

Subconvulsive dose of kainic acid transiently increases the locomotor activity of adult Wistar rats

Short communication

Vladimír Riljak, Dana Marešová, Jaroslav Pokorný, Kateřina Jandová

Institute of Physiology, First Faculty of Medicine Charles University in Prague

Corresponding author:

Vladimír Riljak, MD., PhD., Institute of Physiology, First Faculty of Medicine, Charles University in Prague, Czech Republic, Albertov 5, Prague 2, 128 00, Email: vladimir.riljak@lf1.cuni.cz, Phone: +420 224 968 443

Short title: Subconvulsive kainic acid dose and rat's spontaneous behavior

Summary:

Kainic acid (KA) is a potent neurotoxic substance valuable in research of temporal lobe epilepsy. We tested how subconvulsive dose of KA influences spontaneous behavior of adult Wistar rats. Animals were treated with 5mg/kg of KA and tested in Laboras open field test for one hour in order to evaluate various behavioral parameters. Week after the KA treatment animals were tested again in Laboras open field test. Finally, rat's brains were sliced and stained with Fluoro-Jade B to detect possible neuronal degeneration. Treatment with KA increased the time spent by locomotion ($p<0.01$), exploratory rearing ($p<0.05$) and animals traveled longer distance ($p<0.01$). These parameters tended to increase thirty minutes after KA administration. Week after the treatment we did not found differences in any measured behavioral parameter. Histology in terms of Fluoro-Jade B staining did not reveal any obvious neuronal damage in hippocampus. These results demonstrate that subconvulsive KA dose changes the behavioral parameters only transiently. Clarification of timing of the KA induced changes may contribute to understand mutual relationship between non-convulsive seizures and behavioral/cognitive consequences.

Keywords: Kainic acid, Laboras, behavior, Wistar rat, locomotor activity

Main body of the text:

Kainic acid (KA) is agonist for kainate subtype receptors of excitatory amino acids (Monaghan and Cotman 1982) and its administration was revealed as a good strategy to model the clinical and neuropathological features of temporal lobe epilepsy (Olney et al. 1974). Most commonly, a systemic administration of KA in convulsive dose (10mg/kg) is used to induce status epilepticus leading to massive excitotoxic damage of neuronal tissue (Doble 1999, Riljak et al. 2007). The KA induced status epilepticus is followed by latent period and occurrence of spontaneous recurrent seizures (Albala et al. 1984, Sperk 1994, Drexel et al. 2012). Morphological damage, as a consequence of KA administration is mostly expressed in brain structures rich of kainate receptors like amygdala and hippocampus - degeneration of CA1 and CA3 hippocampal subfields with final hippocampal sclerosis and the mossy fiber sprouting in the inner molecular layer of the dentate gyrus was documented elsewhere (Sperk 1994, Drexel et al. 2012). Mentioned deleterious KA effects are mediated partially by excessive glutamate release, partially by generation free radical species (Doble 1999). On the other hand, systemic administration of KA in doses lower than 6mg/kg induces limbic non-convulsive seizures of variable intensity apparent as scratching and wet dog shakes (Sperk 1994). However behavioral consequences of low dose KA treatment have been described by few authors only (Koryntová and Mareš 1998, Kubová et al. 2001). In order to observe the spontaneous behavior of rat, the open field test is usually used (in many different versions). The time of such studies is intentionally limited (usually ten or fifteen minutes). Therefore we decided to use automated observational system LABORASTM (Metris B.V., Netherland, in the text referred as Laboras open field test, LOFT), to extend this observation to one hour and to catch even the whole behavioral profile of animals placed into the novel environment.

The focus of current study was to further understand the consequences of low dose KA exposure and to determine, whether KA effect on animal behavior is long-lasting. LOFT allows to classified spectrum of different behavioral parameters and characteristics like locomotion, average speed during test session, duration of immobility, distance traveled by animal and rearing occurrence (Schwarz et al 2014, Jandova et al 2013). LOFT took place immediately after KA administration and one week after. Secondly, the immunohistological analysis of hippocampus was made (Fluoro-Jade B) to visualize hippocampal degeneration. We hypothesized, that KA increases the locomotion and average speed of animals, presuming that activation of glutamate receptors could cause excitation and pro-exploratory behavior. Secondly we hypothesized, that behavioral changes caused by low KA dose will have long-lasting effects, as far as recurrent seizures appear.

Experiment was performed in 20 male Wistar rats (purchased from Velaz, Czech Republic). The average weight of animal was 235 ± 2.0 g (8 weeks old as they entered the experiment). All animals were housed 4 animals per cage in a temperature-controlled environment (22-23°C) with free access to food and water on a standard 12 h light/dark cycles (with lights on at 06:00 hr.). All experiments took place between 08:00 and 15:00 in a room with lights on (light intensity between 150 and 200 lx at the level of cages). Animals were weighted prior each session, marked and randomly assigned either to KA treated group (5mg/kg of kainic acid purchased from Sigma, dissolved in saline and injected i.p.) or to second group treated with saline (i.p.) in equal volume. Animals were then placed and tested in Laboras apparatus (Metris B.V., Netherlands) to detect their spontaneous behavior for one hour. During the tests animals had not access to either food or water. After the period of one week the animals were again tested in LOFT for one hour. Experiment was ended day after the second LOFT - animals were transcardially perfused under deep thiopental anaesthesia, brains were removed from the skull and processed for histological examination by Fluoro-Jade B staining (detailed description please see Riljak et al. 2007). All experiments were reviewed and approved by the Institutional Animal Care and Use Committee and are in agreement

with the Czech Government Requirements and Requirements of European Communities Council Directive (86/609/EEC).

Each measured behavioural parameter was analyzed separately. Laboras data were subjected to non-parametric tests (Mann-Whitney test). Firstly, results from whole one hour session were analyzed. Then the same data were analyzed separated in ten minutes intervals. To compare the differences between particular groups within 10 minutes intervals Mann-Whitney test was used. Results were considered significant if $p < 0.05$.

All animals treated with kainic acid (or saline) survived, but the weight of KA animals was lowered (252.5 ± 7.92 g) one week after the injection when compared with saline treated rats (271.1 ± 5.14 g, $p < 0.05$). Immediately after the KA injection animals moved more: they spent 232.1 ± 47.55 s in locomotion (saline treated 52.7 ± 7.15 s, $p < 0.01$) and their average speed was higher (4.852 ± 1.0 mm/s in KA group, saline treated 0.98 ± 0.15 mm/s, $p < 0.01$). Vertical activity (represent as time spent by rearing) was increased as well (KA group 412.7 ± 47.64 s, saline treated group 251.6 ± 42.21 s, $p < 0.05$). If spontaneous behavior was analyzed over 10 minutes intervals (Fig. 1), it showed that the immediate effect of KA-treatment on nearly all tested behavioral parameters became significant after thirty minutes (in 4th analyzed interval for the first time). Proportionally to an increase of locomotor activity, immobility declined and animals tended to explore more even in the vertical sense of direction (rearing). Finally, KA treated rats reached higher average speed during their movement and by this they travelled much longer distance (17.48 ± 3.61 m vs. 3.55 ± 0.55 m, $p < 0.01$) if compared with controls. Week after the KA administration there was no difference in any of tested behavioral parameter. KA treated rats travelled yet longer distance (7.99 ± 3.60 m vs. 3.73 ± 0.64 m, n.s.) but that difference was statistically insignificant. Histological examination of hippocampal area did not reveal Fluoro-Jade B positive cells.

This study shows detailed description of behavioral changes elicited by systemic application of subconvulsive dose of KA. We demonstrated the capability of Laboras system to generate

reasonable and interpretable data that could quantify spontaneous behavior of animals challenged by excitotoxic substance for long period of time. Second important aspect: the setup does not require significant interference of researcher (observation of arena) and might monitor the behavior for days. In this study we were interested how subconvulsive dose of KA influences the immediate and delayed behavioral response of adult rats. Moreover, we were wondering how KA could influence parameters such as average and maximal speed. The effect of KA was observed since 30th minute of the experiment, when KA treated animals started to increase their locomotor activity, while controls in this time period begun to habituate to the novel environment. We hypothesize that this relates probably with pharmacokinetics of KA. KA treated animals were also less immobile during the second half of test session (while controls stopped to explore new environment and became immobile). These effects could be ascribed to excitatory effect of kainic acid and because animals in our experiment never showed severe tonic-clonic convulsions or lost of posture control so their mobility (and possibility to explore) remained spared. These findings are in line with outcomes of other laboratories (Kubová et al 2001). Some authors ascribe the higher horizontal exploratory activity to anxiolytic-like effect of kainic acid (Mikulecká et al. 1999). Such hypothesis seems to be very probable, because rearing duration (sign of exploration) was significantly higher in second half of the test session. Interestingly, one week after the treatment there were no differences between KA treated animals and controls in any of measured parameter, except the weight gain. The finding, that effect of low KA dose on animal's exploratory activity and behavior is not long-lasting is new. In histological part of this study we focused our attention on structures of limbic system, because hippocampus is crucial structure for seizure generation (Spencer 2002). No visible signs of neuronal degeneration were observed as confirmed by Fluro-Jade B staining – it leads us to conclusion, that changes observed in behavioral pattern of animals treated with KA would be rather more functional. To conclude, our data showed that animals treated with subconvulsive dose of KA had higher exploratory activity, which starts to be significant thirty minutes after KA treatment. Spontaneous behavior of KA treated animals was substantially modified. Such effect completely disappeared and

one week after the treatment locomotion duration, rearing duration returned back to the level of controls. Such changes are very probably not caused by direct neurotoxic damage, because histological analysis did not detected any signs of neuronal damage.

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Figure legends

Figure 1

A: Acute effects of systemic administration of subconvulsive dose of KA on locomotor activity (duration), immobility (duration), rearing (duration), average speed and distance travelled. Whole one hour session divided into six 10-minutes intervals. Solid lines represent saline treated rats, dashed lines kainic acid treated rats. * results significant at $p < 0.05$, ** results significant at $p < 0.01$, *** results significant at $p < 0.001$. Error bars were calculated as \pm SEM.

Fig.1

