Fast and Delayed Locomotor Response to Acute High-Dose Nicotine Administration in Adult Male Rats

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Received March 19, 2013
Accepted July 11, 2013

Summary

The aim of the present study was to compare the immediate and delayed locomotor response to high-dose nicotine (NIC) administration in rats. The vertical and horizontal activity of behavior in adult male rats exposed to 1 mg/kg NIC or saline (SAL) were tested in a Laboras apparatus for one hour after drug application. Animals were then returned to their cages and housed for another seven days. After this period all animals were placed in Laboras again and their behavioral pattern was retested for another period of one hour (delayed response). Horizontal activity: immediately after nicotine administration animal were less mobile (first 2-minutes interval), when compared with controls. The immobilization effect of nicotine disappeared within 4 minutes and during whole first 10-minutes interval time spent by locomotion did not differ from controls. Locomotion activity of animals treated with nicotine increased robustly in following 10 minutes and remained significantly higher in 2nd, 3rd and 5th 10-minutes interval. Vertical activity: Rearing frequency was significantly lowered by NIC administration in first two minutes of the experiment and the same was found when the duration of rearing was analyzed. Lower rearing intensity of NIC treated animals disappeared in 4 minutes and was finally higher during whole test session as compared with controls. When duration of rearing was analyzed it was significantly longer in NIC treated animals. In majority of observed behavioral aspects there were no differences between NIC treated rats and controls seven days after NIC or SAL treatment. Our results reflect effect of NIC and we conclude that NIC significantly influences behavior of experimental animals.

Key words

Nicotine • Locomotor activity • Open field test • Adult rats

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Introduction

Nicotine (NIC) is everyday life drug of abuse that possesses variety of properties influencing many physiological parameters (Pelissier *et al.* 1998, Riljak and Langmeier 2005), interferes with learning mechanisms, memory (Ferrea and Winterer 2009, Hralová *et al*. 2011) and cortex excitability (Riljak *et al.* 2010, Riljak *et al.* 2012, Hralová *et al.* 2010) and influences results indifferent sensorimotor tests (Riljak *et al.* 2011b). Low doses of nicotine reduce anxiety, while higher ones cause seizures (Damaj *et al.* 1999, Picciotto *et al.* 2002). Nicotine was even repeatedly reported as drug with certain neuroprotective properties (Riljak *et al.* 2007, Riljak *et al.* 2011a). There is an evidence that the majority of above mentioned NIC properties is related to activation of nicotinic cholinergic receptors (nAChRs) and as NIC activates cholinergic receptors it influences the GABA-ergic system also, by decreasing its inhibitory input to the hippocampus, as well as the system of glutamate-related neuronal signalization (Damaj *et al.* 1999, Dobelis *et al.* 2003). Glutamate receptors mediate the excitatory neurotransmission in the hippocampus and this brain structure seems to play critical role in certain mood disorders, anxiety and depression (Joca *et al*. 2007). There is also an evidence that nicotine is capable to act as either depressive-like (Wallace and Potter 2011) or antidepressant agent (Tizabi *et al.* 2010) influencing *via* this mechanism the locomotor activity of NIC exposed animals.

To evaluate the behavior of experimental animals we decide to use automated observation system

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Laboras^{TM}. Such system allows registering the duration and frequency of rearing (vertical activity) and locomotion, immobility, speed of animal reached during movement in open field and distance traveled in desired time period (horizontal activity). Since vertical activity reflects the exploratory behavior, the horizontal activity might reflect the novelty seeking behavior (Spear 2000). In the present study we have investigated if NIC administered in high dose influences the locomotor behavior of adult rats and whether this effect is long lasting. Dose of NIC chosen by us was rather high, but was chosen purposely to not trigger the seizures and tonic-clonic convulsions. To trigger the seizure pattern higher NIC doses are requested or NIC needs to be administered repeatedly (kindling protocol) (Bastlund *et al.* 2005). We were also interested how NIC influences the animal's habituation to novel environment. We hypothesize, that nicotine administered in high dose causes disruption in locomotor behavior (expressed as higher immobility duration) of treated rats and that this effect disappeared quickly.

Methods

Animals and experiment design

Twenty naive male Wistar albino rats were used in this experiment. The weight of animals entering the experiment was from 282 g to 336 g (test day 1). All animals were housed in standard 12 h light/dark cycles (with lights on at 06:00 h) in temperature-controlled environment (22-23 °C). 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). During the tests animals had not access to either food or water. Immediately after placing the animals in experimental room they were randomly assigned into two experimental groups, weighted prior each session and marked. First group was treated intraperitoneally with 1 mg/kg of (-)-nicotine (Sigma), dissolved in saline recalculated volume 1 mg of nicotine per 1 ml of saline. Second group was sham treated with saline (intraperitoneally) in equal volume. Animals were then placed and tested in Laboras apparatus (Metris B.V., Netherlands) to analyze their behavioral pattern for one hour (test day 1). 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 this period all animals were placed in Laboras apparatus again and their behavioral pattern was observed for period of one hour (test day 8). 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., Netherland)

Laboras^{TM} 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 cm^2) is placed on this platform. Mechanical vibrations generated by animal (locomotion, rearing etc.) are transformed into electrical signal. Such signals are finally processed, classified and compared by with the predetermined characteristic patterns by Laboras software. Following horizontal behavioral activities were analyzed: time of locomotion, duration of immobility, maximal speed reached within tested interval, average speed in tested interval, total distance traveled in tested interval. Vertical activities included rearing time and rearing frequency.

Statistical analysis

Data from Laboras software were in first step analyzed over ten minutes' intervals (0-10 min, 10-20 min, 20-30 min, 30-40 min, 40-50 min, 50-60 min). To gain the detail information of animal´s behavior the very first time interval was analyzed again over two minutes sub-interval. 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 particular groups within 10 minutes intervals Kruskal-Wallis test was used, same test was used to compare differences within NIC-treated group in time (test day 1 vs. test day 8), if $p<0.05$ results were considered as significant.

Results

All animals treated with nicotine (or saline) survived. Weight gain of animals treated with nicotine was not different (22±2.9 g) one week after the injection when compared with saline treated rats (19.75±0.6 g).

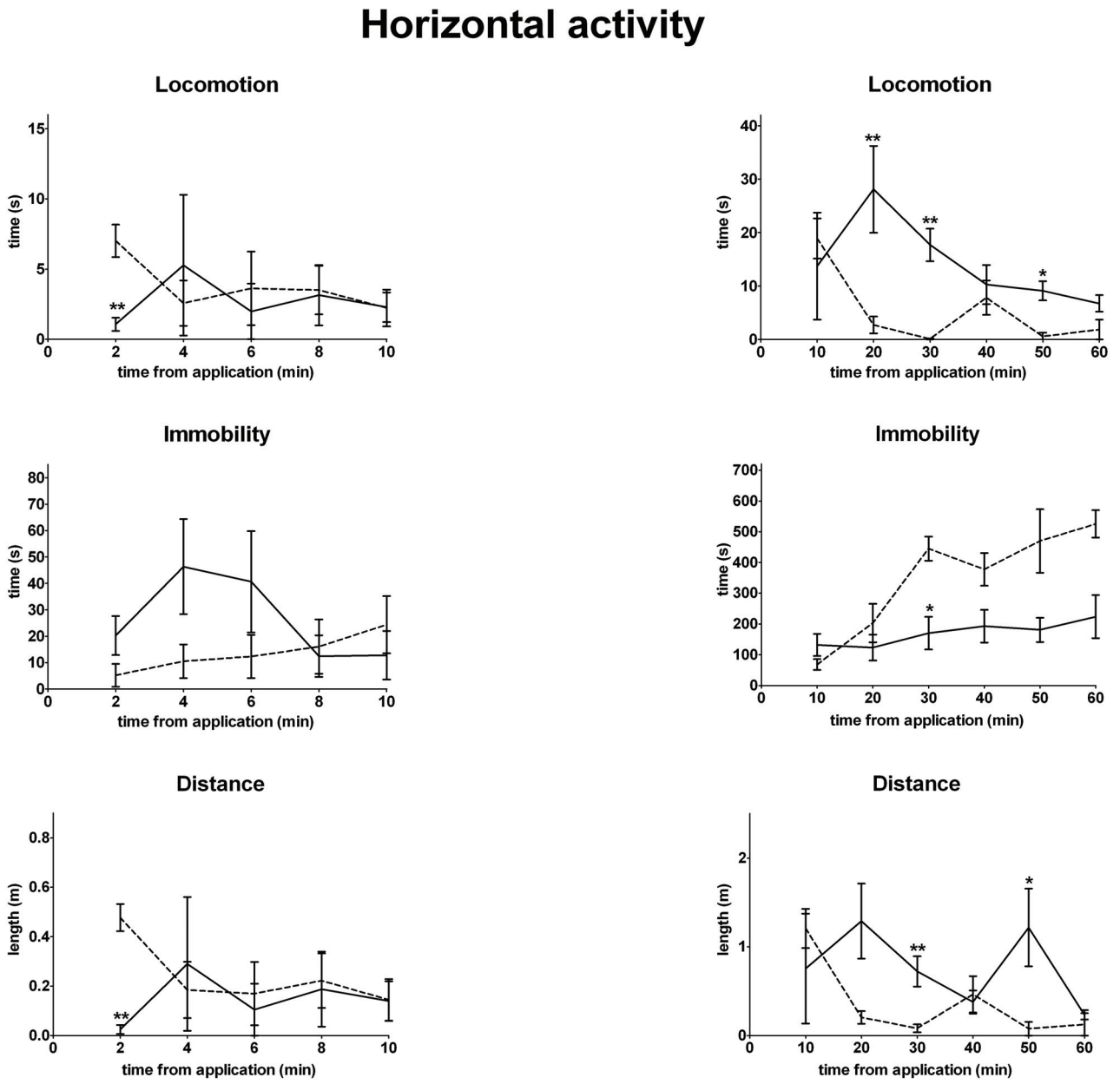

Fig. 1. Immediate NIC-induced effects on horizontal activity: locomotion (duration), immobility (duration) and distance traveled. First 10 minutes divided into five 2-minutes intervals (left part of figure), whole one hour session divided into six 10-minutes intervals (right part of figure). Solid lines represent NIC treated rats, dashed lines saline 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 ± SEM.

Immediate response to nicotine administration

Horizontal activity

Immediately after nicotine administration animal were less mobile (first 2-min interval, $p<0.01$), when compared with controls. The immobilization effect of nicotine disappeared within 4 minutes and during whole first 10-min interval time spent by locomotion did not differ from controls. Locomotion activity of animals treated with nicotine increased robustly in following 10 minutes and remained significantly higher in 2nd, 3rd ($p<0.01$) and 5th ($p<0.05$) 10-minutes interval. In last part of the test session (6th interval) NIC animals did not differ from controls. Increased duration of locomotion was partially followed by shorter time, that NIC treated animals spent as immobile (3rd interval, $p<0.05$) (Fig. 1).

Lower horizontal activity immediately after NIC treatment was reflected by shorter distance traveled in first two minutes ($p<0.01$), but increased within the test session and was significantly longer in 3rd ($p<0.01$) and 5th 10-min time interval ($p<0.05$) (Fig. 1).

Control animals reached significantly higher

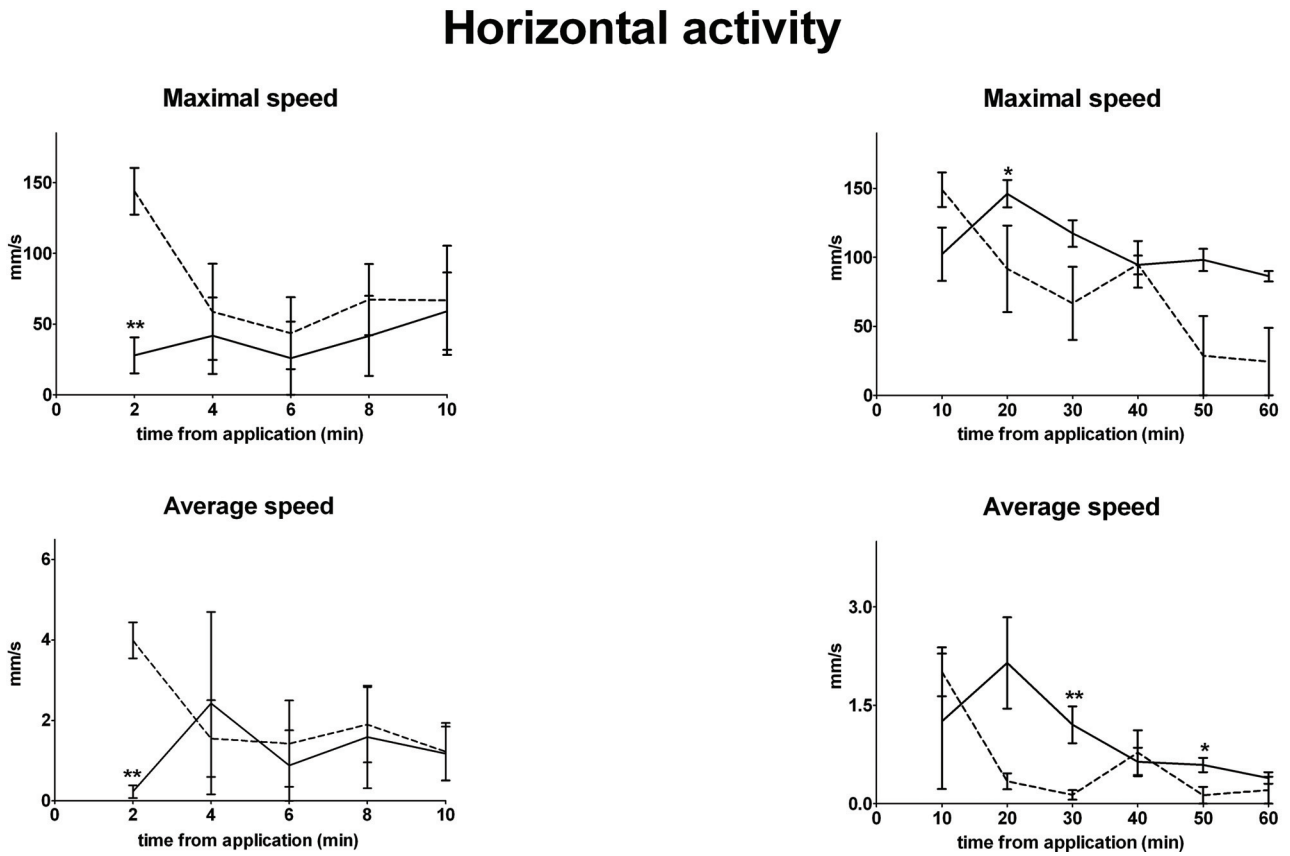

Fig. 2. Immediate NIC-induced effects on horizontal activity: maximal and average speed. First 10 minutes divided into five 2-minutes intervals (left part of figure), whole one hour session divided into six 10-minutes intervals (right part of figure). Solid lines represent NIC treated rats, dashed lines saline 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 ± SEM.

maximal ($p<0.01$) and average ($p<0.01$) speed in first 2 minutes of the test session, but this effect disappeared in 4 minutes and NIC treated reached finally higher values of maximal speed (2^{nd} interval, $p<0.05$) and average speed (3^{rd} interval, $p<0.01$ and 5^{th} interval, $p<0.05$) (Fig. 2).

Vertical activity

Time spent rearing was significantly lowered by NIC administration in first 2 minutes of the experiment ($p<0.01$) and the same was found, when rearing frequency was analyzed ($p<0.01$). Lower rearing intensity of NIC treated animals disappeared in 4 minutes and was finally higher during whole test session (except the interval 5^{th}) as compared with controls. When duration of rearing was analyzed it was significantly longer in NIC treated animals (5^{th} interval $p<0.01$, 6^{th} interval, $p<0.05$) (Fig. 3).

Delayed response to nicotine administration

In majority of observed behavioral aspects there were no differences between NIC treated rats and controls. Week after NIC administration animals embodied only few significantly different values. Duration of immobility was lower in NIC group between 10-20 minutes of data collection ($p<0.01$) and rearing frequency was higher in NIC treated animals between 10-20 minutes of experiment ($p<0.05$) (Fig. 4).

Discussion

In the present study we evaluated behavioral response to nicotine administration to adult male rats. For this purpose we used the automated behavioral observation system Laboras^{TM}. Locomotor activity is widely used to study different behavioral actions, mainly psychomotor stimulant ones in rats (Collins *et al.* 1988, Paulus and Gayer 1991, Slamberová *et al.* 2011, 2012).

Locomotion, especially horizontal activity, has been used to quantify genetically sex, age and event strain based differences in nicotine treated animals. In addition, different aspects of locomotion – horizontal and vertical activity – have been interpreted to reflect different behavioral processes. Horizontal activity has

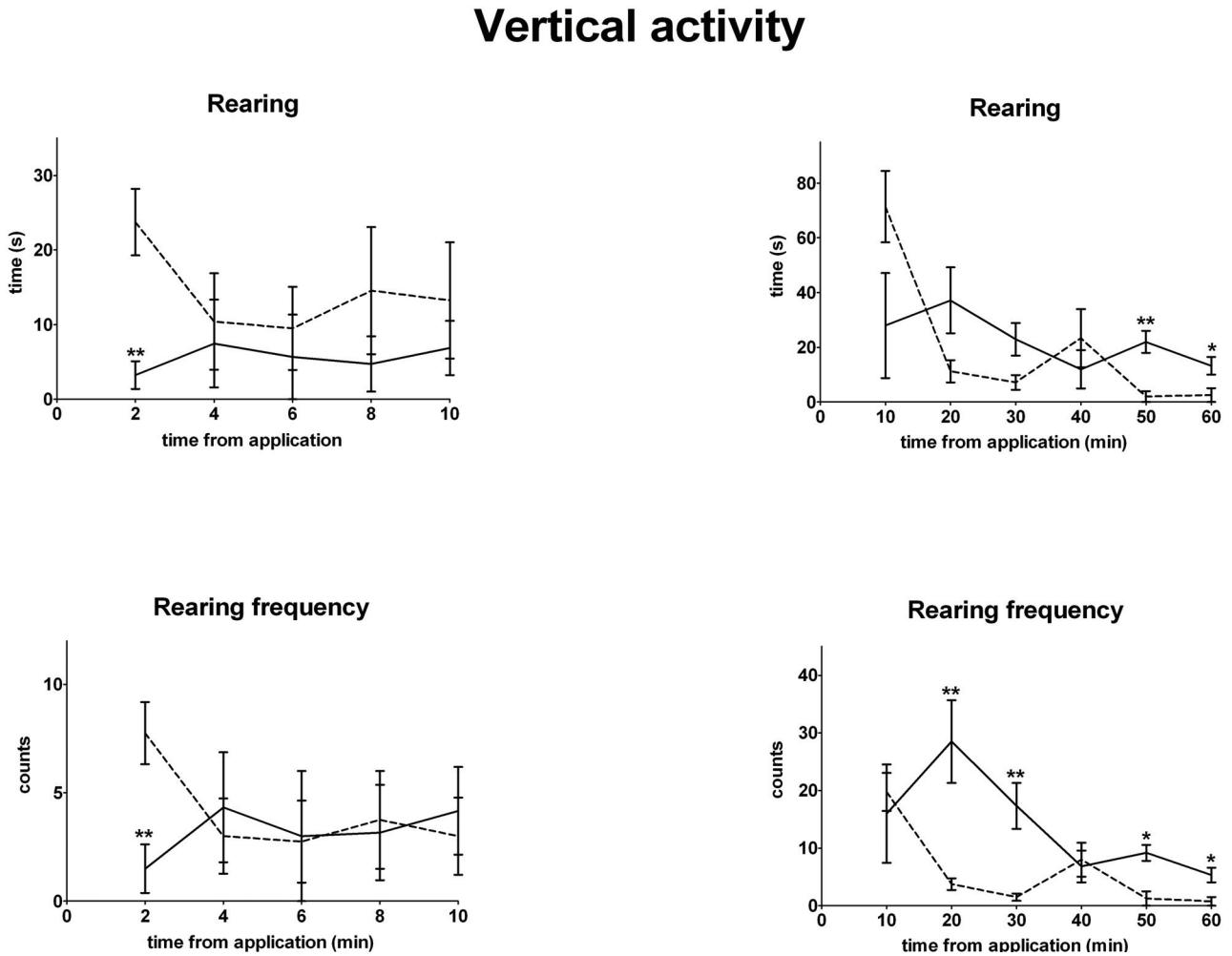

Fig. 3. Immediate NIC-induced effects on vertical activity: rearing and rearing frequency. First 10 minutes divided into five 2-minutes intervals (left part of figure), whole one hour session divided into six 10-minutes intervals (right part of figure). Solid lines represent NIC treated rats, dashed lines saline 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 ± SEM.

been interpreted to reflect general arousal; vertical activity is thought to indicate exploration (Ader and Conklin 1963, Walsh and Cummins 1975, Crawley *et al*. 1997).

Nicotine has significant and complex effects on the locomotor activity of rats depending on dose sex and age (Ksir 1994, Faraday *et al.* 2003). When our data from Laboras were analyzed over two minute intervals we found that immediately after nicotine administration the animals exhibited longer time spent by locomotion, and this effect could be only hardly attribute to injection procedure, because control animals were treated with saline same way. Control animals that have not been habituated to the testing apparatus, demonstrated high initial levels signs of horizontal activity and exploratory behavior (rearing). The decreased horizontal locomotor activity of NIC treated rats could very probably attribute to its action at peripheral nAChRs such as an overload neuromuscular junction (Leonard and Bertrand 2001, MacDermott *et al.* 1999). Nicotine then immediately induced a suppression of locomotor activity as measured horizontal activity and rearing in an open field. This similar effect of NIC was described by us previously in immature rats (Riljak *et al.* 2011b). In this experiment 1mg of NIC was capable to prolong the latency to the surface righting response and prolongs the times in simple negative geotaxis test.

Increased behavioral activity in NIC group rats was appeared in subsequent time intervals. We can speculate this rapidly depressant effect of NIC is replaced by influence of some nicotine-stimulated neurotransmitter systems such as dopamine that is influencing the locomotor activity significantly. Such hypothesis supported by other authors as well, e.g. acute NIC injections have been shown to increase level of plasma adrenocorticotropic hormone (ACTH) (Matta *et al.* 1987),

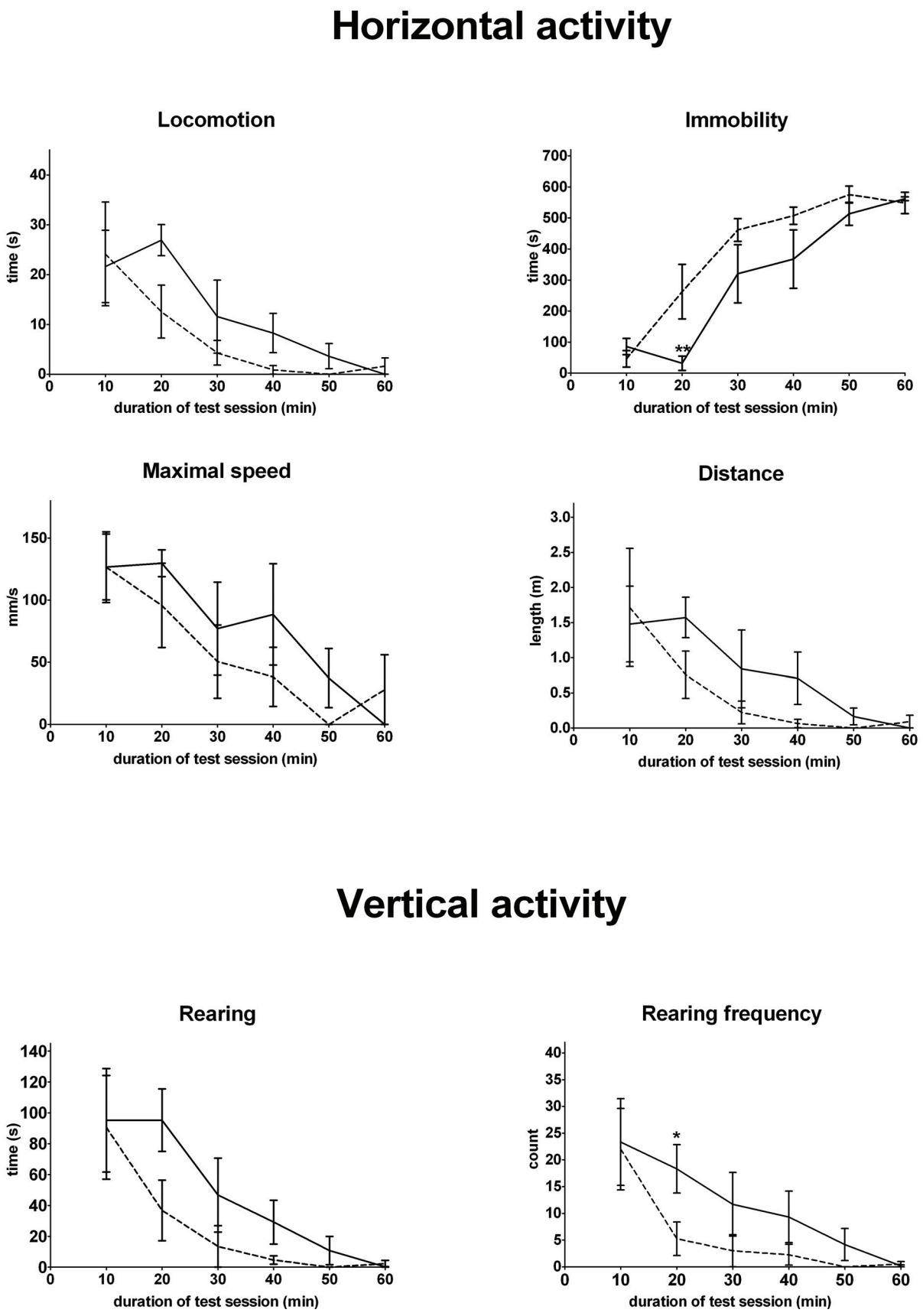

Fig. 4. Delayed NIC-induced effects on vertical and horizontal activity. Whole one hour session divided into six 10-minutes intervals. Solid lines represent NIC treated rats, dashed lines saline treated rats. * Results significant at $p<0.05$, ** results significant at $p<0.01$. Error bars were calculated as ± SEM.

follow by increased plasma corticosterone levels (Cagguila *et al.* 1991). Latter hormone can finally enhance the release of dopamine in nucleus accumbens *via* e.g. NIC (Rouge-Pont *et al.* 1998). It seems to very probably, that the mentioned steroid hormones play important role in nicotine-mediated changes of rats' locomotor activity, because repeated nicotine injection reduces ACTH levels (Benwell and Balfour 1979). So even, experimental design (single vs. repeated nicotine injection) plays crucial role in final locomotion pattern of tested animals. Another important mechanism influencing the outcomes of open field test is the anxiogenic/anxiolytic-like effect of NIC administration. In general it is believed that low doses of NIC (0.1 mg/kg app.) have an anxiolytic effect, while the higher ones have anxiogenic impact (1 mg/kg app.) (File *et al.* 1998). That mechanism should be taken in account when interpreting locomotion-related data.

As can be seen sign of exploratory behavior – rearing activity – was increased by nicotine nearly during the test session, so animals did not demonstrate the process of habituation (as observed in controls).

In conclusion, majority of described effect disappeared completely in one week that leads us conclusion that nicotine affects behavior of animals (in our experimental paradigm) momentarily and transiently.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was supported by grant by PRVOUK-P34/LF1/7.

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