or amygdala is a promising target, although the mechanisms exerting the anticonvulsant effect of DBS are not fully understood.

Epileptic seizures result from the abnormally excessive or hypersynchronous electrical activity of neurons, caused by an imbalance between excitation and inhibition. Hippocampal inhibitory cells, especially parvalbumin-expressing cells and ivy cells play an important role in maintaining this balance. Researching the effects of DBS on epileptic electrical activity and hippocampal interneuronal density could help to decipher the mechanism of DBS and improve stimulation protocols.

We proposed to evaluate the effects of low-frequency deep brain stimulation (LFS) of the amygdala on the seizure pattern, hippocampal electrophysiological activity and inhibitory interneuron density in the pilocarpine model of TLE.

Systemic injection of pilocarpine initiates status epilepticus (SE) that later leads to the onset of spontaneous recurrent epileptic seizures (SRSs), a characteristic of human temporal lobe epilepsy as well. SE was induced in male juvenile Wistar rats by administration of lithium then pilocarpine and was halted 2 hours later with diazepam. Rats were continuously video monitored and SRSs were identified and analyzed offline. Approximately ten weeks after SE two hippocampal recording electrodes, a stimulating electrode in the left basolateral amygdala and four screw electrodes for electrocorticographic recording were implanted. Ten days after surgery regular 4 Hz LFS was applied for 4x50 seconds, with 5 minute pauses between the stimulus trains. Stimulation was performed for 10 days. Before and during stimulation hippocampal and cortical electrical activity was recorded in a freely moving animal setup. The frequency of interictal epileptiform discharges (IEDs, that is spikes, polyspikes and sharp waves), as well as power spectra of theta and delta waves were quantified. The phase-amplitude coupling (PAC) between delta and higher frequencies was determined by a time-resolved phase-amplitude coupling measure (tPAC), which is a novel method to obtain a PAC comodulogram and it detects the peak value of coupling strength between slow rhythm and fast rhythms over optimal sliding time windows. To evaluate the functional connectivity between the left and right hippocampus, a phase-locking value (PLV) was calculated.

At the end of the experiments the animals were sacrificed and the brains were fixated by transcardial perfusion with paraformaldehyde. 60 μm thick sections were labeled by using parvalbumin (PV), neuropeptide Y (NPY), and neuronal nitric oxide synthase (nNOS) as immunohistochemical marker proteins for the identification of interneurons. Sections were scanned with a confocal microscope and the density of the interneurons was determined in the CA1 region of the hippocampus.

The mean frequency and duration of seizures were reduced by the LFS (by 23% and 26.5% respectively). Six animals had fewer seizures while three had more during stimulation; the duration was reduced in eight out of nine rats. The reduction in seizure

frequency had a negative correlation with the frequency of interictal epileptiform discharges.

The rate of mild seizures (Racine grade 3 and 4) decreased significantly. The rate of R6 seizures decreased, but those of R5 increased, so the reduction in the rate of severe seizures (R5 + R6) was not significant. The average seizure rate was not affected by electrode implantation (sham operated without stimulation).

The pilocarpine-treated rats had a significantly higher IED rate compared to control animals. LFS reduced the IED rate in the pilocarpine group when the first and the last day of the stimulation were compared.

The relative theta power was significantly decreased in epileptic animals compared to controls. LFS significantly increased the relative theta power immediately after the application of the stimulus package, restoring the values to the level measured in the control animals. The value of PAC was increased in pilocarpine-treated animals, and the stimulation reduced significantly this increased PAC, but did not bring it back to the control levels. The PLV before the stimulation was significantly lower in epileptic animals in the delta, theta, and gamma bands. The electrical stimulation significantly increased the PLVs in delta and theta bands.

The PV+ cell densities were not changed in pilocarpine treated rats, while the NPY+/nNOS+ cell densities were significantly decreased compared to controls. The volume of the hippocampus was also decreased in the epileptic animals. Amygdala LFS had no effect on PV+ or NPY+/nNOS + cell densities in either the epileptic or the control group.

Detecting IEDs, measuring the relative theta power and PAC values might be helpful in characterizing epileptogenesis. In this study we showed for the first time that LFS could exert its anticonvulsant effect by increasing the theta power and reducing the pathologically increased PAC, as well as improving the altered synchrony between the two hippocampi (by increasing PLVs). Measuring these parameters might help to monitor and improve the efficacy of electrical stimulation.

We had seen that different interneuron types are selectively vulnerable to epileptic insult as PV+ cell numbers remained unchanged and NPY+/nNOS+ numbers were decreased in the epileptic specimens. Decreased ivy cell density (NPY+/nNOS+) could facilitate increased overall excitability and lower seizure threshold. In contrast, the persistence of PV+ cell counts in a shrunken hippocampus could facilitate local hypersynchrony and promote epileptogenesis. Electrical stimulation of the amygdala did not influence the density of PV+ cells and did not improve the decreased density of NPY+/nNOS+ cells.

In conclusion low-frequency stimulation of the amygdala successfully modulated the altered hippocampal electrical activity, but did not influence the changed interneuronal cell densities in the CA1 hippocampal area of epileptic rats. We attribute this limited effect to the short duration of stimulation. Future studies should test longer stimulation periods in order to see if LFS can diminish interneuron loss.

The originality of our work consists in evaluating the effects of deep brain stimulation with low frequency through the complementary use of behavioral, electrophysiological and immunohistochemical methods. The study is the first to evaluate the effect of LFS in *in vivo* conditions, by analyzing seizure patterns, electrical activity and interneuronal densities in an epilepsy model.