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

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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 hour period was compared to the previously assessed open field behaviour. Higher anxiety-like behaviour 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.

Keywords

cortical spreading depression; anxiety; valproate; riboflavin

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

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(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 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 behaviour 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 behavioural 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 behaviour. 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 behaviour in animals [6]. The test sessions were performed just before drug administration. OF activity was assessed during 15 minutes using the MED-OFA-RS (43.2 cm $\times$ 43.2 cm) automatic infra-red beam system (Med Associates Inc., St. Albans, VT, USA). The central 21 $\times$ 21 cm area was defined as the OF center, with a peripheral area 11 cm in width considered as outer zone of the OF (Figure 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 (Figure S1, available online in supplementary materials) during a 2-hour application of KCl over the posterior cortex in anesthetised animals. Cortical DC potential shifts and the electrocorticogram 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 1MKCl application in the most posterior burr hole every 20 minutes. CSDs were counted continuously for 2 hours 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 was used to define time and group effects in behaviour 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 $p < 0.05$. Group values were expressed as means $\pm$ 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 $p < 0.05$.

3. Results

3.1. Between-group differences in behaviour and CSD susceptibility

During the first session of the OFT, animal behaviour 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 behaviour were found between animal groups in the second session of behaviour studies.

The number of anterior CSDs (CSDs propagating from the stimulation site toward posterior and then anterior recording sites, see Figure 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 behaviour 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 behavioural 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 behaviour 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 behaviour

In our study, only chronic riboflavin treatment resulted in novelty-linked increases in locomotion parameters. Riboflavin treatment could be linked to increased novelty-seeking behaviour 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 behaviour 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 et al [8] where high rearing rats show increased habituation in the OFT: on repeated testing, no behavioural differences between high rearing and low rearing animals were found.

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

4.2. Relationship between OFT behaviour and CSD frequency

Our results demonstrating relationships between greater CSD susceptibility and lower activity in the OFT are in line with the observation of co-occurrence of anxiety-like behaviour induced by an acoustic stimulus and CSD recorded in awake rats with a liability to develop spontaneous seizures [11]. It was shown previously that CSD propagation to the amygdala can provoke anxious freezing in rats independently in response to noxious trigeminal activation [12].

It is known that vertical activity and locomotion in the OFT have strong correlations with hippocampal slow wave activity (theta rhythm) in the rat brain. Hippocampal slow wave activity in rat can be enhanced by cholinergic drugs [13] and similar mechanisms are suggested to regulate exploratory rearing activity in rats [14]. Rats with higher cholinergic activation in the hippocampus and frontal cortex have more pronounced rearing responses in the novel OFT [15]. It has been shown that propagation, but not induction, of retinal spreading depression is suppressed by acetylcholine [16]. Increased expression of cholinergic receptors in sensory-deprived cortex mediates inhibition of sensory responses via hyperexcitability of GABAergic inhibitory neurons [17]. This potentially can explain inhibition of CSD in response to high acetylcholine levels. Thus it can be speculated that individual differences in cholinergic system function underlie greater exploratory activity and less efficient CSD propagation from occipital to frontal cortex in control animals due to indirect antagonism between CSD propagation and hippocampal slow wave activity.

Locomotor and exploratory behaviours significantly predicted CSD frequency at the anterior recording site, i.e. frontal cortex in all animals, except for the riboflavin group. The preferential correlation with anterior CSD frequency may suggest that anxiety-like behaviour more likely predisposes to CSD propagation from occipital to frontal cortex than to CSD induction.

4.3. Treatment effect on correlations between behaviour and CSD susceptibility

However, CSD susceptibility correlated differently with three estimated behaviour measures in the saline, valproate and riboflavin groups of animals. Time of vertical activity, i.e. rearing, was related to lower CSD frequency in the saline group, while activity in the OF center (total time spent and time of ambulation) predicted lower CSD susceptibility in the valproate and riboflavin treated animals.

Riboflavin treatment, used in migraine prophylaxis [18], has antinociceptive and anti-inflammatory effects, decreases edema after focal cerebral ischemia and thus can protect against hypoxia and neuronal swelling, which happens during CSD. We found a negative correlation between ambulatory activity in the OF center of riboflavin-treated animals and their CSD numbers recorded under the posterior electrode (proximal to induction site) but not the anterior one. This suggests that under riboflavin treatment, anxiety-like behaviour predicts CSD susceptibility rather than its propagation. In the same dataset of animals, serum levels of riboflavin, flavin mononucleotide and total flavins negatively correlated with the number of CSDs (data not shown). Taken together with the fact that chronic riboflavin treatment did not reduce CSD number in the whole group, it could be an example of differential riboflavin effects due to individual variations in its pharmacokinetics.

Valproate, an anticonvulsant and mood stabilizer that increases GABA neurotransmission, has been used for more than 50 years in migraine prophylaxis and treatment. In spite of the fact that we did not observe any alterations in animal activity caused by valproate treatment, the number of CSDs also correlated with OF behaviour in the first session. Valproate-induced alteration of novelty seeking behaviour [10] may serve as a possible explanation of this association. Interindividual differences may be responsible for discrepancies between the behavioral and electrophysiological efficacy of valproate treatment found in our study. In a subpopulation of animals with increased behavioural responsiveness, valproate treatment attenuates pharmacological induced hyperactivity [19] or normalizes novelty-induced increased locomotor activity in mice that overexpress calcineurin [20]. Valproate also increased immobility in the forced swim test only in animals with low baseline immobility [21]. One possible neurological basis for these interindividual differences arises from the fact that dopamine neurotransmission mediates novelty-seeking and motivated behaviour. High novelty-responder rats have increased dopamine levels in the striatum [8]. Valproate inhibited hyperactivity induced by the dopamine injection into the nucleus accumbens [22], and normalized hyperactive behaviour in hyperdopaminergic dopamine transporter knockdown mice [23], but had no effects on control animals.

Population data indicate that migraine with aura is more strongly associated with anxiety disorder and depression than migraine without aura [24]. Indeed, migraine with aura joined with anxiety are even considered as components of a distinct clinical syndrome associated with allelic variations within the dopamine receptor [25].

In our study design we cannot distinguish if there is a direct causal relationship between anxiety-like behaviour and increased CSD susceptibility, or if they are separate consequences of the same functional brain property. But our results provide a translational background for further human research. Anxiety increases brain excitability in humans and higher CSD susceptibility in anxiety patients may be a potential mechanism for triggering migraine symptoms.

Conclusions

Our work provides evidence for a link between open field behaviour and susceptibility to cortical spreading depression. The results may be contributed to research on the

pathophysiology of migraine and the shared neurological basis of CSD and anxiety deserves further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- 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

Table 1

Correlation coefficients between hourly numbers of anterior or posterior CSDs and time (sec.) of vertical activity, ambulatory episodes in OF center, time spent in OF center in the different treatment groups

animal groups, first session of OFT						
	saline, n=5		valproate, n=10		riboflavin, n=10	
	posterior	anterior	posterior	anterior	posterior	anterior
CSD recording site						
vertical activity	0.00	0.30	-0.30	-0.84 **	-0.02	0.21
ambulatory episodes in OF center	0.50	0.70	-0.03	-0.57 [^]	-0.21	0.14
spent in OF center	0.50	0.70	-0.10	-0.63 [^]	-0.07	0.35
animal groups, second session of OFT						
	saline, n=5		valproate, n=10		riboflavin, n=10	
	posterior	anterior	posterior	anterior	posterior	anterior
CSD recording site						
vertical activity	-0.80	-1.00 ^{n/a}	-0.41	-0.16	-0.40	0.09
ambulatory episodes in OF center	0.10	-0.40	-0.24	-0.83 **	-0.69 *	-0.31
time spent in OF center	0.30	-0.20	-0.04	-0.75 *	-0.58 [^]	-0.29

[^] p<0.1,

* - p<0.05;

** - p<0.005;

n/a - significance level is not estimated if R=1.00.