Influence of Low-Dose Neonatal Domoic Acid on the Spontaneous Behavior of Rats in Early Adulthood

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Summary
Consumption of seafood containing toxin domoic acid (DA) causes an alteration of glutamatergic signaling pathways and could lead to various signs of neurotoxicity in animals and humans. Neonatal treatment with domoic acid was suggested as a valuable model of schizophrenia and epilepsy. We tested how repeated early postnatal DA administration influences the spontaneous behavior of rats in adulthood. Rats were injected with 30 μg DA/kg from postnatal day (PND) 10 until PND 14. Their behavior was observed in the open field test for one hour (Laboras, Metris) at PND 35, PND 42 and PND 112. We did not find any difference between DA treated rats and animals injected with equivalent volume of saline in both test sessions at PND 35 and PND 42. DA rats at PND 112 exhibited significantly higher vertical and horizontal exploratory activity (tested parameters: locomotion, distance travelled, average speed reached during test, grooming and rearing) between the 30th-40th min of the test session and habituated over 10 min later. We conclude that at least in the given experimental design, neonatal DA treatment results in alteration of the spontaneous behavior of rats in adulthood.

Key words
Domoic acid • Locomotor activity • Open field test • Rat

Introduction
Domoic acid (DA) is a water-soluble tricarboxylic amino acid produced by diatom Pseudonitzschia, which contaminates shellfish and other type of seafood. Consumption of them can lead to DA intoxication of sea mammals, birds and finally humans. DA intoxication causes in human the clinical syndrome “Amnesic shellfish poisoning” (ASP) accompanied by gastrointestinal (nausea, vomiting, abdominal cramps, diarrhea) and neurological (headache, disorientation, confusion, coma, seizure and memory loss) symptoms. This diagnosis was first established during human DA-intoxication in 1987 in Canada with 107 people (Wright et al. 1989, Perl et al. 1990, Todd 1993, Pullido et al. 2008).

DA is structurally related to kainic acid. Both excitatory amino acids are analogues of glutamate, the major excitatory neurotransmitter in the brain. DA binds to presynaptic and postsynaptic ionotropic AMPA and kainite (KA) subtype of glutamate receptors and causes then an increase of the intracellular Ca2+ ions resulting in glutamate release and then activation of NMDA receptors. These combined actions are involved in the neuronal death in some sensitive brain regions, preferentially in the limbic system like hippocampus and amygdala (Stewart et al. 1990, Scallet et al. 1993, Berman and Murray 1997, Chandrasekaran et al. 2004, Costa 2010). While higher concentrations of DA induce necrotic cell death, low concentration of DA leads to apoptotic changes in neurons (Giordano et al. 2006, 2007, 2009). In animal model (rats and mice) administration of DA induces neurotoxic syndrome including broad spectrum of the dose-dependent behavioral patterns like scratching, tremor, seizures, wet dog shakes and influences the locomotor activity (Peng
In our experiment we aimed to examine and quantify long-term effect of repeated DA injections in low dose during early development. We hypothesized that locomotor activity of experimental animals will be increased by repeated low dose of DA and that this effect will persist till early adulthood.

**Materials and Methods**

**Experimental animals**

Twenty naive male Wistar albino rats were used in this experiment (ten treated with DA, ten injected with corresponding volume of saline). All animals were bred at the Institute of Physiology, 1st Faculty of Medicine, Charles University in Prague. Dams and litters were housed in standard 12 h light/dark cycles (with lights on at 6:00 h) in temperature-controlled environment (22-23 °C) with food and water provided *ad libitum*. The day of parturition was designed as postnatal day 0 (PND 0). Within 24 h after the birth, male rat were culled to 10 pups per litter and with the exception of routine cage clearing, were left undisturbed until the beginning of experimental procedures. Pups were weaned at 28 days (PND 28) with two to three rats per cage, and then housed PND 112.

On the PND 10, pups were weighed, marked with non-toxic permanent pen for identification and assigned to two experimental groups. Half the males within each litter received a single injection of 30 µg/kg DA over PND 10-14, other half received equal volume of saline. DA (Sigma) was dissolved in sterile saline and injections were administered subcutaneously (s.c.) in volume of 10 ml/kg.

On PND 35 all animals were placed and tested in Laboras apparatus (Metris B.V., Netherlands) for one hour to analyze their behavioral pattern (test day 1 – PND 35). During the measurement animals were left undisturbed. After the test session animals were returned to their home cages and housed for another seven days (food and water *ad libitum*). After that period all animals were placed in Laboras apparatus again and their behavioral pattern was observed for the period of one hour (test day 2 – PND 42). Finally, the last third measurement was conducted on PND 112 under the same conditions (test day 3 – PND 112).

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).

**Laboras apparatus (Metris, B.V., Netherlands)**

Laboras™ is automated system for continuous behavior tracking and analysis. It consists of triangular shaped sensing platform (carbon fiber plate 700 mm x 700 mm x 1000 mm x 30 mm), positioned on two orthogonally placed sensor transducers and third fixed point attached to bottom plate. Makrolon cage (type III, 840 cm²) is placed on this platform. Mechanical vibrations generated by the animal (locomotion, rearing etc.) are transformed into electrical signal. Such signals are finally processed, classified and compared with the predetermined characteristic patterns by Laboras software. We analyzed following horizontal behavioral activities: time of locomotion, average speed in tested interval and total distance travelled in tested interval. Vertical activities included rearing time and rearing frequency (exploratory behavior), the time spent by grooming and the grooming frequency (comfort behavior) were also evaluated.

**Statistical analysis**

Data from Laboras software were analyzed over ten minutes’ intervals (0-10 min, 10-20 min, 20-30 min, 30-40 min, 40-50 min, 50-60 min). Each measured behavioral parameter was analyzed separately. Laboras data were subjected to non-parametric tests (because of non-Gaussian data distribution). To compare the differences between individual groups within 10 min intervals Kruskal-Wallis test was used, if p<0.05 results were considered as significant.

**Results**

All animals treated with DA (or saline) survived. Weight gain of 35-day-old rats treated with DA was not different (92±5.9 g) after the last injection of DA when compared with saline treated rats (88±7.3 g).

**Test day 1 – PND 35**

In the youngest age group (35-day-old animals), monitored at the 21st day after the last dose of DA, no significant changes in the behavior, compared to control offspring, were observed. In the course of six 10 min intervals, not even one of the parameters of horizontal and vertical activity and comforting behavior (grooming) manifested statistically different behavioral
patterns. While both groups of animals in the first half of the experiment were active (getting acquainted with the new environment), in the second part the activity of the offspring was almost zero (sign of habituation) (Fig. 1).

**Test day 2 – PND 42**

Figure 2 shows the results of the spontaneous activity monitoring in 42-day-old animals, 28 days after the last administration of DA. Again, no significant differences in the behavior of animals between the two groups were observed. The graphs indicate nearly identical waveform locomotor activity; distance and average speed during the measurement, as well as the course of rearing (HR explorative behavior) were identical in both groups.

**Test day 3 – PND 112**

Last monitoring was performed 98 days after the treatment with DA in 112-day-old animals. The results (see Fig. 3) bring a different pattern of behavior of DA animals in all parameters. In the first 30 min of the experiment, no significant differences in the horizontal activity were observed, i.e., the locomotor activity, distance, and the average speed of DA animals did not differ from the values of the control group. Between the 2nd to 4th intervals, the measured parameters in DA animals stabilized at a constant value. The activity in the interval between the 30th and 40th min significantly increased compared to control animals (locomotion, p<0.01, the average speed, p<0.01, distance, p<0.01). The same course was observed in indicators of vertical activity with a difference in the second interval where the same course was observed in indicators of vertical activity of young animals (35-day- and 42-day-old). These results correspond with the findings of the study by Doucette et al. (2003) where in a similar experimental protocol no changes in locomotor activity in the open field arena manifested. Significant differences in the behavior of our animals occurred only in adult animals (at PND 112). Experimental rats showed a different pattern of behavior, and habituation occurred 10 min later than in the control animals. While the behavior of DA treated rats in the first 30 min of experiment did not differ from that of control animals (with the exception of rearing), between 30th-40th min DA group of animals showed significantly higher locomotor activity and at a higher average speed they covered a greater distance. The same course was observed in the parameters of exploratory behavior (rearing) and comfortable behavior (grooming), the total time spent and frequency were increased in the second half hour of monitoring in the open field test in comparison with the control group. These findings correspond to the results of work by Burt et al. (2008) that demonstrated the induce by DA treatment depend not only on the route of application, gender, but primarily on the administered dose for the given age (Grant et al. 2010, Costa et al. 2010). Previous studies indicate that the daily treatment with very low doses of DA (20 µg/kg s.c.) in the second postnatal week (between PND 8-14) causes behavioral and cognitive changes and these changes persist into adulthood (Doucette et al. 2003, 2004, Levin et al. 2005, Bernard et al. 2007, Burt et al. 2008, Adams et al. 2008, Adams et al. 2009, Perry et al. 2009, Marriott et al. 2012). Our study extends those works and with the help of automated observation system LaborasTM we analyzed spontaneous locomotor activity, exploratory behavior (rearing) and grooming (comfort behavior) of male rats. In our experiment single doses of DA were higher (five times single dose 30 µg/kg s.c. in PND 10-14) when compared with the above- cited works, but the total dose administered was similar. We hypothesized that the application of the neurotoxin during critical period of CNS development, as the first three weeks of postnatal life are called (brain maturation during the first 2 weeks of postnatal life is likened to the development of the human brain in the last trimester of pregnancy) (Dobbing and Sands 1979) affects the spontaneous behavior during early ontogeny and early adulthood. Results of our experiment show that exposure to low doses of DA in the early postnatal period did not affect any of the monitored parameters of horizontal and vertical activity of young animals (35-day- and 42-day-old). These results correspond with the findings of the study by Doucette et al. (2003) where in a similar experimental protocol no changes in locomotor activity in the open field arena manifested. Significant differences in the behavior of our animals occurred only in adult animals (at PND 112). Experimental rats showed a different pattern of behavior, and habituation occurred 10 min later than in the control animals. While the behavior of DA treated rats in the first 30 min of experiment did not differ from that of control animals (with the exception of rearing), between 30th-40th min DA group of animals showed significantly higher locomotor activity and at a higher average speed they covered a greater distance. The same course was observed in the parameters of exploratory behavior (rearing) and comfortable behavior (grooming), the total time spent and frequency were increased in the second half hour of monitoring in the open field test in comparison with the control group. These findings correspond to the results of work by Burt et al. (2008) that demonstrated the
Fig. 1. Test day 1 – PND 35: Effects of single DA injection over PND 10-14 on the vertical and horizontal activity and comfort behavior. The one hour session was divided into six 10-min intervals. Red lines represent DA treated rats, black lines saline treated rats. Error bars were calculated as ± SEM.
Test day 2 - PND 42

Fig. 2. Test day 2 – PND 42: Effects of single DA injection over PND 10-14 on vertical and horizontal activity and comfort behavior. The one hour session was divided into six 10-min intervals. Red lines represent DA treated rats, black lines saline treated rats. Error bars were calculated as ± SEM.
Fig. 3. Test day 3 – PND 112: Effects of single DA injection over PND 10-14 on vertical and horizontal activity and comfort behavior. The one hour session was divided into six 10-min intervals. Red lines represent DA treated rats, black lines saline treated rats. * Differences significant at p<0.05, ** differences significant at p<0.01. Error bars were calculated as ± SEM.
increased locomotor activity of adult female rats at PND 150 exposed to low doses of DA between PND 8-14. Many studies have confirmed the changes in motor activity and cognitive function after administration of DA, these changes are accompanied by neurological symptoms and neurodegeneration in specific brain regions and the toxicity associated with DA is thought to be attributed to an interaction with kainate receptors (Tryphonas et al. 1990, Sobotka et al. 1996, Appel et al. 1997, Pulido 2008, Schwarz et al. 2014).

Results of our study confirmed that very low doses of DA administered during critical developmental periods have no direct toxic effect (weight gain of the DA animals was not affected), but cause a change in behavioral pattern and these changes depend on the age of animals with the manifestation in the adulthood. The exact explanation for this phenomenon is not simple. On one side in the first weeks of postnatal life excitatory processes predominate and glutamate plays a key role in neurotrophic differentiation, synaptogenesis and manages the development of neuronal cytoarchitecture (McDonald and Johnston 1990). Presence of kainate receptors therefore plays an important role in the normal and abnormal functioning of the CNS (Bahn et al. 1994), and a mild activation of KA receptors in the perinatal period can, together with the absence of inhibitory mechanisms (Leinekugel et al. 1999) significantly interfere with the process of neuroplasticity. On the other hand, it should be noted that NMDA and non-NMDA receptors may cause release of the neurotransmitter dopamine in the mesocorticolimbic pathway, which, according to some authors, is the pathway responsible for the behavior in the open field arena (Crowder and Weiner 2002, Breese et al. 2005). Change in the functional integrity of the dopamine system in the midbrain only, can cause changes in the locomotor activity and habituation that were described in our results.

We conclude that moderate activation of KA receptors by DA during critical developmental period induces behavioral changes that persist into adulthood.

Conflict of Interest
There is no conflict of interest.

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