Effect of the Single-dose of Nicotine-administration on the Brain Bioelectrical Activity and on Behaviour in Immature 12-day-old Rats

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Abstract: A variety of current studies is concentrated on the effect of short-term or long-term administration of nicotine in humans and in animals. The aim of this study was to investigate the effect of nicotine after a single administration in two different doses on the brain bioelectrical activity and on behaviour and motor activity in young, immature rats. Male Wistar albino rats, 12-day-old, were used in the experiment. Two groups were administrated by one intraperitoneal (i.p.) injection of nicotine in two various doses. The last (the third) group, which was given one i.p. saline injection, served as a control group. The group with lower dose of nicotine (0.75 mg/kg body weight) showed only mild alteration of the electrocorticogram (ECoG), and no behavioural or motor changes. In the second group (with higher dose of nicotine – 1.00 mg/kg), epileptiform discharges manifested in about 50% of animals. Those animals showed also changes in motor activity (tremor of hindlimbs), but only slightly expressed within the time when epileptiform changes occurred in the ECoG. Routine behaviour and locomotion was observed only in a part of animals. In the third group (control group) no changes in bioelectrical activity, in behaviour or in motor activity were observed. We conclude that even a single dose of nicotine can evoke alteration in the ECoG, in behaviour and in motor activity of immature rats. On the other hand, the quantity, quality and length of ECoG abnormalities as well as parameters of behaviour were closely related to the dose of nicotine.

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Introduction

Nicotine is a widely used drug of abuse. It is the main part of tobacco smoke. After entering the body (during smoking), it is distributed through the bloodstream and can cross the blood-brain barrier. It takes only a few seconds to reach the brain (Benowitz et al., 2009). Nicotine acts on the neuronal nicotinic acetylcholine receptors (nAChR), placed on cholinergic synapses in the peripheral and in the central nervous system (Barik and Wonnacott, 2009).

Nicotinic acetylcholine receptors (nAChR) are ligand gated ion channels and mediate the neurotransmission in the central and also in the peripheral cholinergic systems. They are present in the brain and have mostly a modulatory role in the development and function of neuronal circuits (Wonnacott, 1997; Doura et al., 2008; Dwyer et al., 2009). The amount, types, subtypes and the function of the nAChR depend on the developmental period of the brain and on the brain region, where are located (Role and Berg, 1996; Benešová, 2003).

Different subtypes of nAChR bind nicotine with various affinity which is in close relation to their subunit composition (Barik and Wonnacott, 2009). Furthermore nicotine by binding to nicotinic acetylcholine receptors influences the levels of several neurotransmitters, as is acetylcholine, dopamine, gamma aminobutyric acid or glutamate and others (Wonnacott, 1997; Benešová, 2003).

The negative effect of nicotine and tobacco smoke on human organism is well known. Negative influence of this drug on human health was demonstrated in all age groups. In the prenatal period maternal smoking can influence the development, bring about the intrauterine growth retardation, preterm birth or even death of foetus and spontaneous abortion (Mathers et al., 2006; Dwyer et al., 2009; Gruslin et al., 2009; Llaquet et al., 2010). Exposure to tobacco smoke in perinatal period can be associated with respiratory disorders, asthma or even sudden infant death syndrome (Rogers, 2008, 2009; Gospe et al., 2009).

Several studies have concentrated on the influence of acute and also chronic treatment by nicotine or nicotinic agonists on cognitive function. This positive effect has been described in experimental animals (Levin, 1992; Rezvani and Levin, 2001; Levin et al., 2006) and as well as in humans, especially in conjunction with neurodegenerative diseases – Parkinson's or Alzheimer's disease. It seems probable, that this alkaloid could play a benefit role in prevention or treatment of those diseases (Rusted et al., 2000; Quik and Kulak, 2002; Quik et al., 2007). Other studies demonstrated nicotine as a possible potent agent for decreasing the changes normally associated with temporal lobe epilepsy in animal models (Riljak et al., 2006). Also other problems normally associated with process of ageing (learning, memory) were reduced by using nicotine (Carrasco et al., 2006).

The effect of nicotine on bioelectrical activity of the brain in animals and on its behaviour has been the topic of many studies for a long time. Longo and co-workers found in the middle of previous century that nicotine applied to the motor cortex caused EEG pathology and motor convulsions in rabbits together with

bronchoconstriction, which was necessary to treat by artificial ventilation in guinea pigs (Longo et al., 1967). Changes in behaviour and EEG abnormalities in sleep and sleep states were described in following years in cats and rats (Yamamoto and Domino, 1965; Riekkinen et al., 1993).

The nervous tissue reactivity after one-shot administration of nicotine in young rats was repeatedly investigated, mainly the effect of nicotine on evoked bioelectric activity of the brain in very young rats. Administration of single dose of nicotine has changed the duration of evoked cortical afterdischarges (Marešová et al., 2009).

Also locomotor activity after nicotine administration has been assessed. One-dose application had no significant effect, but the chronic treatment was accompanied with a reduction in locomotor activity in adult rats (Slawecki and Ehlers, 2002).

With regard to many studies of various influences of nicotine on human health, we decided to compare the influence of two different doses of nicotine on bioelectrical activity of the developing brain, on the behaviour and motor activity in very young, immature, rats.

Material and Methods

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

25 male 12-day-old Wistar albino rats of our own breed were used in this study. They were kept in a temperature controlled room (20–23 °C), on a 12 h light/dark cycle, with food (commercial rat chow) and fresh water available *ad libitum*. The animals were divided in experimental groups with respect to dosage of nicotine (Nicotine Sigma-Aldrich): the first group (n=9) was administrated by one intraperitoneal (i.p.) injection of nicotine in the dose of 0.75 mg/kg body weight (concentration of dilution 0.75 mg of nicotine in 1 ml of saline), the second group (n=8) was administrated by one i.p. injection of nicotine in dose of 1.00 mg/kg body weight (concentration of dilution 1.00 mg of nicotine in 1.00 ml of saline). In the third group (n=8, control group) rats were given one i.p. injection with a pure saline in equal volumes.

Electrophysiological experiments

Under the general inhalation anaesthesia (ether), registration silver electrodes were placed on the right and left sensorimotor cortex and bilaterally on both visual areas. The indifferent electrode was placed on the nasal bone. In the ECoG recording both the bipolar and the reference leads were used. Experiments were performed after at least one hour of recovery on freely moving rats, when righting and placing reflexes had recovered.

The bioelectric activity of the cerebral cortex – electrocorticogram (ECoG) – was registered by EEG EPAS/COMPEG 24 with BRAINLAB 3 program and its

assessment was performed off line in following days by visual analysis. The recording was initiated by 5 minutes long habituation period; in this preliminary part of the ECoG especially the maturity of bioelectrical activity was evaluated. Than the animals were given one nicotine or saline injection i.p. Changes in following 25 minutes after application the drug in the ECoG, behaviour and motor activity were observed. In the ECoG above all the presence of specific epileptiform graphoelements (spikes, sharp waves) and non-specific changes (slow high waves) was evaluated, also the time interval from nicotine application and duration their occurrence. Finally the duration of recovery of abnormity to primary pattern was measured.

Behaviour, motor activity and consequences of the nicotine application of all rats were assessed and noted through the tracing. The ECoG tracing was performed in clear plastic cages (suitable for standard keeping of experimental animals), so all activity of animals could be carefully observed and noted on line. First their consciousness and their ambulation before the nicotine injection, then consciousness (or its failure), moving (or its defect) and other pathology, which can be present after the nicotine application – e.g. convulsions, falls, tremor of the body or limbs, moving discoordination or disorientation, or vegetative symptomathology (cyanosis, dyspnoea).

Results
Bioelectrical activity
All ECoG changes started at the end of the first minute after the application of the nicotine injection.

First – before nicotine application the native ECoG was assessed – low voltage activity about 50 μV mainly with beta frequency with sporadic theta (4–5 Hz) waves during wakefulness and onset of the formation sleep spindles during sleep (Figure 1). This activity was observed almost in all rats. In the 5th minute of the tracing the nicotine injection was given.

The experimental group, which got 0.75 mg nicotine/kg i.p., showed only mild alteration of the ECoG activity. It was only infrequent epileptiform discharges (single
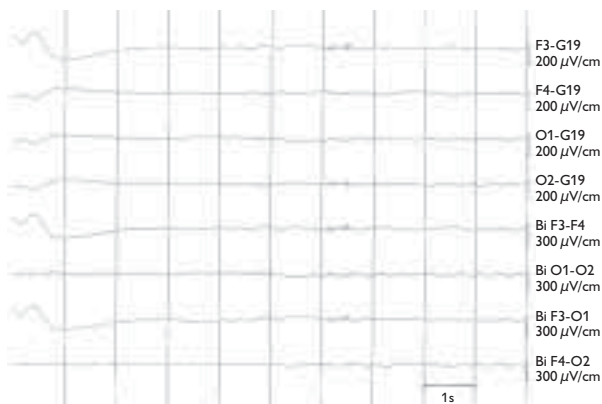

F3-G19
200 μV/cm

F4-G19
200 μV/cm

O1-G19
200 μV/cm

O2-G19
200 μV/cm

Bi F3-F4
300 μV/cm

Bi O1-O2
300 μV/cm

Bi F3-O1
300 μV/cm

Bi F4-O2
300 μV/cm

1s

Figure 1 – Native bioelectrical ECoG recording in 12-day-old rat (before nicotine application) (amplitude calibration 200 or 300 μV, time mark 1 s).

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spikes, sharp waves) within the first two minutes following the nicotine application. More over this pathology was observed only in two rats (22% of this group), which ECoG showed more mature characters as in other rats (Table 1). Also non-specific abnormity (slow high waves) was observed only in this two animals. Only non-specific mild changes – low amplitude, depressed, monotonous activity without sleep characters was seen in all other rats (Table 2). The recovery of this abnormity to primary pattern was recorded after approximately 10–15 minutes after the drug application (Table 3).

The second group of animals (1.00 mg nicotine/kg body weight i.p.) showed after nicotine application epileptiform discharges in about 50% of cases, developing at the end of the first minute after the application of nicotine. Spikes, middle- or high-voltage sharp waves, or later slow waves were observed in very short periods (Figure 2), within three minutes disappeared and the bioelectric activity became also low voltage, depressed, monotonous and without sleep spindles (Figure 3). The primary pattern of the ECoG was observed after approximately 15–20 minutes after the application of the nicotine.

In the third – control group – no abnormal patterns were observed in brain bioelectrical activity after saline application during the whole tracing, the ECoG during wakefulness and during sleep had the same character as before saline application.

Table 1 – Specific changes in the ECoG: percentage distribution of specific changes in the ECoG in dependence on the nicotine concentration

Group	–	±	+
0.75 mg	56%	22%	22%[a]
1.00 mg	25%	75%	0

– = without any changes; ± = only slightly expressed changes; + = clearly expressed changes
Monitored changes: specific changes – spikes, sharp waves; non-specific changes – slow, high waves; depression of bioelectrical activity, monotonous activity; [a] = rats with a more matured bioelectrical activity in native tracing

Table 2 – Non-specific changes in the ECoG: percentage distribution of non-specific changes in the ECoG in dependence on the nicotine concentration

Group	–	±	+
0.75 mg	78%	0	22%[a]
1.00 mg	38%	24%	38%

– = without any changes; ± = only slightly expressed changes; + = clearly expressed changes
Monitored changes: specific changes – spikes, sharp waves; non-specific changes – slow, high waves; depression of bioelectrical activity, monotonous activity; [a] = rats with a more matured bioelectrical activity in native tracing

Table 3 – Recovery of abnormity in the ECoG: percentage distribution of reversion this activity (in minutes) in dependence on the nicotine concentration

Group	10 min	15 min	20 min	>25 min
0.75 mg	33%	56%	11%	0
1.00 mg	0	75%	12.5%	12.5%

Behaviour, motor activity

The animals showed also behavioural and motor changes (tremor of body or hindlimbs, one times poorly expressed convulsion on hindlimbs were observed), but only slightly expressed within the time of specific epileptiform or stronger non-specific changes in the ECoG. Routine behaviour (full consciousness, relaxation or surrounding observation) without disorientation and common locomotion (without discoordination) was observed in major part of animals (Table 4).

All experimental animals in the control group showed routine behaviour and common motor activity before and after the saline application.

Discussion

Nicotine is one of the most heavily abused drugs in human. It is the reason for studying its effects on central and peripheral nervous system for a long time. Small rodents (rats, mice, guinea pigs), small mammals (cats, dogs) but also primates served as models, because these animals are in their reactivity very close to human.
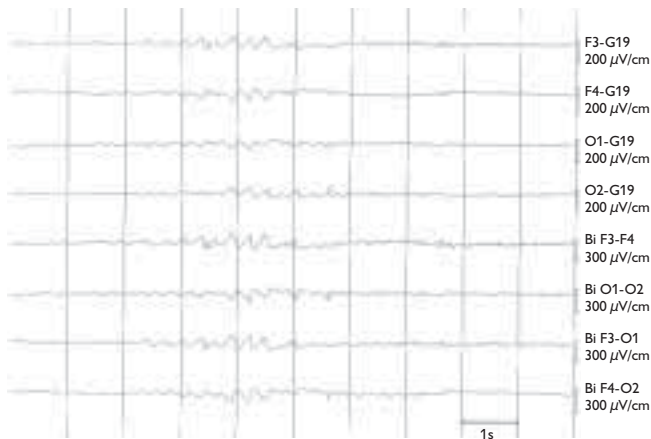
Figure 2 – Bioelectrical ECoG recording 1 min after nicotine application in 12-day-old rat (amplitude calibration 200 or 300 μV, time mark 1 s).
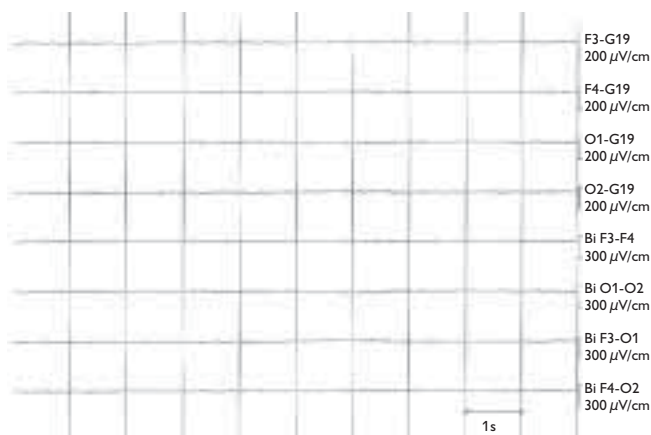
Figure 3 – Bioelectrical ECoG recording 20 min after nicotine application in 12-day-old rat (amplitude calibration 200 or 300 μV, time mark 1 s).

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Tobacco and nicotine exposure has been shown to exert deleterious effect on the health of the foetus, newborn, child, adolescent and of course adults (Mathers et al., 2006; Catassi et al., 2008; Barik and Wonnacott, 2009; Dwyer et al., 2009).

This negative influence of nicotine in the field of neuropathology has been observed in the bioelectrical activity during wakefulness and during sleep, or during assessment sleep states (Yamamoto and Domino, 1965; Riekkinen et al., 1993).

The other point of view is the evaluation of the pathology in behaviour and motor activity; in same case even the occurrence of convulsions (Levin et al., 2006).

Nicotine acts on neuronal nicotinic acetylcholine receptors (nAChR) which are widely spread in the nervous system. These receptors have an important role in brain development – they regulate brain maturation during the prenatal, early postnatal and adolescent periods (Doura et al., 2008; Dwyer et al., 2008) or play a role in cell degeneration during aging (Gotti and Clementi, 2004). Nicotinic acetylcholine receptors (nAChR) are often up-regulated or change its subunits during various developmental phases of the brain development and this fact can influence synaptogenesis during the development (Doura et al., 2008; Dwyer et al., 2008).

A lot of studies have concentrated on effect of long-term or short-term nicotine treatment in experimental animals of different ages. Belluzzi and co-workers reported that the brain in early adolescent, late adolescent and adult rats is differentially sensitive to the nicotine administration. They evaluated mainly the locomotor activity in rats and they did not observe any acute motor response following the nicotine administration in the youngest age group (young adolescent), but this reaction was present in the group of older adolescent and adult rats (Belluzzi et al., 2004). Similar results were published by other authors, when young rats displayed less signs of nicotine-cue conditioning or lower effect of nicotine to their motility (Schochet et al., 2004). The effect of nicotine not only on behaviour but also on bioelectric brain activity differs in dependence on age (Levin, 1992; Rezvani and Levin, 2001; Levin et al., 2006) probably also due to other developmental level of nAChR in early life-period than in more matured animals.

The aim this study was to evaluate the effect of one dose of nicotine on the developing brain, but not in adolescent, but in very young, immature rats. These animals are in a period of an expressive brain development when among others the level and the maturity of nAChR is changing and the synaptogenesis in central

Table 4 – Changes in behaviour and motor activity: percentage distribution of changes in dependence on the nicotine concentration

Group	–	±	+	++
0.75 mg	67%	33%	0	0
1.00 mg	0	0	75%	25%

– = without any changes; ± = only slightly expressed changes; + = clearly expressed changes; ++ = very intensively expressed changes with convulsions and vegetative abnormities
Monitored changes: convulsions; falls; tremor of the body or limbs; discoordination; disorientation; vegetative changes – dyspnoe, cyanosis

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nervous system can be influenced by various external stimuli (administration of various drug, e.g. nicotine). Not only the age is important, but also the level of the maturation, because it could slightly vary in the same age, and it could be in very close relation to different reaction of the organism on nicotine.

That is probably why these very immature rats have not so expressive behavioural reaction and and ECoG response following the application of nicotine as was reported by other authors in adult animals, or why they have different reaction after drug application in lower dose than in higher dose (in our task).

Conclusion

The negative effect of nicotine on immature rat's organism is well shown in the present study.

We can conclude that the administration even the single nicotine injection in experimental animals brings about alteration of bioelectric activity and as well as behavioural changes. These changes were observed very soon after the drug application, within 40–60 s. The quantity, the quality and the length of both was in very close relation to dose of nicotine in the single application. The higher dose of nicotine was connected with higher occurrence of specific epileptic discharges and also non-specific abnormality, but only with minor changes in behaviour or motor activity. Probably the age of animals, i.e. the maturity of the brain, is a very important factor. The adolescent or adult brain could be more sensitive to acute and also to chronic administration of nicotine (Belluzzi et al., 2004).

Further experience will be needed to explore the effect of these two doses of nicotine in older rats or on the evoked bioelectrical activity of the brain.

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