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Alteration of Synaptic Plasticity by Neonatal Seizures in Rat Somatosensory Cortex

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Abstract

Seizures in newborns are associated with a high risk for subsequent epilepsy and adverse neurodevelopmental consequences. Understanding the mechanisms by which neonatal seizures adversely disturb the immature brain is important in developing therapeutic strategies. Using the convulsant agent flurothyl to mimic repetitive neonatal seizures we show that early-life seizures result in long-term alteration in the maintenance phase of long-term potentiation (LTP) in layer IV to layer II/III synapses of the somatosensory cortex without alteration of basal synaptic transmission, the induction phase of LTP and short-term depression. Such alterations may have a role in functional deficits seen following neonatal seizures.

Keywords

early seizures; plasticity; somatosensory cortex

1. INTRODUCTION

Seizures are one of the most common neurological emergencies occurring in newborns and are associated with a considerable risk of long-term sequelae, including epilepsy, cognitive and behavioral issues (Holmes, 2004, Ronen et al., 2007). While the etiology of the neonatal seizures is the most important factor in outcome, there is increasing data from humans that seizures independently contribute to long-term adverse consequences (Glass et al., 2009; 2011, but see Kwon et al., 2011).

To elucidate the effect of neonatal seizure on neuronal plasticity in the present study we used flurothyl model of repetitive seizures. Flurothyl is a volatile convulsant that produces well controlled generalized seizures with no apparent direct drug effect which makes it widely used in basic epilepsy research (Velíšková et al., 2005; Khan et al., 2010). Using the flurothyl model of repetitive seizures on immature rats we previously showed that neonatal

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seizures produce a long-term increase of seizure susceptibility and alteration in excitation/inhibition balance of synaptic transmission in layer II/III neurons of the somatosensory cortex (Isaeva et al., 2009; 2010). As the cerebral cortex is involved in encoding and processing of sensory information and has been shown to express different forms of activity-dependent synaptic plasticity (Castro-Alamancos et al., 1995) here we explored the hypothesis that early life seizures can modify synaptic plasticity in the somatosensory cortex.

2. MATERIAL AND METHODS

All experiments were performed in accordance with the guidelines set by the National Institute of Health and Dartmouth Medical School for the humane treatment of animals. Sprague-Dawley rats (N=8) were subjected to 75 flurothyl-induced seizures using previously described method (Isaeva et al., 2010). To elucidate the effect of neonatal seizure on neuronal plasticity in our animal model we chose the age range from postnatal day 0 to 15 which corresponds to the last trimester gestational period and first year of life in humans (Avishai-Eliner, et al. 2002). Untreated littermate pups (N=9) were used as controls. Brain slices were prepared from P46-P60 rats. The rats were deeply anesthetized with isoflurane and decapitated. Slices (400 μm) were cut in the coronal plane transferred to an incubation chamber where they rested for at least 2 hrs before recordings in oxygenated artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 126, KCl 3.5, CaCl₂ 2.0, MgCl₂ 1.3, NaHCO₃ 25, NaH₂PO₄ 1.2 and glucose 11 (pH 7.3-7.4).

Field potential (FP) recordings were made from LII/III of somatosensory cortex using electrodes filled with ACSF (2-4m Ω). 2-(3-carboxypropyl)-3-amino-6-(4 methoxyphenyl) pyridazinium bromide (SR95531) was included in the recording pipette (50 μM) to block gamma-aminobutyric acid (GABA) A receptors. Synaptic responses were evoked by stimulation of LIV of somatosensory cortex with 100 μsec pulses of 30-80 μA through a concentric bipolar stimulating electrode using a stimulus isolator. Baseline responses were obtained at 0.05 Hz using a stimulation intensity that produced half-maximal response for each recording. To induce LTP we used a primed burst (PB) potentiation protocol repeated 5 times at intervals of 10 sec consisting of a single priming pulse followed 170 ms later by a burst of 10 stimuli at 100 Hz (Diamond et al., 1988). Data were analyzed using the Mini Analysis (version 6.0.3; Synaptosoft, Decatur, GA), Clampfit (Axon Instruments Inc, Union City, CA) and Origin 7.0 (Microcal Software, Northampton, MA) software. Statistical comparison was performed using unpaired Student's t-test. Results in the text and in the figures are expressed as the mean \pm SEM.

3. RESULTS

Stimulation of LIV of somatosensory cortex evoked FPs in LII/III in all slices from flurothyl-treated and control groups of animals. The maximal rising slope of the FP as a measure of synaptic efficiency was not significantly different between groups (0.55 ± 0.11 mV/ms (n=7 animals/16 slices) in control vs 0.41 ± 0.05 mV/ms (n = 6 animals/14 slices) in flurothyl-treated group, p = 0.24). We next examined the effect of repetitive flurothyl seizures on synaptic plasticity using the PB potentiation protocol. In the presence of SR 95531 in the recording electrode the delivery of the PB potentiation protocol to the LIV of somatosensory cortex consistently induced prolonged enhancement of the evoked FP in LII/III in controls as well as flurothyl-treated group. Application of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid blocked the induction of LTP in both groups. LTP maintenance phase was increased significantly in the flurothyl-treated group when compared with controls (FP changes 50 min after PB stimulation: $153.3 \pm 18.6\%$, n = 6 animals/7 slices vs $94.4 \pm 7.4\%$, n = 6 animals/ 9 slices, p = 0.01). This increase in LTP

maintenance occurred without modifications in the induction phase of LTP (Fig. 1). The average maximal response was not significantly different between groups: control (n=6 animals/ 9 slices): 231.8 ± 23.5 % of baseline and flurothyl-treated group (n= 6 animals/7 slices): 212.4 ± 12.3 %, $p = 0.54$. During high-frequency stimulus trains FP exhibit a strong depression. This form of short-term synaptic plasticity has been observed in different cortical areas (Castro-Alamancos et al., 1995, Hernan et al., 2013) and is thought to provide the synapse specific gain control of cortical circuits (Abbott et al., 1997). In our study FP slope decreased to 12.8 ± 3.1 % (n=6 animals/10 slices) of its initial value over the course of a 100 Hz train in control, and to 8.0 ± 1.8 % (n=6 animals/ 9 slices) in the flurothyl-treated group. Figure 2 demonstrates that there was no difference in response to repetitive stimulation between the flurothyl and control groups.

3. DISCUSSION

We previously reported that neonatal seizures resulted in enhanced excitability in somatosensory cortex which can be due to the disruption of the excitation-inhibition balance in sensory pathways in the flurothyl group (Isaeva et al., 2010). In the present study the postsynaptic response in LIV to LII/III network in somatosensory cortex was not changed in the flurothyl-treated group compared to controls, indicating that the basal evoked synaptic transmission in this vertical pathway is unaltered in rats experiencing recurrent neonatal seizures.

In our study neither short-term depression nor induction phase of LTP was modified in the flurothyl-treated group. However, the maintenance phase of LTP was significantly altered. Expression of LTP at layer IV to layer II/III synapses in the somatosensory cortex depends on changes in the postsynaptic NMDA receptors (Bender et al., 2006). Lack of an effect of early life seizures on the induction phase of LTP is in agreement with our previous study where it was shown that amplitude of miniature NMDA-dependent excitatory postsynaptic currents (as indicator of postsynaptic alteration) was not altered in layer II/III synapses in somatosensory cortex after early life seizures (Isaeva et al., 2010).

During high frequency stimulation Ca^{2+} entry through the postsynaptic NMDA receptors activates different Ca^{2+} dependent second messengers which are crucial for the maintenance of LTP. Most factors with established molecular mechanisms responsible for the maintenance phase of LTP interfere with these cascades (Malinow et al., 1988, Matthies et al., 1991). Several studies show that the maintenance phase of LTP could be disrupted by seizures or altered in seizure-prone animals (Hesse and Teyler, 1976, Schubert et al., 2005). However, the lowered threshold for epileptiform activity is not always associated with loss or impairment of LTP and alteration of LTP rather depends on the cellular mechanism underlying the increase in neuronal activity (Luthi et al., 1997).

In summary, the results from the present study demonstrate that neonatal seizures have long-term effects on synaptic plasticity in the somatosensory cortex. Understanding the molecular mechanism of these synaptic changes and determining whether alterations in plasticity reported here interfere with sensory processing in patients with a history of neonatal seizures requires further investigation.

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ABBREVIATIONS

FP	field potential
P	postnatal day
LIV	layer 4
LII/III	layer 2/3
LTP	long-term potentiation
NMDA	N-methyl-D-aspartate
GABA	gamma-aminobutyric acid

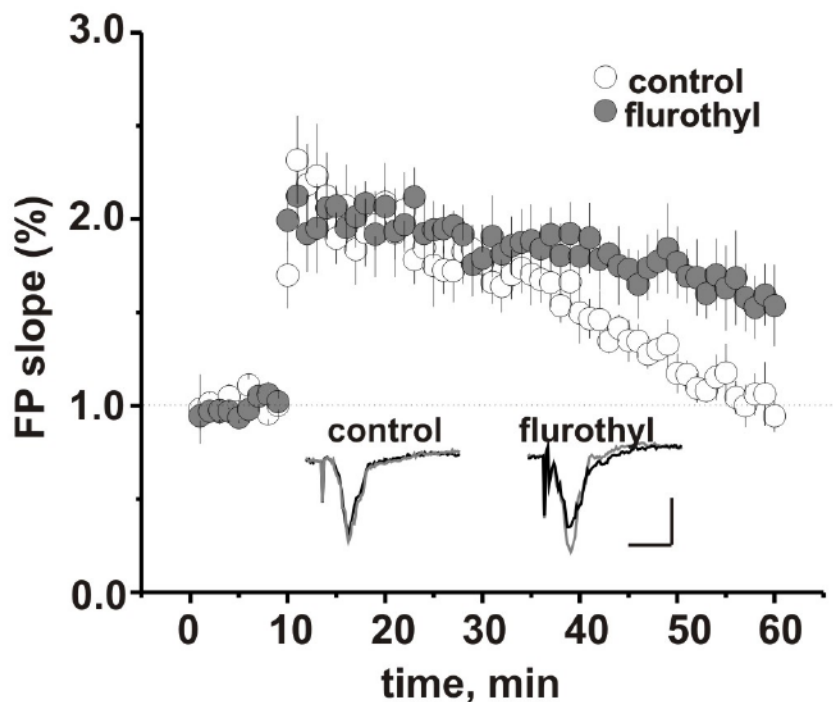


Figure 1. Effect of flurothyl induced seizures on the expression and maintenance of LTP in the somatosensory cortex. The baseline FP in control (white) and flurothyl-treated (grey) group was recorded for 10 min, then the primed burst potentiation protocol was applied and the recording was continued for another 50 min. All data were normalized to baseline. Insert: examples of FPs recorded in LII/III before (black) and 50 min after conditioning stimulation of LIV of somatosensory cortex (grey). Traces scale bars are 0.3 mV by 10 ms. Values are mean \pm SEM.

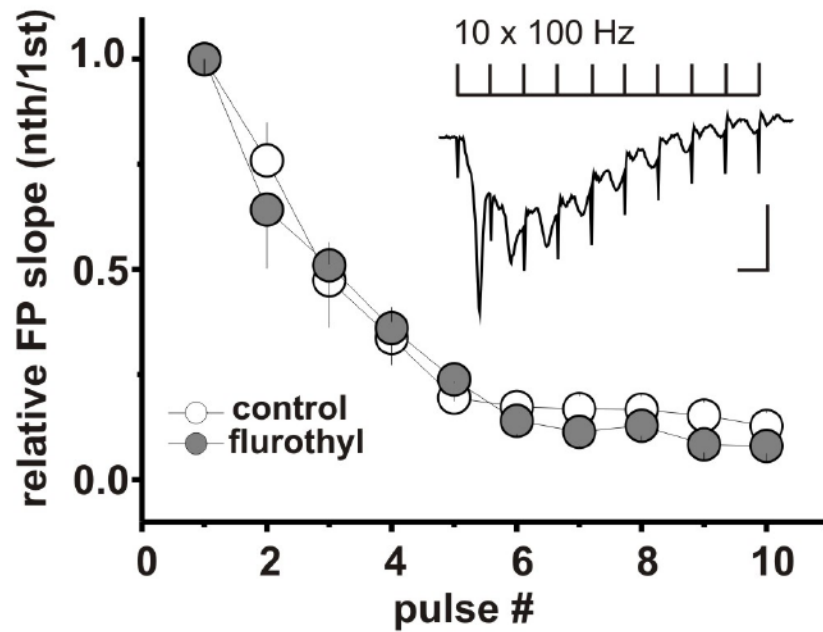


Figure 2. Repetitive neonatal seizures do not affect the short-term depression of synaptic responses during conditioning stimulation. FP slopes were normalized to the FP slope in response to first pulse and graphed versus pulse number. Pulses 1–10 were averaged individually. Insert: trace represent average of five consecutive FP recordings during the conditioning stimulation consisted of 10 pulses delivered at frequency 100 Hz. Traces scale bars are 0.3 mV by 10 ms. All measurements are reported as mean \pm SEM.