BRIEF COMMUNICATION
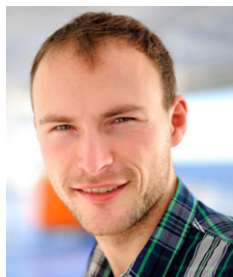
Forebrain-independent generation of hyperthermic convulsions in infant rats

*[1]Alexey S. Pospelov, *[1]Alexey Y. Yukin, †Mark S. Blumberg, *Martin Puskarjov, and *Kai Kaila

Alexey S. Pospelov is a doctoral scholar at the Department of Biosciences, University of Helsinki.

Alexey Y. Yukin is a doctoral candidate at the Department of Biosciences, University of Helsinki.

SUMMARY

Febrile seizures are the most common type of convulsive events in children. It is generally assumed that the generalization of these seizures is a result of brainstem invasion by the initial limbic seizure activity. Using precollicular transection in 13-day-old rats to isolate the forebrain from the brainstem, we demonstrate that the forebrain is not required for generation of tonic–clonic convulsions induced by hyperthermia or kainate. Compared with sham-operated littermate controls, latency to onset of convulsions in both models was significantly shorter in pups that had undergone precollicular transection, indicating suppression of the brainstem seizure network by the forebrain in the intact animal. We have shown previously that febrile seizures are precipitated by hyperthermia-induced respiratory alkalosis. Here, we show that triggering of hyperthermia-induced hyperventilation and consequent convulsions in transected animals are blocked by diazepam. The present data suggest that the role of endogenous brainstem activity in triggering tonic–clonic seizures should be re-evaluated in standard experimental models of limbic seizures. Our work sheds new light on the mechanisms that generate febrile seizures in children and, therefore, on how they might be treated.

KEY WORDS: Brainstem, Precollicular transection, Febrile seizures, Diazepam, Kainic acid.

Febrile seizures (FS) are the most common type of convulsive event in children.[1] The mechanisms underlying FS have been studied extensively in animal models.[2,3] A widely held assumption is that FS are limbic in origin and that the generalization of seizures, which is typical of FS, is caused by invasion of the brainstem by the initial limbic seizure activity. Based on a rodent model of FS where infant rats are exposed to hyperthermia,[4] we examined whether the forebrain is required for the generation of tonic–clonic convulsions. We employed a classical transection approach that has been used to study mesencephalic seizure generation.[5] To this end, 13-day-old rats with their forebrain surgically isolated from the brainstem (via precollicular transection)[6] were exposed to hyperthermia. Strikingly, we found that, upon exposure to hyperthermia, violent tonic–clonic convulsions, similar to those in controls, occurred in transected animals with a shorter latency and at a lower temperature threshold than convulsions in sham-operated littermate controls. Hyperthermia caused a similar increase in respiratory rate and blood pH in the two groups, an important observation given that respiratory alkalosis is a known trigger of FS.[4,7] We further extended our findings to the kainate model of status epilepticus.

Our work demonstrates that the forebrain is not required for generation of hyperthermia- or kainate-induced tonic–clonic convulsions, suggesting that, in standard animal models used to study limbic seizures and seizure generalization, the role of the brainstem in seizure generation may be more independent than previously assumed. Identification of the generators of febrile seizures is critical if we are to devise effective preventive treatments.

METHODS

Animals

The experiments were performed on postnatal day (P) 13 male Wistar rat pups. All procedures were approved by the National Animal Ethics Committee of Finland and the local animal ethics committee of the University of Helsinki.

Precollicular transection

On the day of the experiment, prior to surgery, rat pups were weighed and anesthetized using isoflurane (4% for induction; 2.3% maintenance). Skin covering the skull was shaved and disinfected with 70% ethanol. A small skin incision of ~3.5 mm in length was made over the occipital and parietal skull area. A hole was drilled 2 mm rostral to lambda, and a blunt hypodermic needle (Microlance 30G, BD) was inserted through the hole until the tip touched the bottom of the skull. By performing side-to-side movements of the needle, all connections between midbrain and diencephalon were severed.[6] The incision was closed using Vetbond adhesive (3M). Sham-operated animals underwent the same surgical procedure apart from inser-

tion of the needle through the hole. Following surgery, all pups recovered under thermoneutral conditions (35 ± 1°C) for ~60 min before experiments began. At the end of the experiments, the pups were killed and their brains carefully removed from the skulls. In all transected animals the brain fell immediately apart into two pieces: one containing the forebrain and the other the brainstem and cerebellum. Visual inspection of the transections confirmed that they began dorsally at the location rostral to the superior colliculus and ended ventrally between the pons and hypothalamus.

Induction of hyperthermic convulsions

Hyperthermic convulsions were induced as described in previous articles on experimental FS.[4,8] Briefly, transected and sham-operated littermates were placed together into individual compartments of a chamber preheated to 48 ± 1°C, with continuous behavioral video monitoring for experiment-blind offline analysis. Latency to onset was defined as the time to the appearance of either clonic and/or continuous tonic–clonic convulsions.[8] Both rectal temperature and breathing rate were recorded continuously using a thermocouple probe (Ret-3 coupled to BAT-12 thermometer, Physitemp) and a piezo sensor attached to the animal's chest (Pico movement sensor, TEMEC Instruments), respectively. Additional groups of transected and sham-operated pups were injected intraperitoneally with diazepam (0.15 or 2.5 mg/kg)[8] or saline vehicle (total injection volume: 3.33 μl/g body weight) 15 min before placing the animals into the heated chamber.

Blood analysis

Immediately after verification of the onset of clonic or tonic–clonic convulsions, 80 μl of arteriovenous blood was collected from each pup via rapid decapitation into plastic capillaries coated with 100 IU/ml lyophilized lithium-heparin (Sanguis Counting).

Blood parameters were subsequently measured using a blood gas and electrolyte analyzer (GEM Premier 4000, Instrumentation Laboratory).[8]

Kainic acid–induced seizures

After recovery from surgery, transected and sham-operated littermates were injected with kainic acid (3 mg/kg, i.p.). Following injection, pups were maintained at thermoneutral conditions for 90 min and behavior was recorded using video.

RESULTS

To examine the possible role of the brainstem as an independent generator of hyperthermia-induced convulsions, we took advantage of a preparation where forebrain struc-

tures are fully isolated from the brainstem by means of precollicular transection.[5,6] After recovery from anesthesia, unlike their sham-operated littermates, transected pups displayed sleep-like patterns of behavior, including low motor activity, sleep twitches, occasional spontaneous short locomotor acts, and lack of righting, in line with what has been reported previously.[9,10] Somatosensory stimulation caused

restoration of righting and locomotion, which disappeared immediately after the end of stimulation. Because of the obvious lack of forebrain-dependent early seizure manifestations such as nose rubbing and scratching[2,4] in the transected animals, time of onset was considered here as the time point when clonic or tonic–clonic convulsions were first observed upon exposure to hyperthermia.
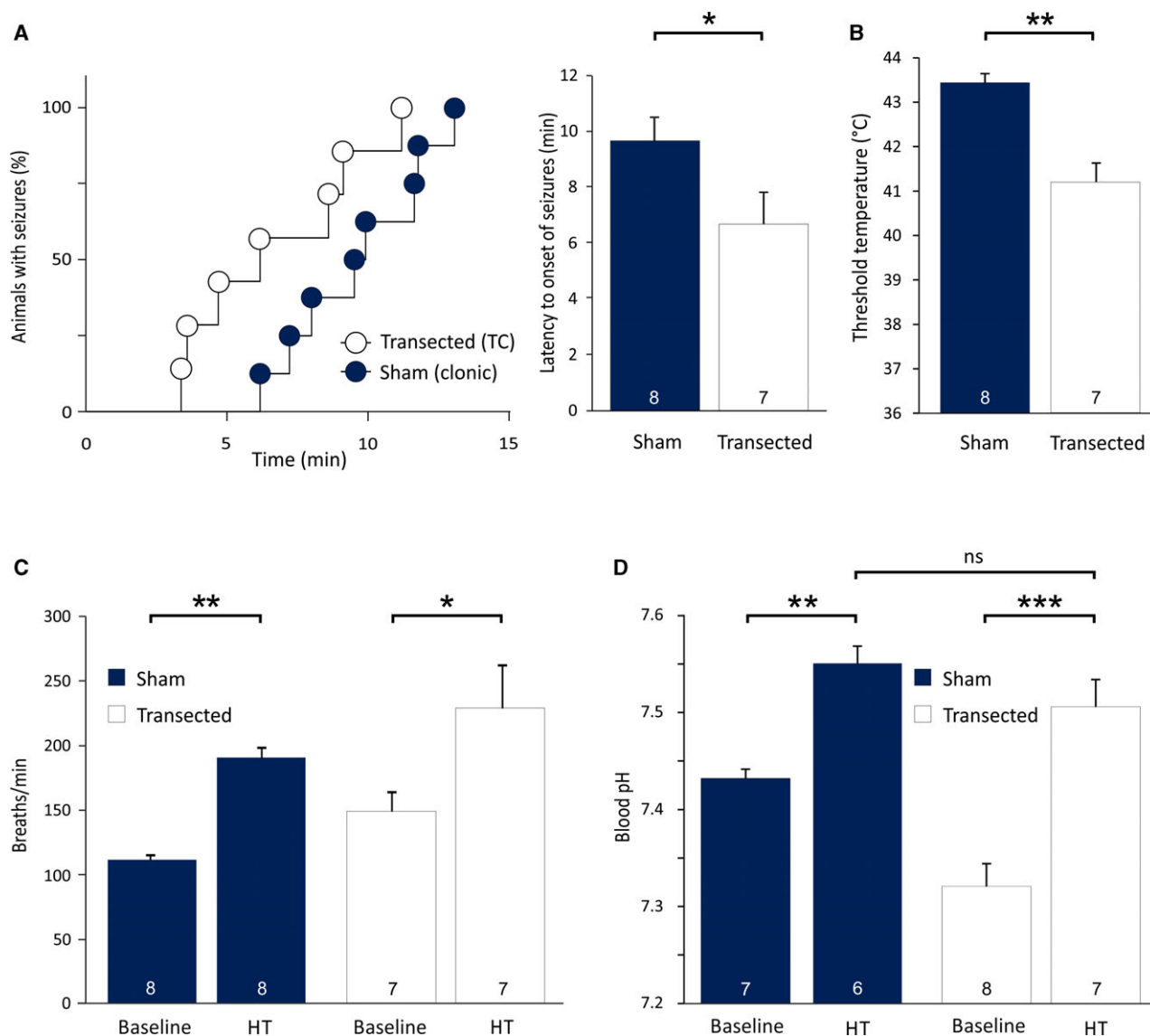
Figure 1.
Hyperthermia induces tonic–clonic convulsions in precollicularly transected P13 rats. (**A**) Left: Kaplan-Meier plots illustrating latencies to seizure onset in transected rats and in their sham-operated littermates. Unlike in sham-operated controls, seizure onset in transected animals was characterized by exclusive occurrence of tonic–clonic convulsions (TC); milder seizure stages in this group were not observed. In sham-operated controls, severe seizures manifested as clonic convulsions. Right: Mean latency to seizure onset in transected pups was shorter than in sham-operated littermates ($p < 0.05$, Mantel-Cox test). (**B**) Rectal temperature at seizure onset in transected pups was lower than in sham-operated pups ($p < 0.05$, Mann-Whitney U test). (**C**) In both transected and sham-operated groups, hyperthermia (HT) induced a significant increase in respiratory rate in relation to baseline ($p < 0.05$, Wilcoxon signed-rank test; HT mean rate derived from data immediate to seizure onset), with no difference in the magnitude of the respiratory rate increase between the two groups. (**D**) Blood pH at seizure onset (HT) was significantly higher than at baseline ($p < 0.05$, Mann-Whitney U test), with a similar change in pH in both groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The values for n are given in the bar diagram. Error bars denote standard error of the mean (SEM).
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Hyperthermia induced violent convulsions in all sham-operated (n = 8) and transected (n = 7) rat pups. In sham-operated pups, onset of behavioral seizures was characterized by severe clonic manifestations (i.e., multiple clonic convulsions of limbs) occurring on average with a latency of 9.7 ± 0.9 min from the start of exposure to hyperthermia. Mean body temperature was 43.4 ± 0.2°C at the time of onset of convulsions. Strikingly, in transected pups, hyperthermic convulsions were much more intense immediately at their onset, and they were exclusively tonic–clonic. Moreover, their onset latency was significantly shorter (6.7 ± 1.1 min; $p < 0.05$ vs. sham; Fig. 1A), and they were triggered at a lower body temperature (41.2 ± 0.4°C; $p < 0.01$ vs. sham; Fig. 1B). Taken together, these data show that in hyperthermia-induced convulsions, tonic–clonic manifestations, which are known to be driven by pathophysiologic activity in the brainstem,[11] are more intense and have a lower threshold in transected pups.

We have shown previously that an experimentally induced increase in body temperature of P8–12 rats leads to respiratory alkalosis, which triggers febrile seizures.[4,12] In line with this, an increased respiratory rate (Fig. 1C) and blood alkalosis (Fig. 1D) were observed in both transected pups and sham-operated controls. Notably, the hyperthermia-induced respiratory alkalosis at onset of convulsions in the two groups was of similar magnitude, ΔpH 0.18 ± 0.03 and 0.12 ± 0.02, with peak alkalosis at pH 7.50 ± 0.03 and 7.55 ± 0.02 ($p > 0.05$) in the transected and sham-operated pups, respectively (Fig. 1D). Thus, the convulsion-promoting action of precollicular transection is not attributable to an enhanced respiratory alkalosis in response

to hyperthermia. Control experiments performed on naive animals that had not undergone any surgery demonstrated that the surgical operation per se did not affect the baseline blood pH (sham pH: 7.43 ± 0.01; naive pH: 7.40 ± 0.01, n = 7 per group; $p > 0.05$; Fig. 1D).

Diazepam administered (2.5 mg/kg, i.p.) to transected rats 15 min before exposure to hyperthermic conditions prevented hyperventilation (onset of hyperthermia: 120 ± 11 breaths/min; at body temperature of 42°C: 141 ± 12 breaths/min; $p > 0.05$; n = 4) as well as the occurrence of convulsions. In contrast, a lower diazepam dose of 0.15 mg/kg (n = 10) or saline vehicle (n = 9) had no effect on the latency to onset of convulsions (11.2 ± 1.4 min and 10.2 ± 1.4 min, respectively). In addition, in transected rats injected with the lower dose of diazepam or saline vehicle, there were no significant differences in respiratory rate at the onset of convulsions (diazepam: 190.8 ± 37/min; vehicle: 197.6 ± 21.8/min; $p > 0.05$), threshold body temperature (diazepam: 39.8 ± 0.3°C vehicle: 39.7 ± 0.6°C; $p > 0.05$), or blood pH (diazepam: 7.51 ± 0.02; vehicle: 7.51 ± 0.02; $p > 0.05$) at the time of convulsion onset.

This prompted us to look at the role of the brainstem in the kainic acid model of status epilepticus, which is frequently used in studies of limbic seizures and epileptogenesis.[13] As was the case for the hyperthermia model, only tonic–clonic convulsions (with no preceding clonic component) were provoked following injection of kainic acid in all transected pups (n = 14), with a latency to convulsions onset of 11.8 ± 1.5 min. In sham-operated littermates (n = 14), latency to seizure onset was significantly longer (17.6 ± 1.5 min;
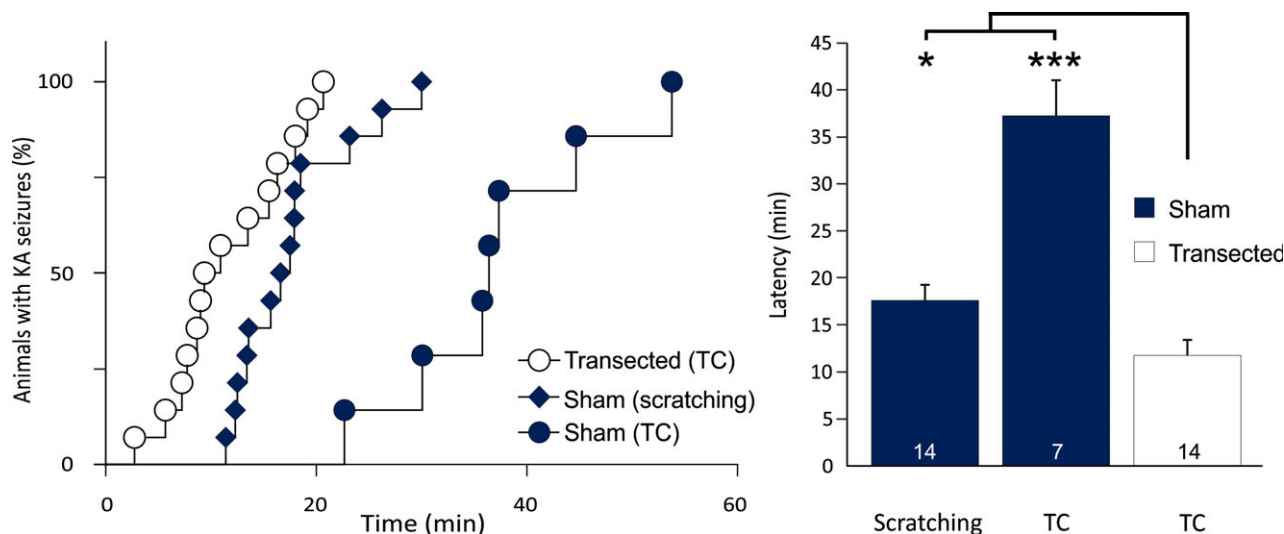
Figure 2.
Kainic acid induces tonic–clonic convulsions in precollicularly transected P13 rats. Left: Kaplan-Meier plots showing that onset of tonic–clonic (TC) convulsions in transected pups occurs at a shorter latency compared to onset of scratching and tonic–clonic convulsions in sham-operated littermates. Right: Mean latency to seizure onset in transected and sham-operated pups. *$p < 0.05$, ***$p < 0.001$, Mantel-Cox test. The values for n are given in the bar diagram. Error bars denote SEM.
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p < 0.05), with seizures initially characterized by scratching that progressed into clonus (Fig. 2). Tonic–clonic convulsions were induced in 7 of 14 sham-operated rats with latencies of 37.3 ± 3.8 min (p < 0.001 versus transected littermates; Fig. 2). Thus, following precollicular transection, the lower threshold and greater intensity of convulsions observed in pups exposed to hyperthermia were also seen in pups injected with kainic acid.

DISCUSSION

Evidence for the role of the brainstem as a seizure generator in convulsive disorders has been derived largely using circumscribed lesions and transections of the adult mammalian brainstem, as well as from human electroencephalography (EEG) recordings.[5,9,14,15] Little is known in this regard about the immature brain[16–18].

Initial seizure behavior induced by hyperthermia in infant rodents involves arrest of movement (i.e., freezing) followed by oral automatisms that are typical for limbic seizures. Based on this, FS generalization in rodents and, by implication, in humans, has been assumed to originate in the limbic circuit.[2] In other words, the most severe stage of FS, characterized by tonic–clonic or purely tonic convulsions, has been attributed to propagation of forebrain seizures to the brainstem.[11] In contrast, we show here using precollicular transections that the brainstem is sufficient for triggering of tonic–clonic convulsions induced in widely used animal models of FS (hyperthermia) and limbic seizures (kainic acid).

The most striking findings in the present work are that not only is the latency to onset of convulsions significantly reduced in transected pups, but that the convulsions are more severe immediately upon onset. These findings support the hypothesis that in the intact brain, brainstem seizure activity is suppressed by forebrain structures.[5] Accordingly, the frequently observed arrest of movement that is commonly seen in rodents during cortical electrographic seizures[11] may, perhaps somewhat paradoxically, reflect active suppression of brainstem activity by hippocampal/neocortical seizures.[8]

We have shown previously that in naive animals, behavioral experimental FS are exacerbated by low doses of diazepam, whereas high doses have a suppressing effect.[8] This seizure-suppressing effect is most likely caused by suppression of breathing by diazepam and the consequent prevention of FS triggering of respiratory alkalosis.[8] Consistent with these findings, we found here that the hyperthermia-induced convulsions in the transected pups were not affected by a low dose of diazepam, whereas an order-of-magnitude higher dose had a marked anticonvulsant effect.

Our previous work has shown that febrile seizures are promoted by hyperthermia-induced respiratory alkalosis in rodents and humans.[4,7,8,12] A similar respiratory alkalosis was presently observed in both transected and sham-operated pups. The current data add strength to the idea[8] that pharmacotherapy of FS by diazepam and related drugs is based on a suppression of breathing and the consequent block of the FS–promoting respiratory alkalosis.

In summary, our results indicate a prominent role for subdiencephalic networks in the triggering of severe convulsions in seizure models that, so far, have been considered prototypical in studies of limbic seizures. The potential identification of the brainstem as a source of seizure generation in humans should help to guide future work aimed at preventing and treating febrile seizures in children.

ACKNOWLEDGMENTS

DISCLOSURE

None of the authors have any conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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