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Short communication

Behavior in the open field predicts the number of KCl-induced cortical spreading depressions in rats

Volodymyr Borysovych Bogdanov[a,c,*], Olena Viktorivna Bogdanova[b,c], Stanislav Vladimirovich Koulchitsky[a], Virginie Chauvel[a], Sylvie Multon[a], Mykola Yukhymovych Makarchuk[b], Kevin Christopher Brennan[c], Perry Franklin Renshaw[c], Jean Schoenen[a]

[a] Headache Research Unit, GIGA-Neurosciences, University of Liege, CHU T4+1, Av de l'Hôpital 1, Liege 4000, Belgium
[b] Taras Shevchenko National University of Kyiv, Volodymyrska str., 64, Kyiv 01033, Ukraine
[c] University of Utah, 383 Colorow Drive, Salt Lake City 84108, UT, USA

HIGHLIGHTS

- Anxiety disorders are frequently comorbid with migraine.
- Cortical spreading depression (CSD) is a culprit for migraine aura.
- Anxious rats have low activity in the open field test.
- Low open field activity predicted higher CSD susceptibility in rats.
- Migraine prophylaxis drugs affected correlation between behavior and CSD numbers.

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ABSTRACT

Anxiety disorders are known to be comorbid with migraine, and cortical spreading depression (CSD) is the most likely cause of the migraine aura. To search for possible correlations between susceptibility to CSD and anxiety we used the open field test in male Sprague-Dawley rats chronically treated with the preventive anti-migraine drugs valproate or riboflavin. Animals avoiding the central area of the open field chamber and those with less exploratory activity (i.e. rearing) were considered more anxious. After 4 weeks of treatment CSDs were elicited by application of 1 M KCl over the occipital cortex and the number of CSDs occurring over a 2 h period was compared to the previously assessed open field behavior. Higher anxiety-like behavior was significantly correlated with a higher frequency of KCl-induced CSDs. In saline-treated animals, fewer rearings were found in animals with more frequent CSDs ($R = -1.00$). The duration of ambulatory episodes in the open field center correlated negatively with number of CSDs in the valproate group ($R = -0.83$; $p < 0.005$) and in riboflavin treated group ($R = -0.69$; $p < 0.05$) as well as total time spent in the open field center in both groups ($R = -0.75$; $p < 0.05$ and $R = -0.58$; $p < 0.1$ respectively). These results suggest that anxiety symptoms are associated with susceptibility to CSD and might explain why it can be an aggravating factor in migraine with aura.

1. Introduction

Migraine is a neurological disorder, characterized by recurrent attacks of severe headache, nausea, photo- and/or phonophobia. In 20% of subjects the migraine headache is preceded by a transient neurological disturbance known as the aura. Cortical spreading depression (CSD) has been suggested to be a trigger for aura, and, possibly, headache. CSD is a wave of neuronal and glial depolarization, slowly propagating in the cortex (3–5 mm/min), and followed by a long-lasting suppression of neuronal activity and excitability.

Anxiety disorders are highly comorbid with migraine [1] and could share neurobiological abnormalities in the same neuronal networks [2]. Anxiety and other psychiatric comorbid disorders are known to aggravate migraine disability and have also been associated with a more chronic course of migraine [3]. The basis for the relationship between anxiety and migraine is not known, and may implicate a range of mechanisms. Whatever the precise relationship may be, it is known that anxiety increases brain excitability in

* Corresponding author. Present address: Brain Institute, University of Utah, 383 Colorow Drive, Salt Lake City 84108, UT, USA. Tel.: +1 801 587 1488; fax: +1 801 581 8075.
E-mail address: vlabogd@yahoo.com (V.B. Bogdanov).

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humans [4] and it could thus in theory augment susceptibility to CSD.

In a previous study we noted that chronic administration of anti-migraine drugs differentially influenced susceptibility to KCl-induced CSD in rats [5]. As part of this study we assessed anxiety-like behavior in the open field test (OFT) in order to search for a possible relationship between level of anxiety and CSD frequency and to verify if previously observed migraine prophylaxis drug effects on CSD susceptibility were accompanied by a change in anxiety levels.

2. Materials and methods

2.1. Animals and drugs

Male adult Sprague-Dawley rats, 287.5 ± 16.1 g average initial body weight, were chosen among the sample of animals used in our previous study on drug effects [5] for the behavioral study: 5 randomly chosen animals in the group treated for 4 weeks with daily i.p. injections of saline (1 ml/kg), 10 animals in the groups treated with i.p. injections of valproate (200 mg/kg, Merck, Belgium) and 10 animals in the group receiving i.p injections of flavin mononucleotide as a donor of riboflavin (Riboflavin 5′-phosphate ester monosodium salt, 12.7 mg/kg, Sterop-Pharmacobel, Belgium). All animals performed two sessions of the open field test (after 2 and 3 weeks of treatment), to detect drug effect and to reduce the effect of novelty on anxiety-like behavior. Two hours after the last drug administration animals were subjected to electrophysiological recording. The study was approved by the University of Liege institutional ethics committee and guidelines for animal care were followed.

2.2. Behavioral studies

We used the OFT, a widely used method of estimation of anxiety-like behavior in animals [6]. The test sessions were performed just before drug administration. OF activity was assessed during 15 min using the MED-OFA-RS (43.2 cm × 43.2 cm) automatic infra-red beam system (Med Associates Inc., St. Albans, VT, USA). The central 21 cm × 21 cm area was defined as the OF center, with a peripheral area 11 cm in width considered as outer zone of the OF (Fig. S1, available online in supplementary materials). The following measures were taken: total time spent in the central zone, time of ambulatory episodes in the central zone, time of vertical activity (i.e. rearings), considered to belong to the anxiety construct [7].

2.3. Surgery and electrophysiological recordings

Cortical spreading depressions were recorded uniformly as previously described [5]; see also supplementary material for procedure details. Briefly, the number of CSD was measured in frontal and parieto-occipital cortices (Fig. S1, available online in supplementary materials) during a 2-h application of KCl over the posterior cortex in anesthetised animals. Cortical DC potential shifts and the electrocorticogam were recorded with Ag/AgCl electrodes. The electrical signals were amplified with an ISODAM-8A bioamplifier at a DC-10 kHz band width (WPI Inc, USA), digitized at a 200 Hz sampling rate and stored for off-line analysis using Micro1401 MKII and Spike2 software (CED Co., UK). CSDs were provoked by 1 M KCl application in the most posterior burr hole every 20 min. CSDs were counted continuously for 2 h and their frequency expressed as mean number per hour.

2.4. Statistical analysis

Statistica 9 (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analyses. Repeated measures ANOVA were used to define time and group effects in behavior and CSD location, one way ANOVA for the group effect in CSD parameters. Fisher's least significant difference post hoc p values are reported. Significance of LSD post hoc tests was evaluated only if the overall ANOVA $p \leq 0.05$. Group values were expressed as means ± standard errors of means, unless otherwise specified. Spearman's rank correlation test was used to compare individual OF test data with the number of CSD in posterior and anterior recording sites. The significance threshold was set at $p \leq 0.05$.

3. Results

3.1. Between-group differences in behavior and CSD susceptibility

During the first session of the OFT, animal behavior differed only in the riboflavin group ($p < 0.05$) demonstrating a 1.8 fold increase in time of vertical activity and 3.2 fold elevation of the time spent in the OF center compared to the control group. No differences in behavior were found between animal groups in the second session of behavior studies.

The number of anterior CSDs (CSDs propagating from the stimulation site toward posterior and then anterior recording sites, see Fig. S1, available online in supplementary materials) was consistently two times lower than the number of posterior CSDs in all the groups ($p < 0.01$ for every group): 7.1 ± 0.9 vs 4.2 ± 0.8 in saline (NaCl) group, 7.1 ± 0.6 vs 2.8 ± 0.4 in valproate treated group and 8.7 ± 0.6 vs 4.5 ± 0.4 in riboflavin group. Valproate treatment had no effect on posterior CSDs, but reduced anterior CSDs by 36% in comparison to the saline treated group ($p = 0.029$). No effect of riboflavin treatment on the number of CSDs was found. These data are comparable to those published previously for the total treatment group [5].

3.2. Within-group relationship between behavior and CSD frequency

In saline-treated animals, there was a strong negative correlation ($R = -1.00$) between time of vertical activity (rearing) during the second session of the OFT and the number of anterior CSDs. Other behavioral parameters did not correlate with CSD number in this group (Table 1).

In the valproate group, negative correlations were found between the number of CSDs in the anterior recording site and all behavior measures during first session of OFT: time of vertical activity ($R = -0.84$; $p < 0.005$), trends for time of ambulatory episodes in the OF center ($R = -0.57$; $p < 0.1$) and total time spent in the OF center ($R = -0.63$; $p < 0.1$). These findings were reproduced in the second session of OFT except for rearings: numbers of anterior CSDs negatively correlated with time of ambulatory episodes in the OF center ($R = -0.83$; $p < 0.005$) and total time spent in the OF center ($R = -0.75$; $p < 0.05$) (Table 1).

In the riboflavin group there was a negative correlation between locomotor activity during the second session of the OFT and the number of posterior CSDs: for time of ambulatory episodes in the OF center ($R = -0.69$; $p < 0.05$) and a trend for a negative correlation between number of posterior CSDs and time spent in the OF center ($R = -0.58$; $p < 0.1$) (Table 1).

4. Discussion

4.1. Between-group differences in behavior

In our study, only chronic riboflavin treatment resulted in novelty-linked increases in locomotion parameters. Riboflavin treatment could be linked to increased novelty-seeking behavior by promoting serotonin turnover via flavin containing monoamine oxidase. Decreased serotonin content in the frontal cortex was reported in a high rearing subpopulation of rats [8]. Conversely, in serotonin transporter knockout mice, an increase in extracellular concentrations of serotonin was accompanied by hypolocomotion and reduction in exploratory activity [9]. In our study, the behavior of riboflavin treated animals during the second session of OFT did not differ from the control group. This is consistent with the findings of Thiel at al [8] where high rearing rats show increased habituation in the OFT: on repeated testing, no behavioral differences between high rearing and low rearing animals were found.

Valproate treatment did not change OFT behavior, consistently with results reported by Barros at al [10], suggesting that suppression of CSD propagation by chronic valproate treatment is not likely to be associated with anxiolitic effects.