Is Learning Ability and Spatial Memory in Rats Influenced by Single Dose of Nicotine?

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Abstract: A lot of studies have been concentrated on an effect of a short or a long-term administration of nicotine in humans or in animals. The negative effects on the human organism have been known for a long time, but these health problems are known especially from observing smokers. The number of tasks in human and in animals with accent on positive effect of nicotine has increased especially with regard to improvement of cognitive functions. The aim of this study was to investigate, how much a single dose of nicotine can influence the learning ability in rats. Male Wistar albino rats, 25-day-old, were studied. Two groups of animals received an intraperitoneal (i.p.) injection of nicotine in two different doses (0.75 mg/kg and 1.00 mg/kg b.w.). The third group received a single i.p. injection of saline in the equal volume (the control group). After the drug application the escape latency and the path length were measured and assessed in two periods of sessions in the Morris water maze. In our study no explicit changes of learning ability after a single nicotine injection was confirmed. Only at the third day of the task the trajectory was shorter ($p<0.05$) but this result appears too isolated. So we cannot exclude that such improvement was caused by other factors than by the nicotine administration.

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Introduction

Nicotine is an alkaloid found in some species of the nightshade family of plants (*Solanaceae*). It is the main part of tobacco smoke. During smoking it enters the body, is distributed through the bloodstream, crosses the blood-brain barrier and during a few second reaches the brain (Benowitz et al., 2009). It influences the brain function by binding to nicotinic acetylcholine receptors (nAChR), placed on cholinergic synapses in the peripheral and central nervous systems (Wonnacott, 1997; Barik and Wonnacott, 2009).

Nicotinic acetylcholine receptors (nAChR) are ligand gated ion channels, which mediate the neurotransmission in the central and peripheral nervous systems. They are present in the brain and have also a modulatory role in its development and its function. The receptors are composed of five subunits; these are divided into four groups (α, β, γ, δ) with respect to their polypeptide structure. Amount, types, subtypes of the nAChR and their function depend on the development period of the brain and as well as on the brain region where they are placed (Role and Berg, 1996; Benešová, 2003). Effect of these receptors on brain function (together with their development) can be observed from perinatal period to old age (Wonnacott, 1997; Doura et al., 2008; Dwyer et al., 2009).

This various level of nAChR development can cause also the various reactivity of the CNS to nicotine administration in dependence on different dose and age (Gotti and Clementi, 2004). Moreover, nicotine by binding to these receptors influences the levels of other neurotransmitters (acetylcholine, dopamine, GABA and others) and thereby also other processes in the organism, such as somatic processes, e.g. heart rate, blood pressure, respiratory rate or intestinal peristalsis, but as well as mental processes, e.g. attention, learning or memory (Benešová, 2003; Barik and Wonnacott, 2009; Placzek et al., 2009).

The negative effect of nicotine and other products resulting during smoking on human health is well known. This effect of tobacco use is evident not only in the self smokers, but also – and it is very important – the non-smokers are influenced by environmental tobacco smoke (Herrmann et al., 2008; Leonardi-Bee et al., 2008). It has been demonstrated in all age groups. Tobacco use can cause maldevelopment or even spontaneous abortion during the intrauterine development (Mathers et al., 2006; Dwyer et al., 2009; Llaquet et al., 2010). Exposure to tobacco smoke in perinatal period is often associated with respiratory disorders, asthma or even sudden infant death syndrome and as well with defects in the neurodevelopment (Rogers, 2008, 2009; Gospe et al., 2009).

The important problems in adult smokers are cardiovascular diseases. The risk of acute myocardial infarction, sudden cardiac death, stroke, aortic aneurysm and peripheral vascular diseases is higher than in non-smokers (Bullen, 2008; Erhardt, 2009). Another complication is the genotoxic effect of tobacco carcinogens. It has

been defined mainly in genesis of lung carcinoma (Catassi et al., 2008). Of course, these negative effects are connected not with the nicotine only, but with other smoke products – mainly tar and nitrosamine (Starek and Podolak, 2009).

Furthermore, a universal problem of tobacco use is the tobacco addiction, starting often in very young people or in children (Vnenková et al., 2009).

Recently, many studies have concentrated on the influence of acute and also chronic treatment by nicotine or nicotinic agonists on cognitive function in experimental animals and as well as in humans. This positive effect has been described in experimental animals, above all in rodents (e.g. rats, mice, guinea pigs, rabbits), when the spatial memory and learning was improved after nicotine treatment (Rezvani and Levin, 2001; Levin, 2002; Gatto et al., 2004; Carrasco et al., 2006; Levin et al., 2006). The outcomes of experimental tests in animals have been used in experimental treatment of neurodegenerative diseases in human (Parkinson's or Alzheimer's disease, schizophrenia, autism etc.) and for treatment for age-associated cognitive disorders (Rusted et al., 2000; Quik and Kulak, 2002; Quik et al., 2007; Toyohara and Hashimoto, 2010).

The neurobehavioral studies are provided by testing the spatial memory and learning ability in experimental animals. The swimming test in the Morris water maze (or other variation of it) is one of the most widely used ways of studying spatial navigation skills in these animals (Stuchlík, 2003; Klement et al., 2008). The principle of the test is very simple – animals are placed in a circular water pool and they are required to escape from water on a small platform hidden under water surface. The repeated finding of the platform is possible due to spatial memory of animals – they can identify various points or symbols in the space around the pool. The ability of learning and the quality of spatial memory and learning ability in rats and mice is evaluated mainly in two aspects – escape latency and path length (see below) but the possibility of utilization this maze is nowadays raising thanks to new and more advanced computer technologies.

This maze was developed by Richard Morris at the University of St. Andrews in Scotland and described by him in the early 1980s (Morris, 1984).

Our previous work was focused on an acute effect of a single dose of nicotine on the bioelectrical brain activity and the behaviour in very young rats (Hralová et al., 2010). We know from our recent studies (Marešová et al., 2009; Hralová et al., 2010; Riljak et al., 2010) and from results of other research groups (Slawecki and Ehlers, 2002; Belluzzi et al., 2004; Benowitz et al., 2009), that effect of nicotine on behaviour and bioelectrical brain activity begins shortly after the application and persist more than 24 hours. This effect of the single dose of nicotine is unambiguous. But experiences in learning ability in rats after one-dose nicotine application (mainly after a longer time interval) are different in various tasks of many other authors. It is the reason, why we extended our observation of single dose nicotine administration on learning

ability and spatial memory in young, 25-day-old rats. It is the first part of a long term study – in following parts the observation in other age groups will be necessary.

Material and Methods

Animals

This study was performed an accordance with the Guide for Care and Use of Laboratory Animals of Central Commission for Animal Welfare (CCAW) of the Charles University in Prague. All efforts were adopted to modify animal discomfort and to reduce the total number of used experimental animals.

Male 25-day-old (on the first day of study) Wistar albino rats, in total number of 27, of our own breed, were used for the experiment. They were maintained in a temperature controlled room (22–24 °C), on a 12 h light/dark cycle, with an access to food (commercial rat chow) and fresh water available *ad libitum*. Twice a week all animals and objects were removed and the cages were cleaned.

The animals were randomly assigned in experimental groups with respect to dosage of nicotine (Nicotine Sigma-Aldrich). The first group (n=9) was treated by one intraperitoneal (i.p.) injection of nicotine in dosage of 0.75 mg/kg body weight, the second group (n=9) was administrated by one i.p. injection of nicotine in dosage of 1.00 mg/kg. In the third – control group (n=9) rats got one i.p. injection with saline in equal volumes (1 ml/kg).

Morris water maze

The spatial memory and the learning ability were tested by using a Morris water maze.

The maze consisted of a circular pool with 180 cm in diameter, filled with fresh clear water of temperature 22–23 °C. The depth of water was 23 cm, a transparent platform 10 cm in diameter was submerged in it, 2 cm below the water surface in the northwest quadrant. The platform was kept in the constant position throughout the whole experiment.

The pool was located in a quiet test room with visual cues on the walls, which were visible from the pool and can be used by the rats for their better spatial location. The pool was imaginary divided in four equal quadrants (north-east, north-west, south-east and south-west) and had four points designed as starting post – north, west, south and east.

The movements of all rats were recorded by a video camera fixed on the ceiling over the maze and connected with a computer.

In this task each rat received two training periods. The first of them consisted of four training days (sessions) in four consecutive days; the other period consisted of two consecutive days (punctual timeline see below). Each session had four trials. The trial began by gentle placing the rat in the water, facing the wall, at one of the four starting position (north – east – west – south). The rat was trained to find the hidden platform within 60 s. When the rat did not reach the platform, it was placed

on it and left there for 15 s observing its surrounding after each unsuccessful trial. When the rat did reach the platform, it was allowed to stay on the platform for 15 s and than it was placed in the water again, but from another start position.

After swimming the rat was dried and returned to its cage.

Escape latency The escape latency is the time needed to find the hidden platform. It was measured in each trial and than the mean latency for every rat and every training day was calculated. If the rat did not find the platform, the latency was evaluated as 60 s.

Path length The path length was also measured. It is the length of the path from the start place (from the wall of the pool) to the platform. The path length in each trial was measured and than the mean length for every rat and every day was calculated. In case the rat was not successful in finding the platform, the length of the path within 60 s was measured.

Obtained data were subjected to nonparametric tests. The statistic analysis of both escape latency and path length was provided by non parametric Kruskal-Wallis analysis and non parametric Friedman test.

Timeline of experimental procedures Nicotine or saline was given to the experimental animals on the 1st task day. Two training periods followed than.

The first period (i.e. the monitoring of acute nicotine effect) took four consecutive days from the 2nd day of this study (i.e. 26th day of life the rats), the second period (i.e. the monitoring of chronic nicotine effect) took two consecutive days from the 30th task day (56th day of their life). In each day (session) the rat received four trials in Morris water maze and path length and escape latency were measured and assessed (Figure 1).

Results

Escape latency – acute effect of nicotine

Time of escape latency in three groups of rats in the first four days of study was measured and evaluated.

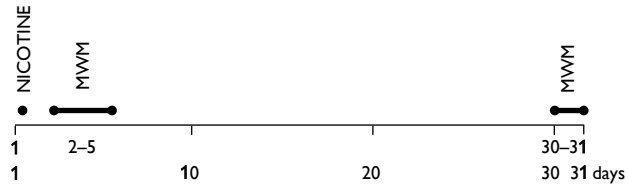

Figure 1 – Time line of the experiment.
1 – the 1st day of experiment – nicotine application; 2–5 – 2nd–5th day of experiment – the first period of trials in Morris water maze; 30–31 – 30th–31st day of experiment – the second period of trials in Morris water maze

No statistically significant difference in the assessment of the escape latencies in each of these three groups of animals within the first training period was found (Figure 2).

Path length – acute effect of nicotine

The length of the path from the start place to the platform in the first four days of study was measured as well and evaluated in all three groups of experimental animals. A statistically significant difference was found between the group treated by saline (group 0) and the group administrated by nicotine in the dose 1.00 mg/kg b.w. (group 2). This difference was present on the third day of the first training period ($p<0.05$), but without any significant improvement in following days (Figure 2).

Escape latency – chronic effect of nicotine

The escape latency in the last two days of study (the second period) was measured and evaluated. No statistically significant difference in the assessment of the escape

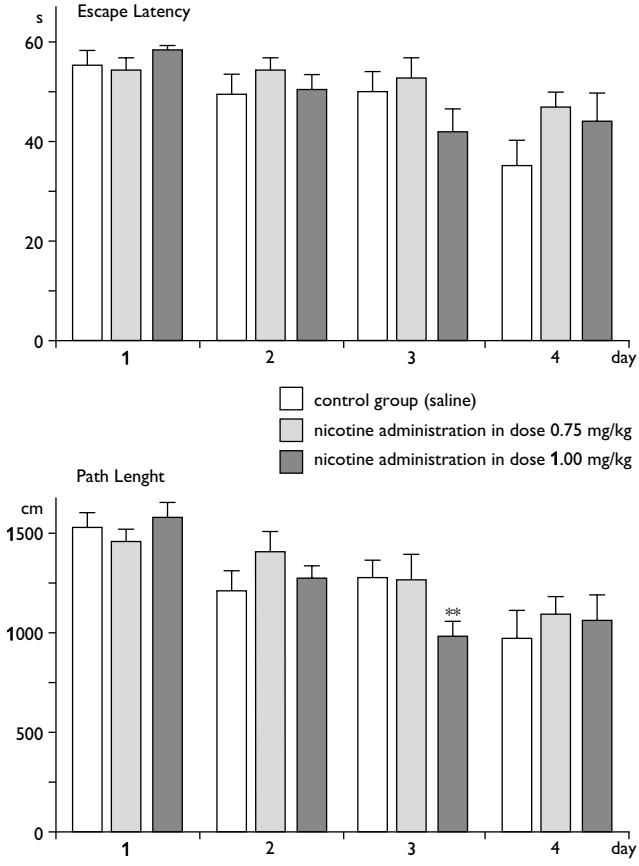

Figure 2 – Acute effect of nicotine in escape latency and path length. No statistically significant difference was found in escape latency observation in all groups. Statistically significant difference was found in path length observation on the 3rd day ($p<0.05$) in the group 2 in comparison with the group 0 (control group). The presented values are means of the latencies and path length of each day.

latencies between any of the three groups of animals within this training period was found (Figure 3).

Path length – chronic effect of nicotine
The path length was as well measured and evaluated in the last two days of study (the second period). No statistically significant difference in the assessment of the path length between any of the three groups of animals within this training period was found (Figure 3).

Discussion

Nicotine has been known as a primary psychoactive agent for a long time. Small rodents, other small mammals (rabbits, cats, dogs), but as well primates have been used as models, because their reactivity is very close to humans.

Tobacco, nicotine and tobacco smoke exposure has been shown to exert deleterious effect on the health of the foetus, newborn, child and of course on adult (Mathers et al., 2006; Leonardi-Bee et al., 2008). This negative influence of nicotine in the field of neuropathology has been observed in various studies – during monitoring and assessment of the native or evoked bioelectrical brain activity, during observation and valuation the behaviour and motor activity (Longo et al., 1967; Slawecki and Ehlers, 2002; Marešová et al., 2009; Hralová et al., 2010). Even convulsions occurred after nicotine-application in various tasks (Longo et al., 1967; Hralová et al., 2010).

Positive influence of nicotine is in focus of many studies which are engaged in the treatment of some neurodegenerative and age-related disorders. Nicotine is suggested to have a neuroprotective effect and to cause an improvement in

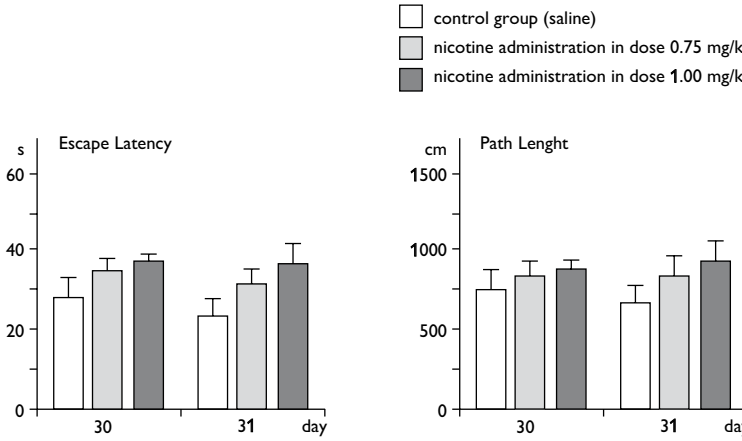

Figure 3 – Chronic effect of nicotine in escape latency and path length.
No statistically significant difference was found in both escape latency and path length observations in all groups. The presented values are means of the latencies and path lengths of each day.

learning ability, spatial memory and other cognitive functions in animals as well as in humans (Carrasco et al., 2006; Levin et al., 2006; Alkadhi et al., 2010). However, the regular application or prolonged administration (long-term treatment, chronic effect) of nicotine is accented very often (Attaway et al., 1999; French et al., 2006; Yamada et al., 2010). Several of these tasks were accompanied by morphological studies, where the effect of nicotine was demonstrated (Riljak et al., 2006; Srivareerat et al., 2011).

A lot of studies have concentrated on the effect of a long-term or a short-term nicotine treatment in experimental animals of different ages. According to other authors we also cannot declare that only a single dose of nicotine in the low age unambiguously improves learning ability in young rats in our task.

Belluzzi and co-workers reported that the brain in early adolescent, late adolescent and adult rats had been differentially sensitive to the nicotine administration. They evaluated mainly the locomotor activity in various age groups of rats (place-conditioning test) following the single nicotine administration in various doses (0.125–0.5 mg/kg). In the youngest age group (young adolescent) no evident locomotor response was present, but reaction (inhibition of ambulatory activity) was found in the group of older adolescent and adult rats (Belluzzi et al., 2004). It is in agreement with Spear (2000), that in this lower maturity the development of the limbic dopamine system was incomplete and that was why the response in early adolescent rat is very slight. Also Badanich and other authors pointed out on a difference in maturity and responsiveness of dopamine pathway during development in rats (Badanich and Kirstein, 2004; Wahlstrom et al., 2010).

Various opinions are in the assessment and the evaluation of acute and chronic effect of nicotine. The acute effect of nicotine has been more connected with evaluation of locomotor activity (Belluzzi et al., 2004; Simmons et al., 2010), learning ability (Attaway et al., 1999; Yamada et al., 2010) or bioelectric brain activity (Hralová et al., 2010). This acute effect of nicotine in a short time after application has been expressed especially in the case of a long-term treatment, less in case of a short-term application. The chronic effect of nicotine has been more examined and evaluated in the long-term treatment of memory deficits (age-related deficits, dementia). The long-term application of lower doses was used in this tasks and the positive effect was observed in a long term interval as well (Carrasco et al., 2006; Levin et al., 2006; Srivareerat et al., 2011). That could be the reason, why no statistically significant differences after only one dose of nicotine in our study were observed.

The investigators in the field of neuroprotective effect of nicotine have divergent opinion regarding to dose of this drug and to application frequency in experimental animals.

A part of experimental studies have shown that single administration of relatively low dose of nicotine (0.1–0.5 mg/kg) significantly improved working memory tested in various test procedures (Rezvani and Levin, 2001; Vicens et al., 2003;

Belluzzi et al., 2004; Levin et al., 2006). On the other side the positive effect of nicotine has been demonstrated in single doses administration only in case of higher doses of nicotine or in lower doses but in chronical treatment (Attaway et al., 1999; Hernandez and Terry, 2005; French et al., 2006; Alkadhi et al., 2010).

Only one nicotine injection was given in our task, but the dose was relatively high (0.75 or 1.00 mg/kg). We know that even a single dose of nicotine causes abnormities in ECoG and in behaviour, in some case loss of righting reflex or convulsions as well (Hralová et al., 2010). Because of this negative impact of nicotine on rat's organism it was not convenient to start the task in the water at the same day, shortly after nicotine application. It could be also the reason, why more the negative effect of nicotine was observed and less the positive influence on the learning ability and spatial memory. Although the statistically significant difference in the third day of this study is present (in the group with the dose of nicotine 1.00 mg/kg), more studies are needed, because no unambiguous improvement was observed in following days.

Conclusion

The aim of this study was to search, how the single dose of nicotine can influence not only the behaviour, but also learning ability and spatial memory in early adolescent rats immediately (or within a short time interval) after nicotine application, and detect, how much this effect persist after a longer time interval, in this task after four weeks. Two important parameters – the path length and the escape latency – were monitored and their changes in these two periods of consecutive days were assessed.

Statistically significant differences in the assessment were found only in the third day of this task (the first time period – the acute effect of nicotine), but not in the following days and not after a longer time interval (the chronic effect of nicotine). So it cannot be explicitly stated, that the administration of the single dose of nicotine injection evocates any significant improvement in learning ability or space orientation in rats. We can say in according with other investigators that more studies with various doses of nicotine and various timeline in administration in rats in various age are needed.

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