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SHORT COMMUNICATION

Prolonged depth electrode implantation in the limbic system increases the severity of status epilepticus in rats

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Summary Chronically implanted intracranial depth electrodes are widely used for studying electroencephalographic activities in deep cerebral locations and for electrical stimulation of such locations. We have previously reported that prolonged implantation of an electrode in the basolateral amygdala (BLA) of rats facilitates subsequent kindling from this site, indicating a pro-kindling or pro-epileptogenic effect. To further characterize this phenomenon, we analyzed data from experiments in which we induced a self-sustaining status epilepticus (SSSE) by BLA stimulation following different periods of post-surgical delay. In a total of 183 Sprague-Dawley rats, three groups with different periods of postsurgical delay to onset of electrical stimulation were compared: group 1 (16 days on average), group 2 (28 days) and group 3 (48 days). Three types of SSSE were observed after BLA stimulation: type 1 (nonconvulsive), type 2 (nonconvulsive occasionally interrupted by generalized convulsive activity), and type 3 (generalized convulsive). While groups 1 and 2 did not differ in the frequency of these SSSE types, the group with the longest interval between electrode implantation and stimulation (group 3) showed significantly more severe SSSE than the two other groups. The data indicate that intracranial electrode implantation may increase the sensitivity of the implanted area to seizure induction, extending previous findings in the kindling model. Potential mechanisms of these findings include the functional consequences of local microhemorrhages and blood—brain barrier destruction.

Abbreviations: ADT, afterdischarge threshold; BLA, basolateral amygdala; SE, status epilepticus; SRS, spontaneous recurrent seizures; SSSE, self-sustaning status epilepticus.

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Introduction

It is generally assumed that the use of intracranial recording with depth electrodes in the presurgical evaluation of patients with medically intractable epilepsy is a reasonably safe method (Van Loo et al., 2011). Similarly, in experimental studies with chronic implantation of depth electrodes into specific brain regions for recording of electroencephalographic activity or electrical stimulation of discrete regions, it is often implicitly assumed that the implantation of electrodes has little if any effect on brain function and related behaviors. However, we and others have previously shown in rats that chronic implantation of electrodes constructed from a variety of materials into different parts of the brain may induce lasting neurochemical, neurophysiological, morphological, and behavioral alterations, including a prokindling effect (Blackwood et al., 1982; Löscher et al., 1993, 1995, 1999; Niespodziany et al., 1999). Boast et al. (1976) demonstrated in rodents that electrode implantation per se may result in functionally significant hemorrhagic vascular damage and consequent release of blood into brain tissue, leading to iron deposits, which may induce chronic focal epileptic discharges (Willmore et al., 1978). In epilepsy patients, the incidence of intracranial hemorrhage associated with depth electrodes is up to 14% (Van Loo et al., 2011). Furthermore, electrode implantation locally destroys the blood—brain barrier, which may lead to extravasation of albumin and, hence, development of an epileptic focus (Friedman et al., 2009). From these data, we recently hypothesized that kindling, i.e., a model in which repeated administration of initially subconvulsive electrical stimuli via a depth electrode in the limbic system induces seizures that progressively increase in severity and duration, may represent a model in which the consequences of traumatic brain injury (by electrode implantation) are facilitated by electrical stimulation (Löscher and Brandt, 2010).

Over recent years, models in which a status epilepticus (SE) induced by electrical or chemical stimuli results in the development of spontaneous recurrent seizures (SRS) have progressively replaced kindling as a model of epileptogenesis (Löscher and Brandt, 2010). We have used such a model, in which a self-sustaining SE (SSSE) is induced by electrical stimulation of the anterior basolateral amygdala (BLA; Brandt et al., 2003), extensively over recent years and had repeatedly the impression that a long interval ($\geq$4 weeks) between electrode implantation and SE induction increases the incidence and severity of SSSE. We therefore performed a retrospective analysis of data to determine whether prolonged electrode implantation in the limbic system alters the sensitivity of rats to SE induction.

Methods

As in previous studies of our group on the SE model used in this study (Brandt et al., 2003), adult female Sprague-Dawley rats (body weight 200—230 g) were obtained from Harlan-Winkelmann (Borchen, Germany) or Harlan (Horst, Netherlands) and adapted for at least one week before onset of the experiments. Maintenance of rats was as previously described (Brandt et al., 2003). All experiments were done in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain or discomfort of the animals used.

At time of electrode implantation, all rats were about 80 days of age. For electrode implantation, rats were anesthetized with chloral hydrate (360 mg/kg i.p.). A bipolar electrode was stereotaxically implanted into the right BLA and served as stimulation- and recording-electrode. As in recent studies of our group, the electrode consisted of two twisted Teflon-coated, 0.2 mm diameter, stainless steel wires separated by 0.5 mm at the tip. The stereotaxic coordinates in millimeter relative to bregma according to the atlas of Paxinos and Watson (2007) were: AP, −2.2; L, −4.7; V, −8.3. One screw, placed above the left parietal cortex, served as the indifferent reference electrode. Additional skull screws and dental acrylic cement anchored the entire headset. After surgery, the animals were allowed to recover for periods of 12—55 days (see below).

Following these periods, 183 rats were electrically stimulated over 25 min via the BLA electrode for induction of SSSE as previously described (Brandt et al., 2003). The stimulus consisted of 100 ms trains of 1 ms alternating positive and negative square wave pulses. The train frequency was 2 Hz and the intra-train pulse frequency was 50 Hz. The intensity of the stimulus was 700 μA. In all rats, the EEG was recorded via the BLA electrode during SSSE. Four hours after the induction of the SSSE, the rats were injected with diazepam (10 mg/kg ip) to terminate the seizure activity.

Sustained BLA stimulation induced three different types of SSSE, as previously reported (Brandt et al., 2003). Type 1, partial (nonconvulsive) SSSE, which was characterized by stage 1 and stage 2 seizures (Racine, 1972); type 2, partial SSSE interrupted by occasional periods of stage 3 and generalized convulsive (stage 4 or 5) seizures; type 3, generalized convulsive SSSE (for further details see Brandt et al., 2003). The typical paroxysmal EEG alterations occurring during these types of SSSE have been described in detail by us previously (see Fig. 1 in Brandt et al., 2003). Previous experiments by our group have shown that both type 2 and type 3 SSSE induce SRS within 2—3 months in >90% of female Sprague-Dawley rats and about the same extent of neuronal damage in the hippocampus, while after type 1 SSSE only about 30% of the rats develop SRS and neuronal damage is much less severe (Brandt et al., 2003). In addition to recording the different types of SSSE, the severity of SSSE was scored as follows: 0, no SE; 1, type 1; 3, type 2; 5, type 3, respectively.

Based on recent studies in the kindling model (Löscher et al., 1993, 1995, 1999), the different intervals between electrode implantation and SE induction were arbitrarily grouped as follows: 12—21 days (group 1; $n = 95$; mean 16 ± 0.3 days), 22—34 days (group 2; $n = 69$; mean 28 ± 0.4 days), and 37—55 days (group 3; $n = 19$; mean 48 ± 1.6 days), respectively. The significance of differences in frequency of SE types between groups was calculated by Fisher's exact test, whereas one-way ANOVA with posthoc Tukey—Kramer multiple comparisons test was used for statistical evaluation of score data. A $P < 0.05$ was considered significant. The authors were blinded to ''time since stimulation'' when they assessed the severity of the SSSE.