Published in final edited form as:

Prog Neuropsychopharmacol Biol Psychiatry. 2010 February 1; 34(1): 62. doi:10.1016/j.pnpbp.2009.09.024.

EFFECTS OF NICOTINE ON DEPRESSIVE-LIKE BEHAVIOR AND HIPPOCAMPAL VOLUME OF FEMALE WKY RATS

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Abstract

The observed high incidence of smoking amongst depressed individuals has led to the hypothesis of 'self medication' with nicotine in some of these patients. The inbred Wistar-Kyoto (WKY) rats exhibit depressive-like characteristics as evidenced by exaggerated immobility in the forced swim test (FST). One aim of this study was to investigate whether nicotine may have an antidepressant-like effect in these animals. Moreover, because of human postmortem studies indicating a reduction of the hippocampus volume in depressed patients, it was of interest to determine whether such an anatomical anomaly may also be manifested in WKY rats and whether it would be affected by chronic nicotine treatment. Adult female WKY and their control Wistar rats were administered nicotine consecutively (0.2 mg/kg, ip, once or twice daily for 14 days) and their activity in an open field, as well as their immobility in FST were assessed either 15 min or 18 hr after the last injection. Another set of animals was treated twice daily with 0.2 mg/kg nicotine for 14 days and sacrificed on day 15 for stereological evaluation of the hippocampal volume. When tested 15 min after the last injection, once or twice daily nicotine exacerbated the immobility in the FST in WKY rats only. When tested 18 hr after the last injection, only twice daily nicotine treatment resulted in less immobility in the FST in WKY rats. Open field locomotor activity was not affected by any nicotine regimen. WKY rats had significantly less hippocampal volume (approximately 20%) than Wistar rats which was not altered by nicotine. These findings further validate the use of WKY rats as an animal model of human depression and signify the importance of inherent genetic differences in final behavioral outcome of nicotine.

Keywords

WKY rats; Depression; Hippocampus; Nicotine; Forced Swim Test; Stereology

INTRODUCTION

It is well documented that the incidence of smoking is much higher in various neuropsychiatric disorders including depression compared to general population (see reviews: Glassman,

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1993; Quattrocki et al., 2000). Although common genetic risk factors may contribute to the co-morbid condition of nicotine dependence and depression (Kendler et al., 1993; Fu et al., 2007; Lyons et al., 2008), a number of preclinical as well as controlled human studies have reported an antidepressant-like effect of nicotine (Semba et al., 1998; Djuric et al., 1999; Tizabi et al., 1999, 2000; Ferguson et al., 2000; McClernon et al., 2006). Moreover, depressed patients are less likely to quit smoking compared to non-depressed individuals (Hughes, 2007; Leventhal et al., 2008; Thorndike et al., 2008). Thus, it has been postulated that at least a subpopulation of depressed patients may be smoking to self medicate with tobacco's nicotine (Cook et al., 2007; Moreno-Coutino et al., 2007; Spring et al., 2008). Although it might be difficult to conceptualize a true animal model of human depression, various rat models with construct and predictive validity have been introduced. Previously, it was reported that Flinders Sensitive Line (FSL) rats exhibit depressive-like characteristics compared to their control the Flinders Resistant Line (FRL) rats. FSL rats are so designated because of their sensitivity to the toxic effects of the organophosphate cholinesterase inhibitors. However, it was later discovered that these inbred rats show remarkable immobility in the forced swim test (FST), reflective of their helplessness and hence depressive-like characteristic (see review: Overstreet et al., 2005). Furthermore, injection or oral administration of nicotine resulted in an antidepressant-like effect in this model (Djuric et al., 1999; Tizabi et al., 1999, 2000).

Wistar Kyoto (WKY) rats represent another putative animal model of depression as these genetically inbred rats also exhibit exaggerated immobility in the FST and are prone to develop stress-induced ulcer or anxiety-like characteristics (Soderpalm, 1989; Paré and Redei, 1993; Pini et al., 1997; Getachew et al., 2008). Moreover, WKY rats have sleep pattern disturbances similar to that observed in depressed individuals (Paré and Redei, 1993). However, unlike FSL rats, WKY rats do not respond to clinically effective selective serotonin uptake inhibitors (SSRIs) whereas both lines respond to tricyclic antidepressants (Griebel et al., 1994; Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003; Getachew et al., 2008). Hence, WKY rats may represent a class of treatment resistant depressive individuals with unique underlying biological bases. One aim of this study was to investigate whether nicotine may also act as an antidepressant in this model.

Although the etiologies of neuropsychiatric and neurodegenerative disorders may be distinct, recent studies suggest that some neuropsychiatric disorders could also involve impairments of structural and functional plasticity in discrete brain regions albeit to a lesser extent than in neurodegenerative disorders (Manji et al., 2003; Fossati et al., 2004). Noteworthy in this regard is the finding of hippocampal volume reduction in depressed humans and volume recovery after antidepressant treatment (Sheline et al., 2003; Czeh and Lucassen, 2007). Thus, another aim of this study was to investigate whether WKY rats show a hippocampal volume reduction, and whether chronic nicotine treatment would affect the hippocampal volume in these rats. Overall our hypotheses were: 1. nicotine would exert an antidepressant-like effect in WKY rats and that this effect would be observable at least a day after the last chronic injection; 2. WKY rats would exhibit a reduction in hippocampal volume and that this reduction would be normalized by chronic nicotine administration.

MATERIALS AND METHODS

Animals

Age matched adult female WKY and Wistar rats (Harlan Laboratories, Indianapolis, IN) were used throughout the study. We selected female rats because of higher prevalence of depression in women, although there have been fewer studies in female than in male rodents (Herzog et al., 2009). Moreover, parallel to what is seen in human population (Kessler et al., 1993; Meagher and Murray, 1997), the female WKY rats show a higher level of depressive-like behavior compared to the male rats of the same strain (Paré and Redei, 1993). Animals were

housed in groups of four in standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on hardwood chip bedding (alpha-dry) in a room designated for female rats. Animals had access to food (Harlan Tek Lab) and water ad libitum. The room was maintained at 24–26°C at 51–66% relative humidity, on a 12-h reversed light/dark cycle (lights on at 19.00 hr). The reversal of time cycle was to allow convenient measurement of the behavior in active (dark) phase of the light cycle. All experiments were carried out in accordance with NIH guidelines as approved by the Institutional Animal Care and Use Committee.

To acclimate the subjects to housing conditions, animals arrived one week prior to testing. During this period, they were gentled once daily in order to minimize any stress that might result from routine handling. Behaviors were evaluated in the early part of the dark phase between 09:00 A.M. and 12:00 P.M using a red light as source of illumination. Different groups of rats were used for various chronic studies. A total of 160 rats (80 Wistar and 80 WKY) were used. Each experiment consisted of 4 groups (8 rats/group).

Nicotine bitartrate, purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) was dissolved in saline and injected intraperitoneally (i.p.) in a volume of 1ml/kg. The reported dose is indicative of pure nicotine base.

Drug Treatment

Initially, a pilot study was undertaken to determine the effects of two acute doses of nicotine (0.1 and 0.2 mg/kg, ip) on locomotor activity and forced swim test. These doses were chosen based on our previous studies with other rat models of depression (Tizabi et al., 1999; Tyler et al., 2000,) with the intention of determining the minimal effective dose of nicotine on a behavioral parameter in WKY rats.

One set of adult female WKY and Wistar rats was treated with nicotine consecutively (0.2 mg/kg, ip, once or twice daily for 14 days) and their locomotor activity in an open field followed by their immobility in the forced swim test was assessed 15 min after the last injection. These two doses of nicotine (0.2 and 0.4 mg/kg/day) were chosen based on reports in the literature and our own previous studies with Flinders Sensitive (FSL) and Fawn-Hooded rats (Matta et al., 2007; Tizabi et al., 1999, 2000, 2009). For once daily treatment the injections were carried out at 10.00 hr and for twice daily treatments the injections were carried out at 10.00 and 16.00 hr.

Another set of animals was treated exactly as above; however, the behavioral tests were carried out 18 hr after the last injection when no blood nicotine would be detectable. A third set of animals was treated twice daily with 0.2 mg/kg nicotine, however, 18 hour after the last injection these animals were perfused as described below for stereological evaluation of the hippocampal volume. Controls in all cases received saline.

These experimental protocols are summarized in the chart below.

Acute pilot studies—Nicotine 0.1 and 0.2 mg/kg

Chronic studies—Nicotine 0.2 mg/kg once daily for 14 days, behavioral tests 15 min after the last injection. Nicotine 0.2 mg/kg once daily for 14 days, behavioral tests 18 hr after the last injection. Nicotine 0.2 mg/kg twice daily for 14 days, behavioral tests 15 min after the last injection. Nicotine 0.2 mg/kg twice daily for 14 days, behavioral tests 18 hr after the last injection. Nicotine 0.2 mg/kg twice daily for 14 days, animals were sacrificed 18 hr after the last injection for histological studies.

Behavioral Testing

The locomotor activity test was always carried out first for 10 min and was followed immediately by the forced swim test for 5 min. The tests were performed alternately on each rat from a different strain and a different treatment group. For all behavioral tests the animals were moved from the housing units to the testing room in their home cage at least one hour before the test to acclimate them to the environment.

Locomotor Activity (LCA) Monitoring

Locomotor activity was measured for each animal during a 10 minute period immediately preceding the swim test. An open-field activity monitoring cage (27 × 27 × 20.3 cm, Med Associates, Inc., St. Albans, VT) was used to assess activity. Ambulatory counts representing the number of infrared beam interruptions were recorded.

Forced Swim Test (FST)

The method of Porsolt et al. (1977) with modification by Detke et al. (1995) was used to assess the immobility of the rats as a measure of their helplessness or depressive-like behavior. Rats were placed individually in a round Pyrex cylinder pool measuring 18 cm in diameter and 60 cm in height for 5 min. The cylinder was filled with 30 cm water (25±1 °C) to ensure that animals could not touch the bottom of the container with their hind paws or their tails (Lucki 1997). The animal's FST activity was video recorded for subsequent analysis. Fresh water was used for each FST in every animal.

A time sampling scoring technique was used whereby the predominant behavior in each 5-s period of the 300-s test was recorded (Detke et al., 1995, Getachew et al., 2007; Tizabi et al., 2009). Inactivity (immobility) and swimming were distinguished as mutually exclusive behavioral states. Swimming behavior was defined as movement throughout the cylinder. Immobility was defined when no additional activity was observed other than that required to keep the rat's head above the water.

It is of relevance to note that most behavioral tests assessing the antidepressant effects of drugs use the model where the rats are exposed to 15 min pretest 18–24 hr before the actual test (Porsolt et al., 1977, Overstreet et al., 2005). However, the WKY rats as well as other genetically selected models of depression (e.g. FSL and Fawn-Hooded rats) have the distinct advantage of exhibiting the depressive-like characteristic without the need for the pretest (Overstreet et al., 2005; Tizabi et al., 1999, 2000, 2009). Thus, these models along with their appropriate controls are used to evaluate the neurobiological substrates of depression or antidepressant effects of various drugs including nicotine (Tejani-Butt et al., 2003; Getachew et al., 2008; Tizabi et al., 2009).

Volume Determination

A. Histology—Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused with approximately 200 ml phosphate-buffered saline (PBS) to flush out the blood, followed by the fixation solution (4% paraformaldehyde in 0.1 M PBS, pH 7.4). Each animal received approximately 150 ml of the fixative solution via manual injection into the right atrium and through the aorta. The descending aorta was clamped to optimize fixation of the brain. The brains were post-fixed in the 4% paraformaldehyde fixative overnight and were then transferred to a 30% sucrose phosphate buffer solution until they sank at which point were frozen in CO₂/isopentane and stored at –80°C until sectioning. Each brain was serially sectioned in the coronal plane on a sliding freezing microtome. Sections were cut at an instrument setting of 50 µm and sampled in a systematic-random manner, i.e., with random start in the first ten sections, then systematic for every tenth section. Thus, the first section was

chosen randomly from the first 10 sections followed by collection of every tenth section such that a total of 8–12 sections per brain were obtained. This approach ensures that all parts of the hippocampus or the sampling region have an equal chance of being sampled. At the microscopic level, this systematic random sampling is achieved using a motorized stage that steps through the hippocampus on each section in a systematic-random manner. For estimation of the hippocampal volume, which included neuronal and molecular layers of dentate gyrus (DG) and CA1–4 regions, sampling was carried out through the entire left dorsal and ventral hippocampus. A similar approach was applied to sampling every tenth section of the total sections through complete left hemisphere for estimation of total hemispheric volume. Sampled sections were stained using routine cresyl violet for Nissl substance and cover-slipped for microscopic visualization.

B. Stereology—Using computer-assisted stereology, volumes for tissue sections were estimated using the Cavalieri principle with point counting (Gundersen and Jensen, 1987; Mouton et al., 2002; O'Neil et al., 2007), as detailed previously for brain and hippocampus volumes in rodent brains (Manaye et al., 2007).

Briefly, the Cavalieri's (Gundersen et al., 1999) principle was applied to analyze the left hemispheric and hippocampal volumes. Every 10th section was selected through the entire structure (total of 10–12 section per region per brain). The reference space on each section was outlined under low power magnification (4x, NA=1.0) using a Nikon 800 Microscope with stereoinvestigator software by MicroBrightfield Inc (MicroBrightField, Williston, VT). The relevant equation for volume estimation was:

$$V_{\text{ref}} = \sum \text{Area}_{\text{slices}} \cdot \text{Average thickness}_{\text{slices}}$$

where, V_{ref} = reference volume (hippocampal formation or hemisphere, in mm³)

$\sum \text{Area}_{\text{slices}}$ = area on slice or section, in mm²

Average thickness_{slices} = mean slice thickness, mm.

In order to capture the majority of variability within- and between-rats for each group, and achieve normalization, data for each brain was collected at a high level of stringency, i.e., the coefficient of error (CE<0.1) was less than one-half of the biological variability (Gundersen et al., 1999).

Statistical Analysis

All behavioral and stereological data were analyzed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test when significant main effects were indicated. All analyses were two-tailed and $P < 0.05$ was considered significant.

RESULTS

In the pilot studies it was revealed that fifteen min after the higher nicotine dose (0.2 mg/kg) but not the lower dose (0.1 mg/kg) there was an increase in immobility in the forced swim test (FST) in WKY rats only. Neither dose had any effect on locomotor activity (LCA) in either strain. On the basis of these findings, the chronic studies were conducted with 0.2 mg/kg nicotine administered once or twice daily.

As expected, WKY rats consistently showed a decrease in open field LCA, $F(1, 28) = 16.9$, $p < 0.01$ and exaggerated immobility in the FST, $F(1, 28) = 12.8$, $p < 0.01$ compared to Wistar

rats (Figs 1). This trend was evident in all the studies with similar statistical significance (Fig 2–Fig 4).

Administration of nicotine (0.2 mg/kg) once daily for 14 days resulted in an increase in immobility in the FST in WKY rats only when tested 15 min after the last injection (Fig 1A), but did not affect LCA (Fig 1B). Consistent with the selective effect of nicotine on FST in WKY rats, there was a significant interaction effect [$F(1,28) = 11.56$, $p < 0.01$]. However, no effect of nicotine on FST or LCA was detected when these behaviors were evaluated 18 hr after the last injection (Figs 2A and 2B).

Administration of nicotine (0.2 mg/kg) twice daily for 14 days also resulted in an increase in immobility in the FST, $F(1, 28) = 6.5$, $p < 0.05$ in WKY rats only (Fig 3A) without any effect on LCA (Fig 3B) when tested 15 min after the last injection. However, when the behavioral tests were carried out 18 hr after the last injection a significant reduction in the FST immobility was observed in WKY rats only, $F(1, 28) = 5.9$, $p < 0.05$ (Fig 4A). This selective effect in WKY rats was reflected in significant interactions [$F(1, 28) = 6.0$, $p < 0.05$ for test after 15 min, and $F(1, 28) = 6.9$, $p < 0.05$ for test after 18 hr]. Again, open field LCA was not affected by nicotine in either strain (Fig 4B).

Interestingly, the effects of nicotine on FST in WKY rats when tested 15 min after the last injection were very similar to what had been observed in the pilot study with acute (0.2 mg/kg) nicotine. Moreover, nicotine-withdrawn WKY rats for 18 hrs had very similar scores to saline-treated Wistar rats. Wistar rats were not affected by nicotine withdrawal in this test (Fig 1–Fig 4). The effects of 18 hr nicotine withdrawal (0.2 mg/kg twice daily) on FST immobility in WKY rats had disappeared when the animals were tested a week later (data not shown).

Fig 5 depicts the hippocampal volume and the effects of chronic nicotine treatment. There was a significant reduction (approximately 20%) in the hippocampal volume of WKY rats compared to Wistar rats, $F(1, 28) = 6.2$, $p < 0.05$. The total hemispheric volume of WKY rats ($622 \pm 14.0 \text{ mm}^3$) was very close to that of Wistar rats ($640 \pm 18.4 \text{ mm}^3$), suggesting that the observed reduction was specific to the hippocampus, and not due to a decrease in total brain volume. There was no detectable effect of nicotine on hippocampal volume. The measurement of hippocampal volume was carried out only following nicotine withdrawal because this was the instance when an antidepressant effect of nicotine in WKY was observed.

A repeat of the hippocampal volume determination in a set of naïve female Wistar and WKY rats yielded almost an identical result to that observed in saline-treated Wistar and WKY rats (data not shown).

DISCUSSION

The results of this study indicate that WKY rats, a putative animal model of depression may show further exacerbation of their depressive-like characteristic or an antidepressant-like response to nicotine depending on temporal evaluation of the behavior post nicotine administration. Thus, an exacerbation of the depressive-like behavior may be obtained when nicotine is present in plasma or an antidepressant-like effect may be manifested during drug withdrawal. These findings are in contrast to previous reports where an antidepressant-like effect of nicotine in other animal models of depression (e.g., FSL and Fawn-Hooded rats) was observed regardless of such temporal consideration (Djuric et al., 1999; Tizabi et al., 1999, 2000, 2009). These differences are unlikely to be due to gender effect or estrous cycle variations as studies with female Fawn-Hooded rats using a similar housing condition or female FSL rats did not show any “depressogenic” effect of nicotine (Djuric et al., 1999; Tizabi et al., 2009).

Although the majority of human studies indicate an antidepressant-like effect of nicotine and exacerbation of the depressive symptoms following smoking cessation, WKY rats may represent a unique population where withdrawal and not the presence of nicotine may result in antidepressant-like effect. These individuals would be unlikely to develop addiction to nicotine or if they did, an antidepressant-like effect may be reported upon nicotine withdrawal. Curiously, at least one recent human study indicates alleviation of the depressive-like symptom upon smoking cessation in smokers with threshold or subthreshold depressive disorders (Blalock et al., 2008). Moreover, there are reports of antidepressant effects of nicotinic antagonists but not agonists in mice (Rabenstein et al., 2006; Andreasen et al., 2009a). Indeed, a “depressogenic” effect of nicotine in some mouse strains has recently been reported (Hayase, 2007, 2008; Andreasen and Redrobe, 2009b). Thus, depending on the species, strain, gender and the behavioral test, differential outcome of nicotine effect may be observed (Tizabi et al., 1999, 2000, 2009; Andreasen and Redrobe, 2009b).

It is known that nicotinic receptor may be desensitized upon chronic nicotine administration and can recover following termination of exposure (Buccafusco et al., 2008; Yu et al., 2009). It is also possible that some of the nicotinic receptor subtypes may become supersensitive following withdrawal from nicotine. Such super-sensitivity can render the receptors more responsive to the endogenous ligand acetylcholine (Buisson and Bertrand, 2002) which in case of WKY strain may lead to normalization of neurotransmitters or circuitries involved in mood regulation and hence result in an antidepressant-like effect. Alternatively, a delicate balance between receptor subtype activation and desensitization in discrete brain circuitries in WKY rats may be responsible for the observed effects (Picciotto et al., 2008). Interestingly, WKY rats which appear to experience a depressogenic effect of nicotine upon its administration and antidepressant-like effect upon its withdrawal, do exhibit significant reduction in nicotine self-administration (De La Garza II, 2005), which may be related to a deficit in nicotine reward in these animals (Rauhut et al., 2008).

The effects of nicotine in the FST appear to be independent of its effects on general locomotor activity as this parameter was not consistently affected by nicotine treatment. However, the very low baseline locomotor activity (LCA) in WKY rats might be responsible for the high degree of immobility of these rats in the FST. Moreover, LCA in WKY rats might have been at a floor level where further decrease in this parameter could not be detected despite an increase in FST immobility. It is also of relevance to note that the effect of nicotine on open field locomotor activity is quite varied and depends on a number of factors including dose of nicotine, the duration of the treatment, the testing paradigm, strain and sex (Stolerman, 1990; DiFranza and Wellman, 2007; Andreasen and Redrobe, 2009b). That nicotine did not have any behavioral effects in Wistar rats is most likely due to genetic differences between this strain and WKY rats. However, at this point contribution of differential nicotine pharmacokinetics in these two strains cannot be ruled out as pharmacokinetic differences in nicotine between various rat strains have been observed (Sziraki et al., 2001). Moreover, it would be difficult to observe any antidepressant-like effect of nicotine in Wistar rats using the forced swim test without the pretest due to their low level of immobility. However, no “depressogenic” effect of nicotine in Wistar rats was observed either. This together with our previous findings where nicotine did not affect the FST in control strains for FSL or Fawn-Hooded rats (Tizabi et al., 1999, 2009), strengthen the contention that inherent genetic differences are critical determinants of final behavioral outcome of nicotine.

The differential responses of various individuals or various rat strains to nicotine may be reflective of inherent differences in nicotinic receptor distribution and/or differential responses of these receptors to nicotine (see reviews Fowler et al., 2008; Tizabi, 2007). Furthermore, nicotinic receptor stimulation may result in release of a variety of neurotransmitters including biogenic amines as well as glutamate (Wonnacott, 1997). As these neurotransmitters are

considered to be major players in mood regulation (see reviews: Charney, 1998; Maeng and Zarate, 2007; Mathew et al., 2008; McNally et al., 2008; Nutt, 2008), differential release of such neurotransmitters by nicotine in different population may contribute to the diverse behavioral outcome of nicotine. In addition, differential distribution of brain derived neurotrophic factor (BDNF) and nicotinic interaction with this system can play a role in observed behavioral differences as a role for BDNF in mood regulation and interaction of nicotine with this system has been documented (Rot et al., 2009; Son and Winzer-Serhan, 2009).

The data also provides further justification for use of WKY rats as a suitable animal model of depression as these rats similar to depressed humans show a reduction of hippocampal volume compared to Wistar rats. However, unlike the reported study in humans where some antidepressants may normalize the volume reduction of the depressed patients (Sheline et al., 2003; Czeh and Lucassen, 2007) we did not observe any effect of nicotine on hippocampal volume in this study. Although a neuroprotective effect of nicotine in various in-vitro and in-vivo studies have been reported (Tizabi et al., 2003, 2004, 2005; Copeland et al., 2005, 2007; Toborek et al., 2006; Picciotto and Zoli, 2008; Quik et al., 2008; Das and Tizabi, 2009), the lack of nicotine effect on hippocampal volume in WKY rats might not be so surprising given that the WKY rats actually show an exacerbation of their depressive-like behavior when tested 15 min after nicotine administration. Hence, it would be unlikely that volume recovery would occur during the short (18 hr) withdrawal from nicotine. Alternatively, it might be suggested that hippocampal volume recovery is not a requisite for antidepressant effectiveness. Recent findings indicate that not only depression subtype, but also the chronicity of depression might be important factors associated with loss of hippocampal or amygdalar volumes in depression (Keller et al., 2008). Thus, it would be of significant interest to evaluate the hippocampal and amygdalar volume in various animal models of depression at basal as well as following long term nicotine or other antidepressant treatments.

CONCLUSION

In summary, nicotine may exacerbate or alleviate the depressive-like characteristics of WKY rats depending on temporal evaluation of the behavior post nicotine administration. Moreover, loss of hippocampal volume in this strain further validates their use as an animal model of human depression. The findings also signify the importance of inherent genetic differences in final behavioral outcome of nicotine.

Acknowledgments

Supported by NIH/NIGMS (2 SO6 GM08016-39)

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Fig. 1.

Effects of chronic nicotine treatment on the forced swim test (1A) and open field locomotor activity (1B) of female Wistar and WKY rats. The animals were treated once daily with 0.2 mg/kg nicotine for 14 consecutive days and were evaluated for LCA and FST, 15 min after the last injection. Values are mean $\pm$ SEM. N=8/group, * p <0.05, ** p <0.01

Fig. 2.

Effects of chronic nicotine treatment on the forced swim test (2A) and open field locomotor activity (2B) of female Wistar and WKY rats. The animals were treated once daily with 0.2 mg/kg nicotine for 14 consecutive days and were evaluated for LCA and FST, 18 h after the last injection. Values are mean $\pm$ SEM. N=8/group, **p<0.01

Immunity in the Forced Swim Test

Locomotor Activity

Fig. 3.

Effects of chronic nicotine treatment on the forced swim test (3A) and open field locomotor activity (3B) of female Wistar and WKY rats. The animals were treated twice daily with 0.2 mg/kg nicotine for 14 consecutive days and were evaluated for LCA and FST, 15 min after the last injection. Values are mean $\pm$ SEM. N=8/group, * p <0.05, ** p <0.01

Fig. 4.

Effects of chronic nicotine treatment on the forced swim test (4A) and open field locomotor activity (4B) of female Wistar and WKY rats. The animals were treated twice daily with 0.2 mg/kg nicotine for 14 consecutive days and were evaluated for LCA and FST, 18 h after the last injection. Values are mean $\pm$ SEM. N=8/group, * p <0.05, ** p <0.01

Hippocampus Volume

Fig. 5.

The total left hippocampal volume in Wistar and WKY rats following 14 consecutive days of daily treatment with nicotine or saline. Rats were injected twice daily with 0.2 mg/kg nicotine and were perfused for histological studies 18 h after the last injection. N=8/group, * $p < 0.05$