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Transition from *status epilepticus* to interictal spiking in a rodent model of mesial temporal epilepsy

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ABSTRACT

Status epilepticus (SE) is a serious, life-threatening condition requiring immediate care to prevent neuronal injury and long-term functional deficits. SE is modeled in rodents by systemic injection of chemoconvulsants such as pilocarpine, which induces EEG and behavioral activities similar to what seen in humans. Combined injection of diazepam and ketamine is commonly used to terminate SE in rodents but, to date, no study has analysed the EEG activity and behavior during SE and after diazepam + ketamine administration. We therefore performed EEG recordings from the hippocampal CA3 region of mice before and during pilocarpine-induced SE as well as for 24 h after injection of diazepam + ketamine. We found that although convulsive behavior disappeared within 5.5 min (± 1.12 min; n = 5) after diazepam + ketamine treatment, EEG epileptiform activity resembling what seen during SE persisted up to 278.8 min (± 262.0 min). The end of this SE-like EEG pattern was characterised by transition to high amplitude, persisting interictal spikes. Our findings show that (i) administration of diazepam and ketamine stops behavioral but not EEG epileptiform activity associated to pilocarpine-induced SE; and (ii) such SE-like EEG pattern persists for approx. 4 h to be replaced by interictal spikes that predominate during the so called latent period in this model of mesial temporal lobe epilepsy.

1. Introduction

Status epilepticus (SE) is a life-threatening, neurological condition that is characterised by seizure activity with loss of consciousness lasting for more than 5 min, and that requires immediate care to prevent neuronal injury and long-term cognitive deficits (Treiman, 2007, 2001; Trinka et al., 2015). SE can lead to mesial temporal lobe epilepsy (MTLE), an epileptic disorder in which seizures initiate from the hippocampus, amygdala or entorhinal cortex (Gloor, 1997). In line with this clinical evidence, MTLE is modeled in animals by inducing a SE with the systemic injection of chemoconvulsants, such as pilocarpine or kainic acid; this initial SE is then followed, a few days later, by spontaneous focal seizures with EEG and behavioral features similar to those observed in patients with MTLE (Lévesque et al., 2015).

The concomitant injection of diazepam and ketamine is often used to stop pilocarpine-induced SE in rodents (Martin and Kapur, 2008). However, it remains unclear whether the behavioral arrest of SE following administration of these drugs is paralleled by normalization of the EEG activity. Therefore, we analysed the behavior and the EEG activity recorded from the hippocampal CA3 region in mice that were first injected with pilocarpine (to induce SE) and later with diazepam + ketamine (to stop it).

2. material and methods

All procedures were performed according to the protocols and guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee. C57BL/6 mice (The Jackson Laboratory) mouse colonies were bred and maintained under controlled environment (22 ± 2 °C, 12 h light/dark schedule) with food and water provided *ad libitum.* Mice from age 58 to 96 days (n = 5) were anaesthetized with 3% isoflurane in 100% O_2. Heads were then fixed onto a stereotaxic frame for the implantation of electrode and reference. An incision was made in the skin and one anchor screw (2.4 mm length) was fixed to the skull. Two small holes were then made to allow the implantation of a bipolar electrode and a reference. A recording electrode (20–35 kΩ) was implanted into the CA3 subfield of the hippocampus (ML: -3, AP: -2.85, DV: -4). The reference electrode (5–10 kΩ) was bent at 90° and inserted under the skull to overlie the cortex. The screw, bipolar electrode and reference were covered with

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dental acrylic cement. For 72 h post-op, chloramphenicol (Erfa, Qc, Canada) and lidocaine (5%; Odan) were applied around the surgery site, carprofen (20 mg/kg s.c.; Merail, Qc, Canada), enrofloxacin (5 mg/kg, s.c., CDMV, Qc, Canada) and 1 mL of 0.9% saline were injected every 24 h while buprenorphine (0.1 mg/kg s.c., CDMV, Qc, Canada) was injected every 8 h.

After surgery, mice were housed in custom-made 30 × 30 x 40 cm boxes for one week. They were then connected to a multichannel cable and electrical swivel (Commutator SL 18C, HRS Scientific, Qc, Canada). Continuous 24/7 recordings were performed from one day before induction of SE to 24 h after injection of diazepam and ketamine. EEGs were amplified with an interface kit (Mobile 36 ch LTM ProAmp, Stellate, Qc, Canada) and low-pass filtered at 500 Hz. The sampling rate was set at 2000 Hz. Data was collected by monitoring software (Harmonie, Stellate, Qc, Canada). Scopolamine methylnitrate (1 mg/kg i.p.; Sigma-Aldrich, Qc, Canada) was injected 30 min before the injection of pilocarpine. A dose of 200 mg/kg i.p. of pilocarpine (Sigma-Aldrich, Qc, Canada) was administered followed by doses of 100 mg/kg every 30 min until the onset of SE, which was characterised by continuous spiking activity on the EEG without a return to baseline. Diazepam (10 mg/kg, s.c.; CDMV, Qc, Canada) and ketamine (25 mg/kg, s.c.; CDMV, Qc, Canada) were administered 1 h after the onset of SE.

Ictal spikes associated to SE as well as interictal spikes after the administration of diazepam and ketamine was performed using custom-built algorithms in Matlab 7.11.0 (The Mathworks, MA, USA). Events having amplitude 5 SD above the mean of the baseline, defined as a 10 min time-period after behavioral recovery, were considered as epileptiform events. Continuous spiking activity was considered as SE, whereas isolated irregular spikes were considered as interictal spikes.

3. Results

Fig. 1 shows representative EEG recordings from a mouse before (Fig. 1A) and after the onset of SE (Fig. 1B). The administration of pilocarpine first induced interictal spikes and isolated seizures that were associated to body tremor, forelimb clonus and head bobbing (not illustrated). A transition to SE was then observed; this was characterised by continuous ictal spiking (Fig. 1Ba and b) that were associated to severe tonic-clonic seizures with intense salivation, jumping, rearing and falling. The administration of diazepam and ketamine rapidly abolished convulsive behavioral symptoms since, within 5.5 min (± 1.12 min), all mice showed complete muscle atonia. However, in all animals, ictal spikes - punctuated by episodes of flattening on the EEG - were observed (Fig. 1 Ca); such epileptiform pattern persisted up to 278.8 min (± 262.0 min) after injection of diazepam and ketamine.

As illustrated in Fig. 2, ictal spiking was still observed 2 h after diazepam and ketamine. This pattern of electrographic SE was then followed by interictal spiking activity that was recorded in our experiments up to 24 h after the injection of these drugs. Fig. 2B shows the quantification of spike frequency and its relationship to behavioral recovery for all 5 animals. In all animals, a return to normal behavior - which was characterised by grooming and exploration - was observed on average 792.4 min (± 476.5 min) after the end of the electrographic SE. However, once more, the EEG recorded from these mice continued to be characterised by interictal activity.

4. Discussion

The main findings of our study can be summarised as follows: (i) administration of diazepam and ketamine abolishes convulsive behavior associated to pilocarpine-induced SE in mice but does not efficiently suppress electrographic epileptiform activity recorded from the CA3 region of the hippocampus, which can persists up to 278.8 min (± 262.0 min) after treatment; and (ii) termination of the electrographic SE is associated to interictal spikes that continue to occur in this limbic area, which is often the onset zone of spontaneous seizures in this model of MTLE (Gloor, 1997).

Our findings show that injection of diazepam and ketamine is effective in abolishing convulsive behavior that occurs during SE induced by pilocarpine in rodents but fails in suppressing the electrographic

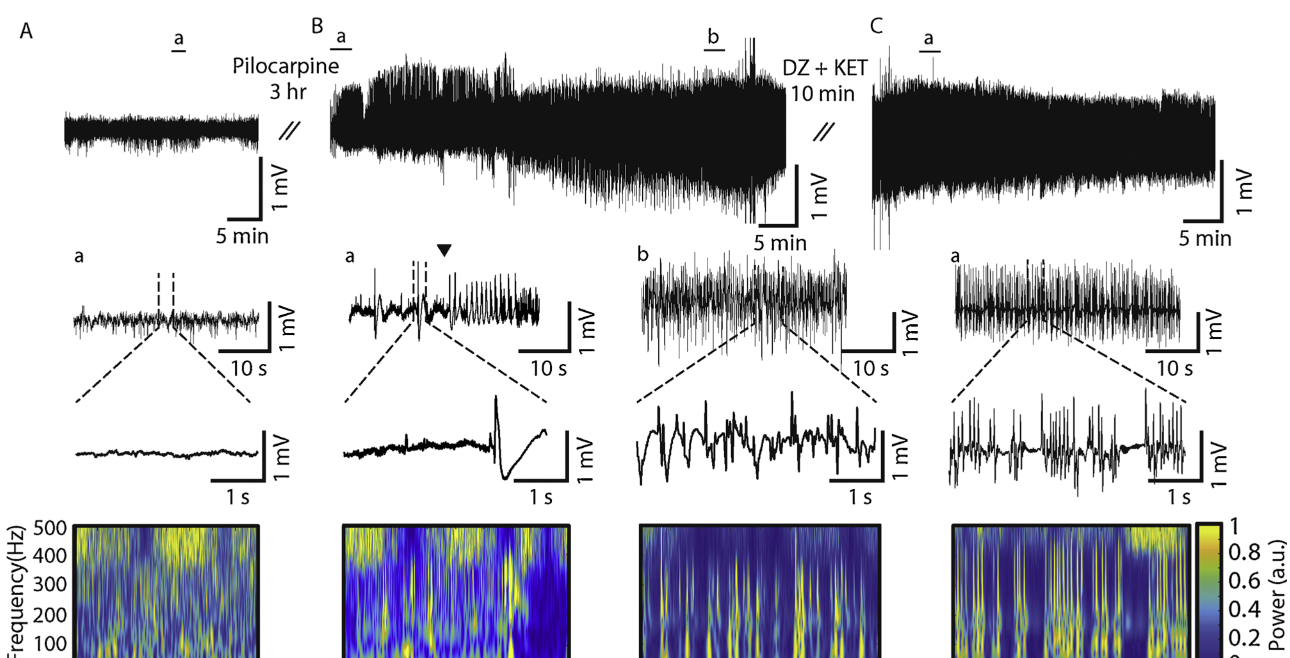

Fig. 1. Representative EEG recordings from the CA3 region of the hippocampus in a pilocarpine-treated mouse. **A-C:** EEG recordings were obtained before the onset of SE (**A**) and after the administration of pilocarpine (**B** and **C**). The onset of SE (**Ba**, arrowhead) was characterised by continuous ictal spiking, that persisted for one hour (**Bb**) after onset. Ictal spiking was still observed after the administration of diazepam and ketamine (**Ca**). Corresponding power spectra are shown to illustrate the changes in EEG activity. DZ = diazepam, KET = ketamine.

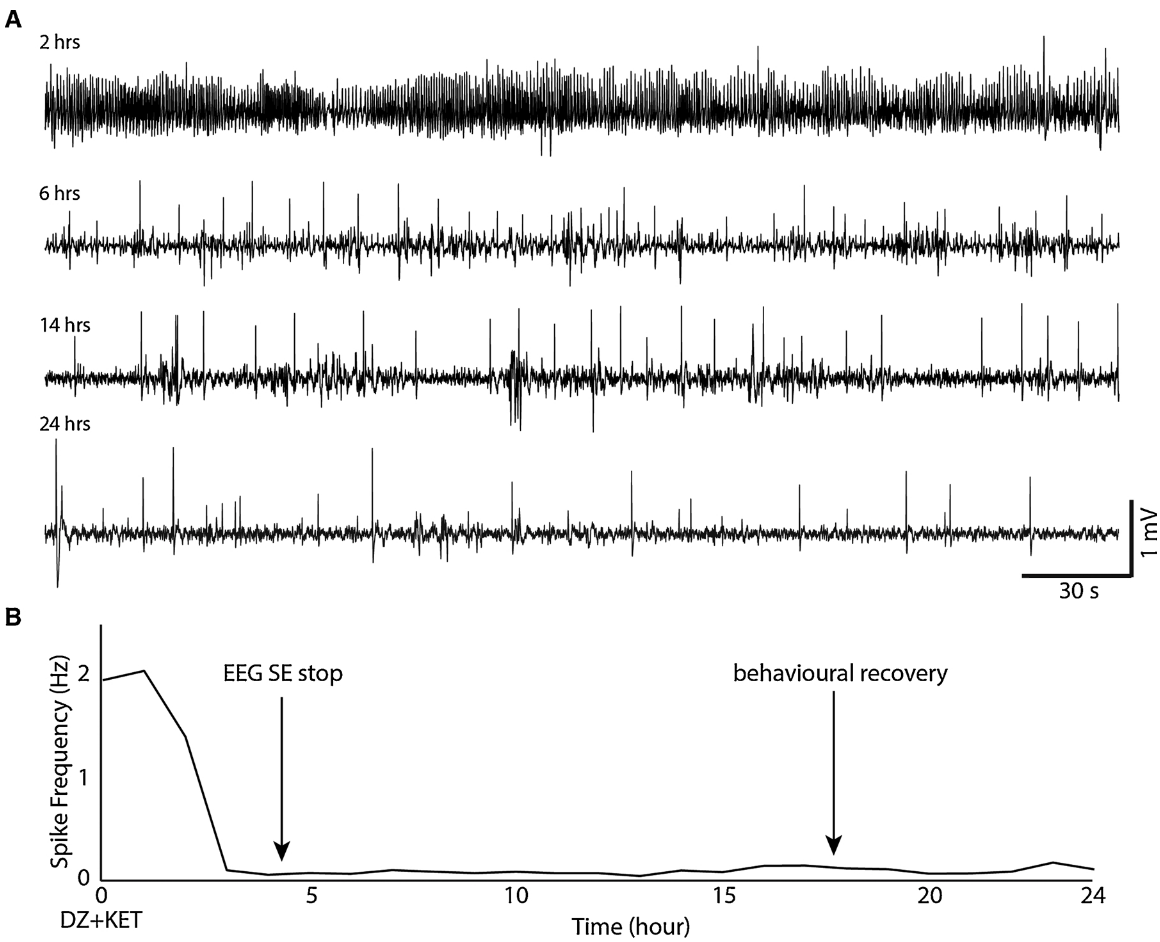

Fig. 2. Representative EEG recording and quantification of spikes after injection of ketamine and diazepam. **A:** Representative EEG recordings obtained from another pilocarpine-treated mouse 2, 6, 14 and 24 h after the administration of diazepam and ketamine. Note that 2 h after diazepam and ketamine, continuous ictal spiking is still observed on the EEG, and it is followed by irregular interictal spiking activity that persisted up to 24 h after. **B**: Frequency of spikes over time from the injection of diazepam and ketamine (time 0) to 24 h after. End of SE occurred 278.8 min (± 262.0 min) after ketamine and diazepam. Behavioral recovery occurred 792.4 min (± 476.5 min) after the end of electrographic SE.

counterpart that consists of continuous ictal spiking. The effects induced by diazepam and ketamine on the SE induced by systemic injection of pilocarpine in rodents, are therefore characterised by a clear dissociation between EEG activity and behavior; this evidence indicate that evaluating the duration of SE based on behavior could be biased when no depth EEG recordings are performed.

We have also observed that the end of the electrographic SE is followed by irregular interictal spiking persisting up to 24 h later, after which EEG recordings were stopped in these mice. Therefore, in the pilocarpine model of MTLE, a rapid transition to an epileptogenic condition occurs after SE. These results are similar to what has been previously reported by Puttachary et al. (2015), who found that nonconvulsive seizures occur approximately 140 min after the administration of diazepam in kainic-acid treated animals. Continuous recordings in pilocarpine-treated mice also showed irregular interictal spiking activity 24 h after diazepam injection to stop SE, as well as hippocampal spontaneous seizures on the following day (Mazzuferi et al., 2012; Pitsch et al., 2017). The pathophysiologic mechanisms triggering early spontaneous seizures after such a short delay are currently unknown, although it was recently proposed that pilocarpine could have immediate neurotoxic and inflammatory effects that favor neuronal hyperexcitability (Pitsch et al., 2017); such mechanisms could also explain the occurrence of irregular interictal spikes early after SE.

Our findings challenge the notion of a latent period in rodent MTLE models and support the hypothesis that epileptogenesis is a continuous process during which epileptiform activity can be generated in temporal lobe regions early after an initial brain insult. Further studies should however investigate whether interictal spiking activity between the end of SE and the first spontaneous seizure reflects residual epileptiform activity or the development of a chronic epileptic state.

Declarations of interests

None

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