Enriched environment palliates nicotine-induced addiction and associated neurobehavioral deficits in rats

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Abstract: This study was designed to investigate the role of enriched environment in preventing and/or reducing the neurobehavioral deficits produced after nicotine administration in albino Wistar rats. Equal numbers of rat in two groups were either placed in social environment (control group) or social along with physically enriched environment for four weeks before the administration of nicotine. Exposure to different environmental conditions was followed by the intraperitoneal injection of nicotine at the dose of 0.6 mg/kg for seven consecutive days during which addictive behavior was monitored using conditioned placed preference paradigm. Behavioral responses to locomotor activity, anxiety and retention of short term memory were investigated in control and nicotine injected groups exposed to different environments. Results of this study showed that the rats pre-exposed to physical along with social enrichment exhibited a decrease in drug seeking behavior, hyper locomotion, anxiogenic effects along with improvement of working memory as compared to control and nicotine injected groups that were kept in social environment alone. This behavioral study suggests that the exposure to physical enrichment along with socialization in young age can later reduce the chances of compulsive dependence on nicotine and related neurobehavioral deficits.

Keywords: Enriched environment, nicotine, addiction, locomotor activity, anxiety, memory function.

INTRODUCTION

Enriched environment is a broad term use to describe environ that consists of social surroundings and a regularly changing combination of physically inanimate objects. This kind of environment has shown to stimulate and enhance sensory, cognitive and motor functions in various animal studies (Fischer, 2016; Nithianantharajah and Hannan, 2006). It has also been suggested that physically and socially enriched environment benefits the brain development indicating an important role in the treatment of a wide range of brain disorders including neurological, neurodegenerative and addictive disorders (Simpson and Kelly, 2011; Solinas *et al*., 2010). Animals that are grown up from an early age in a physically, socially, and mentally triggering and safe environments, with bigger housing capacity, social interactions, variety of activities and colorful regularly changing objects have shown improved cognitive function as compared to the animals reared in isolation or without physical enrichment (Leary *et al*., 2017; Diamond, 2001). The enriched animals have also shown enhanced process of neurogenesis in specific brain areas that improve the physiological health along with improvement in acquisition of learning and retention of memory (Klein *et al*., 2017). Behavioral and biochemical studies have shown greater chances of recovery in animals that are exposed to enriched environment from various neurological and neuropsychological disorders such as stress, clinical depression, and addiction (Bekinekinschtein *et al*., 2011; Will *et al*., 2004).

Physically and socially enriched environments has been shown to regulate neurobehavioral and neurochemical effects related to drug dependence by minimizing the activation and reinforcement of psychostimulants and providing protection against the development of drug dependence (Arndt *et al*., 2015). Social isolation, food restriction or lack of exercise on the other hand can increase the activating and reinforcing effects of drug abuse. In previous literature the preventive and curative effects of environmental enrichment against cocaine and amphetamine addiction have been well demonstrated (Solinas *et al*, 2010; Noonan *et al*, 2010) but the preventive effects of physically and socially enriched environment in reducing the chances of the development of nicotine addiction and psycho-behavioral despairs related to it are very limited, despite the fact that it is far more abundantly used drug among adults as compared to cocaine and amphetamine around the world (Park *et al*., 2006).

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When nicotine is administrated in the body it reaches the brain within seconds by crossing blood brain barrier where it produces stimulation and sedation depending upon the dose and route of administration (Benowitz, 2010). Chronic adverse effects caused by use of tobacco containing nicotine, like high blood pressure, cancer, anxiety, depression, heart attack and tuberculosis cause thousands of deaths each year and hence necessary preventive measures are indispensable to have protection against deleterious effects produced by prolonged drug abuse (Bryant *et al*., 2012; Alouf *et al*., 2006). Furthermore, there is a dire need to provide a healthy stimulating environment from an early age which might help an individual in abstaining from drug addiction and associated neurobehavioral deficits.

Earlier studies mainly focused on the attenuation of nicotine-induced behaviors such as hyperlocomotor activity in rats following the exposure of enriched environment (Green *et al*., 2003). Here we emphasized and focused on the importance of pre-exposure of physical along with standard social environment from an adolescence age in attenuation of nicotine-induced addiction using conditioned place preference (CPP) paradigm. Neuropsychological behaviors such as locomotor activity, anxiety and memory functions which are said to be affected by drug dependence were also investigated in this study. This study also investigated whether social enrichment alone can be as beneficial as social plus physical enrichment in preventing and/or reducing the chances of nicotine addiction and the related behavioral deficits in rats.

MATERIALS AND METHODS

Animals

Locally bred young and naive (3-4 mo old) albino Wistar rats weighing about 200 g were used in present study. Upon arrival rats were housed in a temperature and humidity controlled room and maintained on a 12-hour light/dark cycle. Rats were also given standard laboratory diet and water *ad libitum* during entire phase of study.

Drug preparation

Nicotine Hydrogen Tartrate (Merck) was used to prepare a dose of 0.6 mg/kg nicotine as intraperitoneal injections.

Experimental protocol

Upon arrival rats were kept in standard laboratory cages for habituation period of 3 days after which rats were divided into four equal groups; Control, Nicotine, EE (enriched environment) and EE+Nicotine (n=6 each), control and stress group were kept socially in standard cages having 59 × 38 × 20 cm dimensions in a group of 3 rats per cage. While EE and EE+Nicotine group were kept socially in a group of 3 rats/cage in enriched cages that consisted of housing capacity of 120 × 100 × 60 cm and equipped with continuous enrichment in the form of three to four colorful synthetic toys, tunnels and running wheels (fig. 1). Toys were replaced every twice per week while tunnels and running wheels were kept constant for entire phase of study. The rats were kept in respective environment for four weeks after which nicotine was injected to Nicotine and EE+Nicotine groups for seven consecutive days. Rats were subjected to behavioral testing during the course of drug administration. CPP testing was carried out before drug administration and at 3^{rd}, 5^{th} and 7^{th} day of nicotine injection. Open field test (OFT), light/dark transition (L/D) test and elevated plus maze (EPM) test were conducted before and after 7^{th} day of nicotine injection.

Conditioned place preference (CPP)

CPP was performed according to the method defined earlier with slight modifications (Le Foll and Goldberg, 2005). A three-chambered CPP apparatus was used (fig. 2). One of the compartments had transparent walls and the opposite compartment had black walls. The compartments were separated by a door and neutral area considered as the third neutral chamber with white colored walls and floor. In the present study the test was comprised of three sessions including pre-conditioning (pre-CPP), conditioning and testing. During pre-CPP the rats were placed in the middle of the neutral area and allowed to explore both compartments freely. The time spent in each compartment was observed using a stopwatch during a cutoff time of 600 sec. After 24h of pre-CPP rats were subjected to conditioning phase during which nicotine and EE+nicotine rats were treated with nicotine at a dose of 0.6 mg/kg i.p. The rats were then immediately confined to light compartment of CPP apparatus for 600 sec. This treatment was continued for seven consecutive days. Respective control of each group received saline every day and was confined to light compartment in the similar manner. After CPP procedure rats of each group were placed in their respective environments. Testing phase was conducted on 3^{rd}, 5^{th} and 7^{th} day of drug administration of nicotine and in similar manner to the pre-CPP phase with free access to both compartments. The time spent in light or drug-preferred compartment was monitored for each rat during the cutoff time of 600 sec. A significant increased time spent in light compartment was considered as index of increased nicotine dependence.

Locomotor activity

OFT box was utilized to monitor locomotor activity. The method used in this study was same as mentioned by Haider *et al*., 2015.

Anxiety

L/D box was utilized to assess anxiolytic activity in rats. The procedure and dimensions of apparatus were same as described previously (Haider *et al*., 2015).

Working memory

EPM test was used to perform the assessment of working memory as explained earlier (Liaquat *et al*., 2017).

STATISTICAL ANALYSIS

Statistical analysis for results was accomplished by Bonferrroni test after performing three-way (weeks × environment × nicotine) ANOVA using repeated measure design. Pearson correlation test was employed to assess the relation between days of nicotine injection and time spent in drug-preferred area of CPP. Values of $p<0.05$ were considered significant.

RESULTS

Effect of environment on nicotine addiction

Effects of pre-exposure of standard and enriched environment following nicotine administration in CPP paradigm are shown in fig. 3a. Analysis of data by three-way ANOVA using repeated measure design indicated a significant effect of weeks [F (3,60)= 128.628; p<0.01], environment [F (1,20)= 316.864; p<0.01], nicotine [F (1, 20)= 698.668; p<0.01] and significant interaction between weeks × environment [F (3,60)= 50.157; p<0.01], weeks × nicotine [F (3,60)= 97.616; p<0.01], environment × nicotine [F (1,20)= 347.462; p<0.01] and weeks × environment × nicotine [F (3,60)= 58.089; p<0.01].

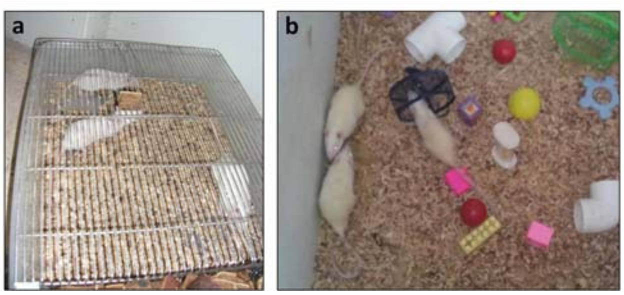

Fig. 1: Rats exposed to social environment alone (a) and socially and physically enriched environment (b).

Bonferroni post-hoc test revealed that the nicotine injected group exhibited significantly increased time spent in nicotine-preferred area as compared to control group on day 3 (p<0.01), day 5 (p<0.01) and day 7 (p<0.01) of nicotine administration. However, rats pre-exposed to physically enriched environment showed significantly decreased time spent in nicotine-preferred light box as compared to nicotine group (p<0.01) during the entire period of assessment. Pearson correlation test was conducted to observe the relation between days of nicotine administration and addiction and it was found that there was a strong positive correlation in nicotine injected rats reared in non-enriched environment (r=0.827; p<0.01) representing that these rats exhibited significant addiction to nicotine. This correlation was not observed in nicotine injected rats placed in enriched environment (r= 0.359; p>0.05).

Fig. 2: Conditioned place preference apparatus used in the present study.

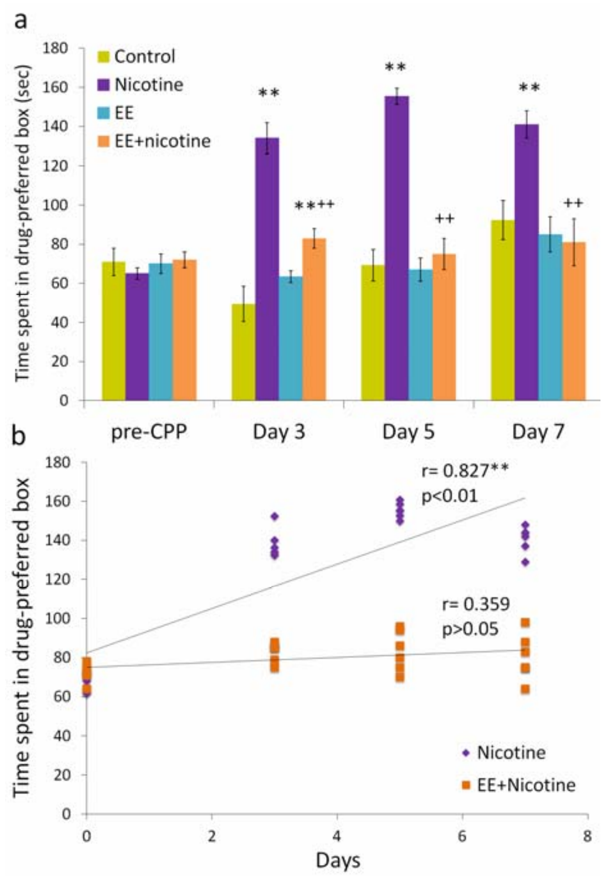

Fig. 3: Conditioned place preference (CPP) test before and during nicotine administration in rats subjected to different environmental conditions (a). Data was analyzed by three-way ANOVA with repeated measured design followed by Bonferroni test. ** p<0.01 versus control group; ++ p<0.01 versus nicotine injected rats kept in non-enriched environment. Correlation between days of nicotine administration and CPP activity observed in this study (b).

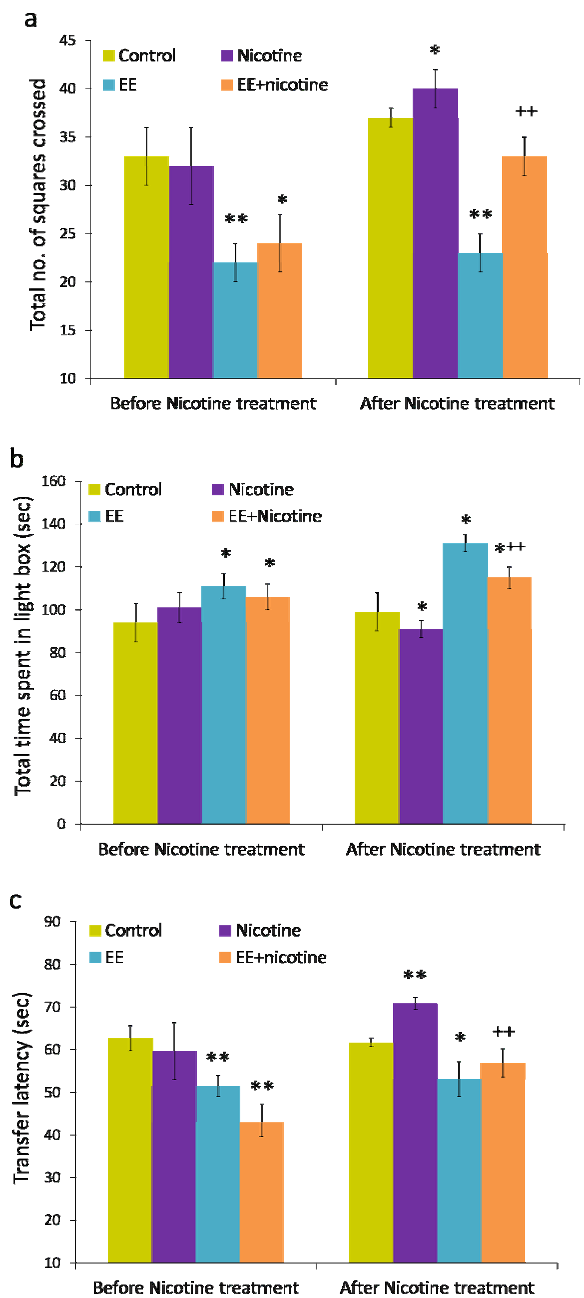

Fig. 4: Effects of different environmental conditions on open filed (a), light/dark transition (b) and elevated plus maze (c) activities before and after the drug injection. Data was analyzed by three-way ANOVA with repeated measured design followed by Bonferroni test. * p<0.05, ** p<0.01 versus control group; ++ p<0.01 versus nicotine injected rats kept in non-enriched environment.

Effect of environment and nicotine on locomotor activity

Effects of pre-exposure of standard and enriched environment following nicotine administration on locomotor activity are shown in fig. 4. Analysis of data by three-way ANOVA using repeated measure design indicated significant effect of environment [F (1,20)= 95.17; p<0.01], nicotine [F (1,20)= 15.67; p<0.01], weeks [F (1,20)= 27.39; p<0.01] and significant interaction between weeks × nicotine [F (1,20)= 10.802; p<0.01]. Other interactions were found to be non-significant. Bonferroni post-hoc test showed that nicotine addiction significantly induced hyperactivity in rats kept in standard environment in OFT as compared to control rats (p<0.05). Whereas, pre-exposure to enriched environment significantly reduced hyperactivity in EE+Nicotine rats as compared to nicotine injected group kept in non-enriched conditions (p<0.01). Moreover, rats that were exposed to physically enriched environment exhibited significant hypo-locomotor activity than control group (p<0.01) after four weeks of environmental exposure.

Effect of environment and nicotine on anxiety

Effects of pre-exposure of standard and enriched environment following nicotine administration on anxiety are shown in fig. 5. Analysis of data by three-way ANOVA using repeated measure design indicated significant effect of weeks [F (1,20)= 13.46; p <0.01], environment [F (1,20)= 147.48; p<0.01], nicotine [F (1, 20)= 24.79; p<0.01] and significant interaction between weeks × environment [F (1,20)= 14.24; p<0.01], weeks × nicotine [F (1,20)= 16.29; p<0.01], environment × nicotine [F (1,20)= 9.92; p<0.01] and weeks × environment × nicotine [F (1,60)= 6.07; p<0.05]. Bonferroni post-hoc test showed that nicotine addiction induced significant anxiogenic effects in rats kept in non-enriched environment as compared to control animals (p< 0.05). However, the rats that were exposed to physically enriched environment for four weeks exhibited significant anxiolytic activity in L/D box as compared to control group (p<0.05). This anxiolytic effect also persisted after the administration of nicotine when EE+Nicotine group was compared to nicotine group that was kept earlier in non-enriched environment (p<0.05).

Effect of environment and nicotine on working memory

Effects of pre-exposure of standard and enriched environment following nicotine administration on working memory are shown in fig. 6. Analysis of data by three-way ANOVA using repeated measure design indicated significant effect of weeks [F (1,20)= 33.60; p< 0.01], environment [F (1,20)= 152.83; p<0.01] and significant interaction between weeks × nicotine [F (1,20)= 30.33; p<0.01] and environment × nicotine [F (1,20)= 7.07; p<0.05]. Other interactions were found to be non-significant. Bonferroni post-hoc test showed that the rats that were exposed to physically enriched environment showed a significant increase in short term memory retention as compared control group (p<0.01). Nicotine administration significantly induced decrease in escape latency (p<0.01) as compared to controls indicating impaired memory retention in these rats. However, nicotine-induced impaired memory was not observed in

EE +nicotine group as evident by significant decreased escape latency as compared to nicotine injected group (p <0.01) that was kept in non-enriched conditions.

DISCUSSION

This present study was designed to analyze whether early exposure to environmental enrichment would have a protective role in the development of nicotine addiction. Neurobehavioral parameters including locomotor activity, anxiety and spatial working memory were also investigated. The total output of the results obtained from socially and physically enriched group was then compared with rats that were pre-exposed to social environment alone.

In previous studies the role of environmental enrichment related to the addiction of cocaine and amphetamine mainly focus the post-environmental effects of enrichment (Lafragette *et al*., 2017; Solinas *et al*., 2010). However, the exposure of enrichment from young age in prevention of nicotine addiction, a drug which is however more abundantly used among adolescents and one of the major causes of deaths due to chronic side effects, has been given limited attention (Park *et al*., 2006). According to the previous findings nicotine has its addictive property intact when administered at the dose 0.6 mg/kg of nicotine intraperitoneally in adolescent rats. Hence, a dose of 0.6 mg/kg was chosen in current study because in accordance with previous studies it has shown to produce reliable preference in rats for nicotine in CPP paradigm (Ahsan *et al*., 2014). The observations of present study indicated that the preference of rats to spend more time in nicotine preferred area was significantly high in nicotine injected group as compared to control group during every place preference assessment. However, the rats that were injected with nicotine but pre-exposed to physical enrichment exhibited significantly low drug seeking behavior during the place preference assessments as compared to nicotine group kept in non-enriched environment.

The phenomena of nicotine addiction revolve around mesolimbic pathway (reward's pathway) of the brain. This is a specific area condensed with dopaminergic neurons that produces the feeling of pleasure and euphoria. Increased concentration of dopamine in brain is considered to be the main cause of eventual development of nicotine dependence and addiction (Gozen *et al.*, 2016). Unhealthy and stressful life conditions, social isolation and absence of enrichment have shown to increase the activating and reinforcing effects of psychostimulants which is considered to play an important role in the development of stress related anxiety and drug addiction (Sinha and Jastreboff. 2013). Whereas, physically enriched environment reduces the activating and reinforcing effects of psychostimulants and may provide protection against the development of drug addiction by reducing the stress and stress related anxiety (Benaroya-Milshtein *et al*., 2004). Our results here demonstrate that social interaction alone may not effectively prevent the chances of the development of nicotine addiction unless combined with social and physical enrichment both.

Administration of psychoactive stimulants has normally shown to produce hyperlocomotion in rats (Wellman *et al*., 2008; Maric *et al.,* 2009). Nicotine administration has also shown to induce significant hyperactivity in rats exposed to novel environment (Schneider *et al*., 2012). The underlying mechanism involves the stimulatory effects of nicotine on dopaminergic neurons in nigrostriatal pathway of brain. Increased functional concentrations of dopamine have shown to induce hyper locomotion of rats in novel environments (Zhang *et al*., 2016). However, exposure to enriched environment has shown to attenuate the psychostimulant-induced hyper locomotion in rats due to increased adaptation and habituation to novel environment that ultimately decreases the locomotor activity of the rats. It has also been shown that nicotine-induced hyperactivity was attenuated in rats that were exposed to enriched conditions as compared to social and impoverished conditions (Green *et al*., 2002). The present observation of attenuation of drug induced hyperactivity in socially and physically enriched rats are also in accordance with previous findings. The decreased locomotor activity in EE rats observed in this study may be attributed to increased process of habituation to novel environment as well as anti-anxiety effects following the exposure of enriched environment (Hammami-Abrand Abadi and Miladi-Gorji, 2017).

Nicotine has shown to produce both anxiolytic and anxiogenic effects in previous studies (Cohen *et al*., 2009). When rats experience the signs of anxiety or any other form of stress the behavioral response to stay in dark side of L/D box is increased. Nicotine has shown to activate the response of hypothalamic pituitary adrenal (HPA)-axis that enhances the functions of corticotrophin factor (CRF) (He *et al*., 2017). Physically enriched environment on the other hand has shown to slow down the response of CRF system by down regulation of CRF receptors thus creating an anti-stress and anxiolytic effect in rats (Sztainberg *et al*, 2010). Hence, it can be assumed in the present study that the rats that were kept in social along with physical enrichment exhibited significant anxiolytic activity in L/D transition box paradigm as compared to socialized rats via decreasing the response of CRF in brain. It also indicates a potential role of enrichment along with socialization to attenuate the response of anxiety that was induced by the administration of nicotine.

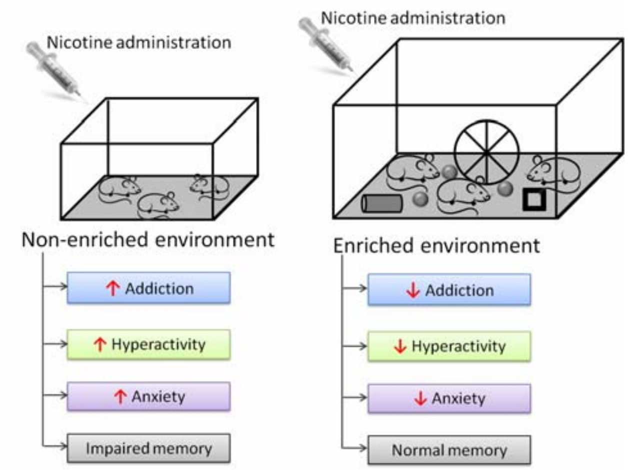

Fig. 5: Schematic representation of findings obtained from current study.

Regarding the exposure of social along with physical enrichment on acquisition and retention of learning and memory, it has been suggested previously that, it improves information processing in organisms particularly the memory acquisition in rats (Arai and Feig, 2011). On the other hand nicotine intake has shown to impair the working memory despite enhancing the spatial attention (Parks *et al.*, 2000). Physically enriched environment has shown to enhance the learning and memory via increasing the neuronal plasticity as well as neurogenesis in specific brain areas such as hypothalamus, hippocampus and cerebral cortex that are mainly involved in memory acquisition and learning process (Monteiro *et al.*, 2014; Livingston-Thomas *et al.*, 2016). Previous studies have also found growth of neurons as well as expansion of dendrite connections in these regions during the brain analysis animals that were housed in social and physical enrichment rather than social or impoverished conditions. Moreover, increase in concentration of neurotropic growth factors which are shown to enhance the learning and memory process were also found to be increased in cerebral cortex and hypothalamus (Diamond *et al.*, 2001). In light of the above literature it may be suggested that four weeks exposure of physical along with social enrichment improved retention of working memory of rats as compared to control group that was kept earlier in social environment alone. Moreover, after treatment with nicotine the non-enriched rats demonstrated impairment in retention of working memory as compared to the control group as well as socially and physically enriched group.

CONCLUSION

Hence, it may be concluded from present neurobehavioral study that presence of physical enrichment in socialized environment for a minimum period of four weeks may significantly reduce the nicotine addiction and minimize the behavioral deficits produced as a result of nicotine administration. This study emphasizes that the presence of physical along with social enrichment might plays an important role in minimizing the chances of impaired memory, anxiety and hyperlocomotor activity that are frequently associated with compulsive use of nicotine. The findings of this study are summarized in fig. 5.

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