Performance of motor associated behavioural tests following chronic nicotine administration

Omamuyovwi M. Ijomone[1,2], Olayemi K. Olaibi[1], Ifechukwude J. Biose[2], Christian Mba[2], Kenneth E. Umoren[2], Polycarp U. Nwoha[1]

[1]Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun Nigeria; [2]Neuroscience Unit, Department of Human Anatomy, Cross River University of Technology, Okuku, Cross River, Nigeria

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Corresponding Author:

Omamuyovwi M. Ijomone, MSc
Tel : +2347031354971
E-mail : godmamus@gmail.com

ABSTRACT

Background: Nicotine has shown potential therapeutic value for neurodegenerative diseases though there are concerns that it may induce behavioural deficits. **Purpose:** The present study sought to determine the effect of chronic nicotine administration on overall motor functions and coordination. **Methods:** Forty adult female and male Wistar rats were randomly grouped into 4 groups. Treated groups were administered nicotine via subcutaneous injections at doses of 0.25, 2 and 4 mg/kg body weight for 28 days. Control groups received normal saline. All animals were monitored for the first few minutes after each injection for any observed immediate effect of drug administration. Motor associated behavioural tests performed include: open field test, string test for grip strength and limb impairment, movement initiation and step test. **Results:** Nicotine induced muscular convulsions within the first 1-5 minutes following daily subcutaneous injections, throughout the period of administration. This was observed to be more severe in females. Nicotine did not produce major alterations in overall motor functions and coordination in both females and males. **Conclusion:** The present study shows chronic nicotine treatment produces muscular convulsion but no major deficit in overall motor function and coordination and that any observed alterations may just be transient effects.

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Introduction

Nicotine, a major constituent of tobacco smoke is widely abused drug with strong addictive tendencies.[1] This highly addictive substance is mainly responsible for relapse of most smokers within a year of quitting smoking.[2] While there exist many documented side effects of tobacco smoke which has many other constituents apart from nicotine,[3,4] potential therapeutic benefit for management of neurodegenerative and neurologic diseases have been associated with nicotine. This beneficial effect of nicotine is due to its interaction with wide range nicotinic acetylcholine receptors (nAChR) in both the central and peripheral nervous system.[5,6] nAChR, which can either be homo pentamers or hetero pentamers, are allosteric membrane protein that respond to the neurotransmitter acetylcholine (ACh) by the fast opening of cationic channels which are permeable to Na^+, K^+ and sometimes C^{2+} ions.[7,8] nAChR are located both as presynaptic or postsynaptic receptors. While presynaptic nAChR could influence neurotransmitter release in synpases, postsynaptic nAChR facilitate excitatory neurotransmission.[9,10] These receptors are also stimulated by nicotine to produce responses similar to ACh stimulation, hence the name nAChR as opposed to another type of acetylcholine receptor (AChR) called muscarinic acetylcholine receptors (mAChR).[11]

Nicotine via its action on nAChR has been demonstrated to be neuroprotective against major neurodegenerative disorders including, Alzheimer's disease,[12] Parkinson's disease[13] and Huntington' disease.[14] Also, administration of nicotine alone as non-prescription nicotine replacement therapy (NRT) is the most popular method of smoking cessation aid. [15] Nicotine (in the form of chewing gum or a transdermal patch) is also being explored as an experimental treatment for Obsessive-Compulsive Disorder (OCD).[16,17] Even with these potential benefits of nicotine, concerns still exist that it may induce behavioural deficits especially in non-pathologic states.[18]

Therefore, the present study has investigated the effect of chronic nicotine administration on overall motor functions and coordination, in both adult female and male rats, as well as in determining any sexual dimorphic responses following nicotine administration.

Methods

Animal management and treatment

Forty adult albino strain (20 females and 20 males) Wistar rats (150-200 g) were used for this study. Animals were housed in clean plastic cages in a clean environment of 12 hours day/light cycle, at room temperature. Animals in all groups were allowed access to standard laboratory rat chow and water *ad libitum*. All animals were handled in accordance with the guidelines for animal research as detailed in the Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health, 2011).

The animals were randomly grouped into 4 groups; a control group and 3 treated groups each for both female and male rats. Treated groups were administered nicotine via subcutaneous injections at doses of 0.25, 2 and 4 mg/kg body weight for 28 days. Control groups received normal saline, – as a vehicle for nicotine. All animals were monitored for the first few minutes after each injection for any observed immediate effect of drug administration.

Nicotine was obtained in free base form as (–)-Nicotine also called (−)-1-Methyl-2-(3-pyridyl) pyrrolidine from Sigma Chemicals, USA. The selection of nicotine dose is based on previously published studies.[14,19,20]

Neurobehavioral Studies

Behavioural tests were carried out before, during and after nicotine administration; on days 0, 15 and 29. Behavioural tests

during and after administration were performed approximately 24 hours after a previous nicotine administration. All behavioural tests were recorded live using a digital camcorder and later scored manually by at least two independent trained observers. The following motor associated behavioural tests were performed:

Open-field test: This is commonly used to access locomotor and exploratory activities in experimental rats and mice. The apparatus consists of a box (72x72x36 cm) with the floor divided into 18x18 square units. The interior of the apparatus is painted white and the floor is covered with Plexiglas. The animals were placed in the centre of the box and allowed to freely explore the area for 5 min. The following parameters were obtained throughout the test; locomotion frequency (number of crossings from one square to the other), rearing frequency (number of times the animals stood on their hind paws), rearing against the wall (no of times the animals stood on their hind paws against the wall), hinding (calculated by adding the rearing frequency to rearing against the wall) and immobility time (number of seconds of lack of movement during the test). The apparatus was cleaned with 5% ethanol before testing a new animal to eliminate possible bias due to odour left by previous animal.[21,22]

String test: This was used to measure grip strength and limb impairment. The rat is allowed to hold with the forepaws a steel wire (2mm in diameter and 60 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire till it fell was recorded, with cut-off time of 180 seconds. This latency to the grip loss is considered as an indirect measure of grip strength.[14] To access limb impairment, rats were scored 3 for gripping the wire with both hind paws, 2 for gripping the wire with 1 hind paw, and 1 for not gripping the wire with either hind paws. The results were expressed as the total score.[23]

Movement initiation test: The rat is held by its trunk with its hindlimbs and one forelimb lifted above the surface of a table so that the weight of the animal's body is supported by one forelimb alone. The time to initiate one step is recorded for each forelimb. Initiation times for both forelimbs is averaged together to make one score.[24]

Step test: The step test is used to measure postural stability. In this test, animals are held in the same manner as in the movement initiation test where one forelimb bears the weight of the animal. The animal is then moved laterally across a distance of 90 cm on a table top over 5 s. The number of adjusting steps made as the animal is moved across the table is recorded for each forelimb. The average number of steps in three trials for each forelimb is used for analysis.[24]

Statistical Analysis

All behavioural studies were analysed using Two-way Repeated Measures (2-RM) ANOVA followed by Bonferroni post-tests; with time as the repeated-measures variable. Data for females and males were analysed separately and compared to their respective controls. GraphPad Prism 5 (Version 5.03, GraphPad Software, USA.) was the statistical package to be used for data analysis. Significant difference was set at $p<0.05$.

Results

Behavioural Changes

Nicotine induced spontaneous and stereotypic activities within the first 1-5 minutes following daily subcutaneous injections throughout the period of administration. The activities included rotational and jerky muscular convulsive movements associated with tremors of the limbs. Rotational movements with few limb tremors were observed in all nicotine administered groups. Convulsive movements mostly accompanied with vigorous limb tremors were observed following 0.25, 2 and 4 mg/kg nicotine treatment for females, for the first 3 weeks of nicotine administration but absent in the fourth week for only 0.25 mg/kg treatment. However, only 2 and 4 mg/kg nicotine treatment produced convulsive movements for males (Figure 1). The duration of convulsive movements was recorded daily and weekly average determined for all nicotine treated groups, apart from control since no such behaviours were observed. A two-way repeated measures (2-RM) ANOVA analysis of the duration of convulsive movements in females

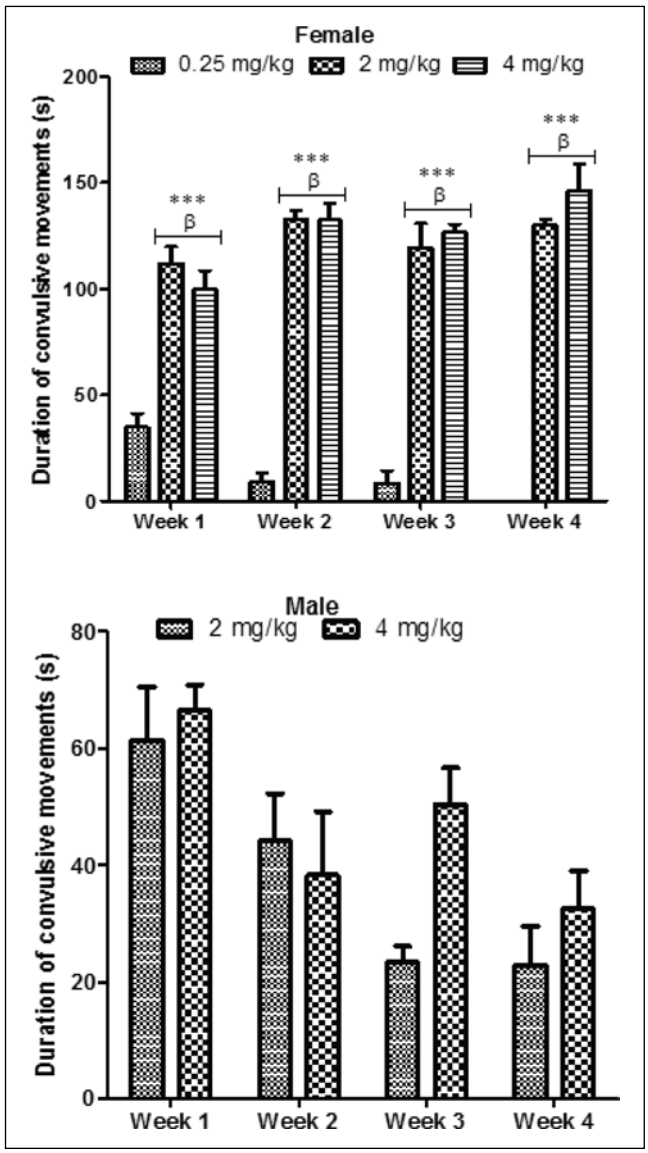

Fig. 1: Duration of convulsive movements following nicotine treatment in females (top) and males (bottom). Values are expressed as mean±SEM. ***$p<0.001$. β is significant difference compared to 0.25 mg/kg treatment. 2-RM ANOVA followed by post-tests.

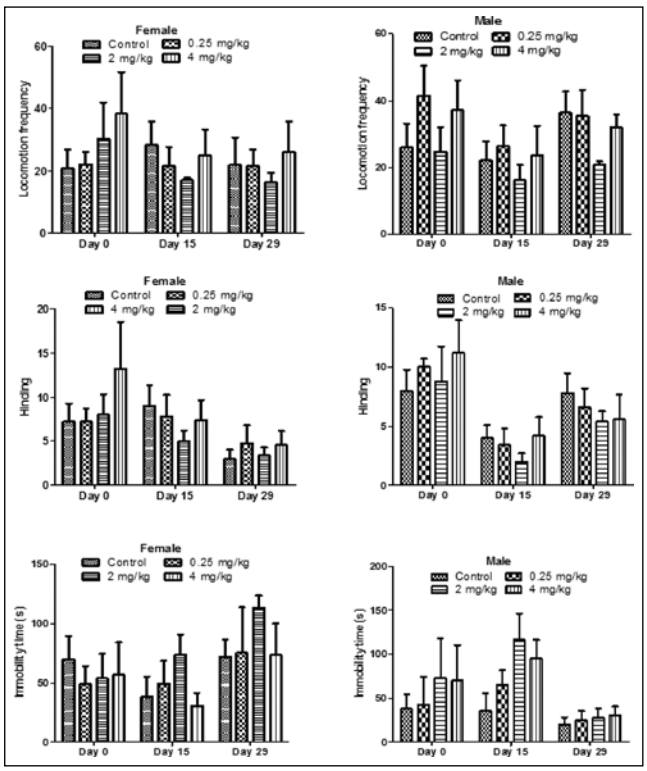

Fig. 2: Open field tests of controls and nicotine treated for females (left) and males (right). Values are expressed as mean±SEM. 2-RM ANOVA followed by post-tests.

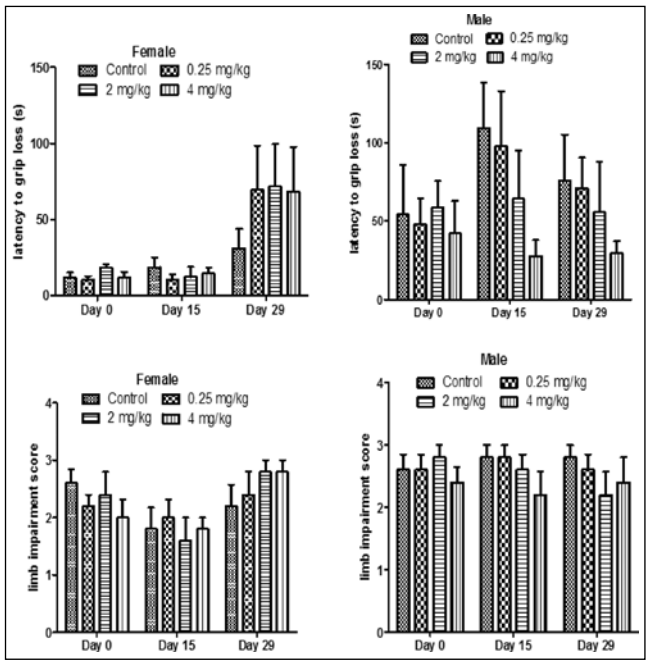

Fig. 3: String tests of controls and nicotine treated for females (left) and males (right). Values are expressed as mean±SEM. 2-RM ANOVA followed by post-tests.

for the three groups (0.25, 2 and 4 mg/kg) showed significant ($p<0.001$) interaction [$p=0.0001$] and group factor [$p<0.0001$], but no significant time factor [$p=0.2519$]. Consequently, post-test revealed a significant increase ($p<0.001$) in the duration of convulsive movements following 2 and 4 mg/kg treatment compared to 0.25 mg/kg, throughout the duration of administration. Duration of convulsive movements in males for the two groups (2 and 4 mg/kg) showed no significant interaction [$p=0.0911$] and group factor [$p=0.2305$], but a significant ($p<0.001$) time factor [$p<0.0001$] was observed, showing decrease in the duration of convulsive movements for both groups, as the administration progressed (Figure 1).

Open field tests

Locomotion frequency for females had no significant effect in interaction [$p=0.4569$], group factor [$p=0.7698$] and time factor [$p=0.2392$]. Likewise in males, locomotion frequency showed no significant effect in interaction [$p=0.9129$], group factor [$p=0.0636$] and time factor [$p=0.0987$]. Post-test indicated no significant difference between treated and control groups in both females and males (Figure 2).

Hinding in females showed no significant effect in interaction [$p=0.4887$] and group factor [$p=0.6643$], but there was significant effect ($p<0.01$) in time factor [$p=0.0047$]. Similarly, in this parameter for males, there was no significant effect in interaction [$p=0.8132$] and group factor [$p=0.7554$], but there was significant effect ($p<0.001$) in time factor [$p<0.0001$]. Post-test did not show any significant difference between treated and control groups in both females and males (Figure 2).

Immobility time in females also showed no significant effect in interaction [$p=0.5839$] and group factor [$p=0.6722$], but there was significant effect ($p<0.05$) in time factor [$p=0.0112$]. Similarly, immobility time in males had no significant effect in interaction [$p=0.8678$] and group factor [$p=0.1819$], but there was significant effect ($p<0.05$) in time factor [$p=0.0200$]. Post-test indicated no significant difference between treated and control groups in both females and males (Figure 2).

String tests

Grip strength (latency to grip loss) for females showed no significant effect in interaction [$p=0.6816$], and group factor [$p=0.7330$], but there was significant effect ($p<0.001$) in time factor [$p<0.0001$]. Males on the other hand, had no significant in neither time factor [$p=0.1021$], nor in interaction [$p=0.3448$] and group factor [$p=0.4512$]. Post-test indicated no significant difference between treated and control groups in both females and males (Figure 3).

Similarly, there was no significant effect in interaction [$p=0.5120$] and group factor [$p=0.9906$] in limb impairment for females, though a significant effect ($p<0.01$) in time factor [$p=0.0072$] was observed. On the other hand, limb impairment for males had no significant effect in interaction [$p=0.4027$], group factor [$p=0.6108$], and time factor [$p=0.7035$]. Post-test showed no significant difference between treated and control groups in both females and males (Figure 3).

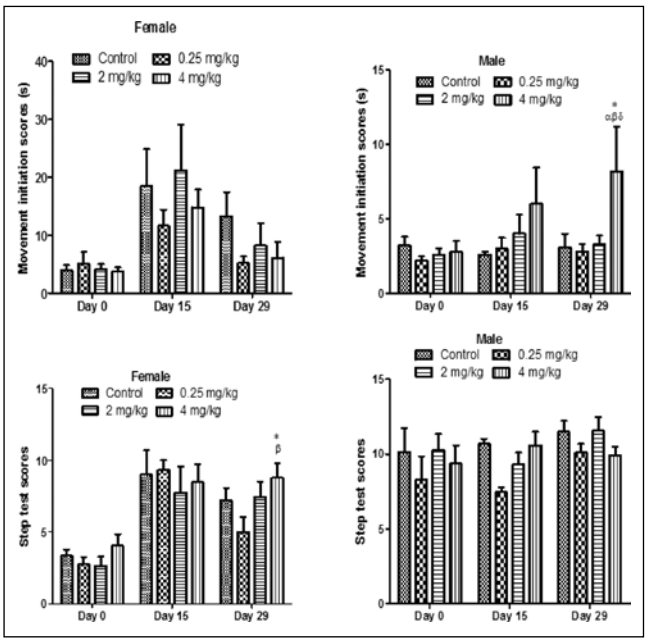

Fig. 4: Movement initiation and step tests of controls and nicotine treated for females (left) and males (left). Values are expressed as mean±SEM. *p<0.05. α, β, and δ is significant difference compared to control,0.25 mg/kg, and between 2 and 4 mg/kg respectively. 2-RM ANOVA followed by post-tests.

Movement initiation and step tests

In females, time taken to initiate movement had no significant effect in interaction [p=0.7404] and group factor [p=0.3955], however time factor [p=0.0002] had a significant effect (p<0.001). Males on the other hand, no significant effect was noted in interaction [p=0.1395], group factor [p=0.1637], and time factor [p=0.0675] for time taken to initiate movement. Post-test showed no significant difference between treated and control groups in females, however, in males, post-test revealed a significant increase (p<0.05) in time taken to initiate movement on day 29 for 4 mg/kg treatment as compared to control and other nicotine treated groups (0.25 and 2 mg/kg).

In the step tests, number of adjusting steps in females had no significant interaction [p=0.2719] and group factor [p=0.5932], but there was significant effect (p<0.001) in time factor [p<0.0001]. Number of adjusting steps in males had no significant effect in interaction [p=06654.], group factor [p=0.0845], and time factor [p=0.1147]. Though post-test revealed significant difference (p<0.05) between 0.25 and 4 mg/kg treatments for females, there was no significant difference between treated groups compared to control. Post-test showed no significant difference between treated and control groups in males (Figure 4).

Discussion

The results of the present study indicate that nicotine induces jerky muscular convulsions few seconds following subcutaneous administration at the doses given. These convulsive movements seem to be more serious in females than in males. While males exhibited convulsions at the higher doses of 2 and 4 mg/kg treatment, females on the other hand exhibited such convulsions even at the lowest dose of 0.25 mg/kg. Also, while males were able to habituate to such convulsions as indicated by significant effect in time factor showing a decrease in the duration of convulsive movements, as the administration progressed, females on the other hand demonstrated no such habituation. It is also obvious from these results that higher doses are more likely to produce such convulsive movements as seen in males response and even in females where there was significantly reduced duration of convulsive movements following 0.25 mg/kg treatment compared to 2 and 4 mg/kg treatment. The data also suggest that the severity of nicotine induced convulsions at higher doses is similar as there was no significant difference in duration of convulsive movements between 2 and 4 mg/kg nicotine treatment, in both males and females.

Nicotine has been reported to produce convulsions and tremors in human studies.[25] Hralova et al. reported that even a single dose injection of nicotine could produce marked alteration in bioelectric activities as well as elicit epileptiform discharges in immature rats.[3] In that study, single intraperitoneal injections of nicotine at a dose of 1 mg/kg produced significantly increased epileptic discharge by the first minute after administration, with a lower dose treatment of 0.75 mg/kg producing infrequent epileptiform discharges by the end of the second minute. In view of this, the present study thus indicates that nicotine induces convulsions in adult male and female rats, though females show more severity in the convulsive movements induced. Also, it is likely that beyond a certain dose, no dose dependent differences exist in nicotine induced convulsions.

The utmost basic and common outcome of interest in the open field test is movement, which is greatly influenced by motor output and exploratory drive.[26] Increase in locomotion frequency and rearing, as well as decrease in immobility time, are indicative of the improved locomotion and willingness to explore.[21,22,27] In the present study, nicotine did not alter any of these parameters measured at any time point compared to controls, in both males and females.

The string test can be typically used to measure grip strength and as a traction apparatus for accessing limb impairment. Reduced latency to grip loss is indicative of compromised muscle strength and ability to grasp and hold onto objects,[28,14] while reduced scores on limb impairment indicate compromised limb function and strength.[23] The present study showed no alteration in grip strength and limb impairment, at the time point accessed following nicotine administration in both male and female rats compared to their respective controls.

Increased time taken to initiate movement and a fewer number of adjusting steps respectively in the movement initiation and step test are often indicative of compromised central control of motor functions. Specifically, these parameters are used to access nigrostriatal damage in rodent Parkinson's disease model.[24] In the present study, nicotine produced no alteration in movement initiation and step test in female rats compared to control, Nevertheless, male rats showed a significantly longer time to initiate movement following 4 mg/kg treatment at day 29 compared to control, but no alteration in step test. This indicates that males but not females may be susceptible to compromised nigrostriatal functions at higher doses of long-term nicotine treatment. The nigrostriatal system is an essential target of nicotine action in the CNS.[29]

Taken together, this data suggests that, in relation to motor associated functions, nicotine is well tolerated even in chronic

doses, though minimal possibility of causing motor deficits still exists. Acute exposure to nicotine has been earlier reported to produce increased exploratory activities. This is possibly via increase activity of midbrain dopaminergic neurons of nigrostriatal system[29] however, the present study indicates that chronic treatment at high doses may compromise the motor functions associated with this system. On the other hand, chronic nicotine treatment was reported to significantly increase locomotor activities during treatment but this was lowered 12-14 hours after treatment withdrawal, and by 23-25 hours after no significant difference was observed in locomotor activities compared to control.[30] In another study which evaluated the effect of nicotine on motor function and coordination in mice, nicotine administered at sub-lethal dose produced significant loss in motor function and coordination few minutes after administration but full normal motor function and coordination was recovered by 30 min post-dosing.[28]

The present study, therefore, has thus shown that chronic nicotine treatment produces muscular convulsion which are more severe in females than males. Also, it has shown that nicotine produced no major deficit in overall motor function and coordination and that any observed alterations may just be transient effects.

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