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# NMDA receptor-mediated long-term alterations in epileptiform activity in experimental chronic epilepsy

Jennifer L. Hellier [a,1], Andrew White [b,1], Philip A. Williams [c], F. Edward Dudek [d], Kevin J. Staley [e,*]

[a] Neuroscience Program, University of Colorado Health Sciences Center, United States
[b] Pediatrics Department, University of Colorado Health Sciences Center, United States
[c] Physiology Department, Case Western University, United States
[d] Department of Physiology, University of Utah School of Medicine, United States
[e] Neurology Department, Harvard University School of Medicine, United States

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ABSTRACT

When epileptiform activity is acutely induced in vitro, transient partial blockade of *N*-methyl-D-aspartic acid (NMDA) receptor-mediated calcium influx leads to selective long-term depotentiation of the synapses involved in the epileptic activity as well as a reduction in the probability of further epileptiform activity. If such selective depotentiation occurred within foci of epileptic activity in vivo, the corresponding long-term reduction in seizure probability could form the basis for a novel treatment of epilepsy. Continuous radiotelemetric EEG monitoring demonstrated modest acute anticonvulsant effects but no long-term reductions in the probability of spontaneous seizures after transient partial blockade of NMDA receptors (NMDAR) during ictal and interictal activity in the kainate animal model of chronic epilepsy. In vitro, depotentiation was induced when NMDAR were partially blocked during epileptiform activity in hippocampal slices from control animals, but not in slices from chronically epileptic rats. However in slices from epileptic animals, depotentiation during epileptiform activity was induced by partial block of NMDAR using NR2B- but not NR2A-selective antagonists. These results suggest that chronic epileptic activity is associated with changes in NMDA receptor-mediated signaling that is reflected in the pharmacology of activity- and NMDA receptor-dependent depotentiation.



## 1. Introduction

Synchronous activation of pre- and postsynaptic neurons is a necessary condition for long-term modification of synaptic strength (Sjöström and Nelson, 2002). The necessity of synchronous activation has been supported by a variety of experiments, perhaps most specifically by the observation of spike timing-dependent long-term synaptic plasticity (Caporale and Dan, 2008). Pathologically high levels of neuronal synchronization occur during epileptic activity, including both seizures and interictal electroencephalographic (EEG) spikes. The synchronization during epileptiform activity suggests that interictal spikes and seizures may be a robust means of inducing long-term synaptic plasticity. In brain slice preparations, long-term potentiation of active synapses can be readily induced by seizures (Ben-Ari and Gho, 1988) as well as by interictal spikes (Bains et al., 1999; Abegg et al., 2004; Debanne et al., 2006; Behrens et al., 2005). Strengthening of the synaptic connections between neurons in epileptic foci as a consequence of ongoing interictal activation of the neurons in the focus has been proposed as a means of maintaining the increased probability of seizure activity and thereby stabilizing the epileptic state (Staley and Dudek, 2006).

Because long-term synaptic plasticity is bidirectional, it should also be possible to weaken synapses in epileptic foci. Such weakening reduces the probability of synchronous activity (Chamberlin et al., 1990; Traub and Miles, 1991; Bains et al., 1999; Staley et al., 2001) including seizures, which would be of significant therapeutic interest. The calcium hypothesis proposes that large increases in postsynaptic calcium lead to long-term synaptic potentiation (LTP), but smaller increases lead either to de novo long-term depression (LTD) or reversal of previously established LTP (depotentiation) (Lisman, 2001). Reduction in postsynaptic calcium influx by partial blockade of NMDA receptors with competitive antagonists induces robust depotentiation when synapses are activated by either tetanic stimuli (Cummings et al., 1996) or epileptiform activity in acute slice preparations (Bains et al., 1999; Hellier et al., 2007). This depotentiation is specific for synapses that participate in the

* Corresponding author. MGH Department of Neurology, 114 16th Street, B114-2625, Cambridge, MA 02129, United States. Tel.: +1 617 643 0363; fax: +1 617 643 0141.
*E-mail address:* kstaley@partners.org (K.J. Staley).
[1] These authors contributed equally to this manuscript.

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epileptiform activity (Bains et al., 1999), making it an attractive potential mechanism to selectively reduce the strength of synapses in epileptic foci in order to prevent further seizures.

Although the data from acute slice experiments support the possibility that synapse-specific reductions in the strength of synaptic connections in epileptic foci could lead to long-term reductions in seizure probability, there are several possible complications. First, depotentiation of recently potentiated synapses is much more robust than LTD of synapses that presumably were potentiated in the more distant past (Montgomery and Madison, 2004). Supporting this observation, LTD induction is accompanied by anatomical alterations manifest primarily in the smallest synapses (Bastrikova et al., 2008) that are presumably the weakest (Baude et al., 1995; Zhou et al., 2004). This suggests that in long-standing epilepsy, where synapses have been repeatedly potentiated by interictal and seizure activity, depotentiation or long-term depression of synaptic strength may be less robust. Second, the mechanisms of synaptic plasticity in vivo may represent a superset of the mechanisms observed to date in vitro. For example in contrast to what has been observed in vitro, NMDA antagonists have increased the strength of active synapses in vivo (Clem et al., 2008). Third, activity-dependent changes in NMDA receptor subunit expression (Galvan et al., 2003; Yang et al., 2006) or the linkage of NMDAR to second messenger systems (Chen et al., 2007) may alter the pharmacology of activity-dependent depotentiation in chronic epilepsy. Finally, activity- and NMDAR-dependent synaptic weakening is accompanied by reductions in the fraction of membranous NMDA receptors (Hellier et al., 2007), which may limit further depotentiation.

In this study, we find that transient partial antagonism of NMDA receptors with the nonselective competitive NMDA antagonist SDZ 220-581 (Urwyler et al., 1996) in chronically epileptic animals does not lead to long-term reductions in seizure activity. In vitro slice experiments demonstrate that in contrast to acute slices from naïve animals, partial NMDA antagonism with nonselective competitive antagonists does not induce depotentiation during synchronous network activation in slices from epileptic animals. However, in slices from epileptic animals, partial blockade of NMDA receptors using an NR2B- (but not an NR2A-) selective antagonist during synchronous CA3 network activity *does* reduce the probability of further epileptiform activity in a pattern, consistent with depotentiation of the active synapses.

## 2. Methods

### 2.1. Surgery: freely behaving rats

The methods have been described previously (for a detailed description see Williams et al., 2006). Briefly, male Sprague–Dawley rats (180–200 g; Harlan, Indianapolis, IN) were injected subcutaneously (SC) with atropine (2.0 mg/kg), dexamethasone (4.0 mg/ml), and 0.2 ml penicillin (300,000 IU); anesthesia was induced and maintained with isoflurane. The head was placed in a stereotaxic apparatus, a mid-sagittal incision made on the scalp and the skin reflected with hemostats. Using bregma as a reference, holes were bored through the skull with a Dremel (#105 drill bit) for implantation of a single dural and bilateral intrahippocampal recording electrodes (rostral–caudal −4.0 mm, medial–lateral ±2.5 mm, dorsal–ventral −3.3 mm), a ground electrode, and three support screws placed behind the hippocampal sites. The intrahippocampal electrodes were placed in the granule cell layer of the dentate gyrus and confirmed by increased spike activity with an audio monitor. All electrodes were permanently fixed by dental cement. The radiotelemetry unit (Transoma Medical, Arden Hills, MN) was placed subcutaneously in the flank region through a 2-cm skin incision behind the scapula.

Both incisions were closed using 4–0 Dermalon (American Cyanamid Co., Danbury, CT). The animal was given 4–6 ml of warmed Ringer's solution (SC), buprenorphine (0.01 mg/kg, SC), removed from anesthesia and placed back in its cage under a heat lamp for 30 min. All animals were given buprenorphine (0.01 mg/kg, SC) and 0.2 ml penicillin (300,000 IU, SC) for 3 days following surgery. The toenails of each rat were cut while the rat was anesthetized, and if necessary were trimmed once per week to decrease scratching and irritation at the incision sites.

### 2.2. Kainate treatment

The kainate-treatment protocol has been previously described (Hellier et al., 1998; Hellier and Dudek, 2005). If the animal was implanted with electrodes, kainate treatment was performed 1–2 weeks after surgery. Rats ($n = 20$ for slice experiments and $n = 9$ for implantation) were given hourly injections of kainate (5 mg/kg, intraperitoneally, IP; Sigma, St. Louis, MO or Ocean Produce International, Nova Scotia, Canada) diluted in sterile 0.9% saline at 2.5 mg/ml. Motor seizure activity was rated according to a modified Racine's scale (i.e., class III, class IV, and class V seizures; Ben-Ari, 1985; Racine, 1972). To minimize mortality, injections were reduced (2.5 mg/kg) or eliminated if an animal showed excessive inactivity or activity (for details see Hellier and Dudek, 2005). Kainate treatment was continued until class IV and class V seizures were elicited for ≥3 h. Implanted rats were continuously monitored for both electrographic and motor seizures throughout kainate treatment. Control rats ($n = 10$ for slice experiments) were treated with an equivalent volume and number of injections of sterile saline. All rats were given 3–6 ml warmed lactated Ringer's (SC) and apple slices following treatment.

### 2.3. Radiotelemetry and video monitoring: freely behaving rats

This study used the Dataquest A.R.T. Analog software provided by Transoma Medical (Arden Hills, MN). The radiotelemetry unit was specifically designed to transmit physiological data from conscious, freely behaving animals to the analog system. Rats were housed individually after surgery, given food and water ad libitum, and exposed to a 12-h light/dark cycle. Each cage was placed on an individual radio receiving plate (RPC-1; Transoma Medical, Arden Hills, MN), and captured data signals from the transmitter was sent to an input exchange matrix and then to a computer. Custom-made software (KS and AW) was used to acquire the data with routines written in VisualBasic 6.0 (Microsoft, Seattle, WA), and the data were written to DVD for analysis offline (for a detailed description see White et al., 2006).

Two Color Quad Observation Systems (SOD14C4LN; Samsung, Korea) were used to continuously videotape eight individually housed rats, and the time stamp for each system was synchronized to the digitizing computer. Night recordings were performed with a Kodak 1A filter (Eastman Kodak, Rochester, NY) over a safelight and daytime recordings with a diffuse fluorescent light. The behavioral data were used in this study for identifying motor seizures from nonmotor seizures and for differentiating EEG seizure activity from electrical noise generated by jaw artifact and grooming.

### 2.4. EEG analysis: freely behaving rats

The seizure-detection analysis was performed in an automated manner with custom-written software that greatly minimizes the potential for bias (White et al., 2006), and also visually analyzed (AW and PAW). The investigators were not blinded to treatment or time after kainate-induced status epilepticus. Electroencephalographic seizures were differentiated from background noise by the appearance of large-amplitude (>three times baseline noise), high-frequency (≥5 Hz) EEG activity, with a sufficiently high temporal correlation and progression of spike frequency (White et al., 2006).

### 2.5. Chronic seizures

To ensure that kainate-treated rats used for slice experiments were epileptic, both kainate- and saline-treated rats were viewed directly for seizure activity during random 1–2 h intervals, totaling 6–8 h/wk (Hellier et al., 1998). These observational periods were initiated approximately 3 months after treatment until euthanasia and occurred during the 12-h interval when lights were on. Behavioral observations of seizure activity were recorded, applying the same modified Racine scale used during kainate treatment. When a kainate-treated rat was observed to have at least two spontaneous motor seizures, the animal was defined as epileptic. Only rats with kainate-induced epilepsy were used for slice experiments. No saline-treated rats were observed to have motor seizures and were used as controls for slice experiments.

### 2.6. Acute whole-animal LTP surgery and electrophysiological recordings

Adult male Sprague–Dawley rats (200–350 g, Harlan) were initially injected with atropine (1 mg/kg, IP) to prevent cardiorespiratory complications associated with surgery and general anesthesia. Subsequently, rats are anesthetized with urethane (1.8–2.0 mg/kg, SC) and animal body temperature was maintained between 37 and 40 °C. Once a level plane of anesthesia was obtained, the head was placed in a stereotaxic apparatus (bite bar = −3.0 mm), a mid-sagittal incision made on the scalp, and the skin reflected with hemostats. Using bregma as a reference, 3 holes are burred with a Dremel (#105 bit) in the skull for implantation of recording (rostral–caudal −4.0 mm, medial–lateral −2.0 mm, dorsal–ventral −2.4 mm; 3 MΩ, Fredrick Haer, Co), stimulating (rostral–caudal −4.5 mm, medial–lateral −3.1 to −3.5 mm, dorsal–ventral −2.5 to −3.0 mm; wire size = 0.0045″, Teflon-coated, A-M Systems, Inc.), and grounding electrodes (rostral–caudal +2.0 mm, medial–lateral ±2.0 mm, on dura; wire size = 0.013″, Teflon-coated, A-M Systems, Inc.). Grounding electrodes were glued in position (Instant Krazy Glue, Advanced Formula Gel) prior