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Does Methylphenidate Administration To Juvenile Rats Cause Altered And Addictive Behavior In Adulthood?

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ABSTRACT

Methylphenidate (MPH) is commonly prescribed drug for attention deficit hyperactivity disorder (ADHD) in children and these symptoms known to persist during adolescence and adulthood. The neural consequences like emotionality, anxiety like behavior and even addictive behavior in adolescence and adulthood after early-life exposure to MPH is not adequately addressed. Hence this study was designed to test whether MPH administration to juvenile rats cause altered and addictive behaviour in adulthood. Twenty three day old male rats were given either 2 or 5mg/kg dose of MPH for 22 consecutive days and another group of rats received saline. Four weeks after cessation of treatment rats were subjected to open field and dark/bright arena test to evaluate emotionality and anxiety like behavior and two choice ethanol tests for addictive behavior. Further rats were sacrificed and histomorphological studies of prefrontal cortex were performed. The present study demonstrates an increased emotional activity and anxiety like behavior which is associated with addictive behavior in rats during early adulthood after juvenile MPH treatment. A considerable neuronal loss was also observed in prefrontal cortex. MPH is been extensively and inappropriately used children with ADHD and the addictive behavior observed in the present study are of concern. Studies further directed towards addictive behavior and anatomical regions involved are warranted.

Keywords: Addictive behavior, Anxiety-like behavior, Attention deficit hyperactivity disorder & Methyl phenidate

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a childhood disorders of behaviour and it is known to continue into adulthood in about 17% cases [1]. In this spectrum of behavioural disorders the hyperactivity more often disappears in adulthood but anxiety like symptoms may persist in adulthood. Methylphenidate (MPH) is the most extensively used drug in ADHD in children.MPH alleviates the three major symptoms of ADHD which includes hyperactivity, inattentiveness and impulsivity, in all age groups [2]. MPH is known to act on norepinephrine and dopamine transporters by blocking re-uptake into presynaptic neurons. This intern causes an increase in extracellular concentrations of norepinephrine and dopamine which alters the neuronal circuits that control the behavior [3,4 & 5]. It is well known that psycho stimulants also enhances dopamine content in the regions of the brain concerned with reward and reinforcement which is a common mechanism to all drug abuse. Hence serious concern has been emerged about effect of MPH on alcohol abuse vulnerability in children with ADHD [6]. However some clinical studies suggest that MPH does not increase the risk of drug or alcohol abuse in children with ADHD when they were followed into adulthood [6,7].

As far as the animal model studies are concerned with respect to addictive behavior, the literatures available are conflicting. In one study, MP reduced the rat's preference for cocaine in adulthood while in another study it has increased reinforcing effects of cocaine, making adult rats more vulnerable to cocaine self-administration than untreated controls [8,9]. It is also described that preadolescent administration of MPH might be beneficial because of MPH treatment at adolescence increased the risk of drug abuse [6]. In the present study the juvenile rats were treated with MPH and end parameters were studied when rats were 73 days old. In rats the transition period from pubertal period to adulthood begins after eighth week of postnatal life [10].

Despite of inconsistencies between the studies mainly due to methodological differences, it is obvious that MPH treatment during early development results in long-lasting neurobiological adverse effects. Addictive behaviour mainly involves nucleus accumbens, the hippocampus (lays down memories) and the amygdala (creates a conditioned response to certain stimuli). The prefrontal cortex is responsible for driving force for go after it (something with wanting it/addiction). Considering limited number of studies with respect to substance abuse in adulthood and also morphological changes in rat brain, the study objective is to evaluate the sequel of repeated MPH treatment on locomotor and exploratory behavior, anxiety like behavior and susceptibility to alcohol abuse during early adulthood. We also further evaluate the morphological changes in medial prefrontal cortex, because ADHD and other similar conditions are linked to sub-performance of dopamine and norepinephrine primarily in prefrontal cortex which is responsible for self-regulatory functions [11].

MATERIALS AND METHODS

Animals

In-house bred male albino *Wistar* rats were used in the present study. Rats were fed with water and food *ad libitum*. The rats were maintained under controlled conditions of light-dark cycle (12:12), temperature (22±3°C), humidity (approximately 50±10%) and pathogen free environment. Polypropylene cage with paddy husk as bedding material was used for housing the rats. Institution animal ethics committee approval was obtained before commencing the experiment.

Animal groups

The experiment consists of following animal groups (n=6).

Group 1: Control rats
Group 2: Rats received 2mg/kg MPH twice daily from postnatal day 23 (PND23) to 45(PND45)
Group 3: Rats received 5mg/kg MPH twice daily from PND23 to PND45

MPH is administered (i.p) twice daily to the rats. The rats are subjected to behavioral studies during early adulthood (4 weeks after cessation of MPH administration - PND 73), followed by histomorphological studies.

Behavioural studies

Open field test

Open-field test is one of the most widely used methods to assess the motor and exploratory activities and emotional reactivity of rodents.

Apparatus: A rectangular box (100x100x40 cms) with the floor consisting 25 equal squares (5x5 cms) of fine unit wire mesh was used. Illumination was provided with 100 watts bulb fixed 60 cm above the center of field.

Procedure: The rats were placed in one corner of the chamber. The number of peripheral and central crossings in a-ten-minute duration was recorded. Rearing (elevated hind limb & pelvis with elevation of fore limb) and grooming (use of head, tongue and fore limb for the process of cleaning various part of the body) activities were recorded [12].

Dark-Bright arena test

An increased exploratory activity in the bright area is taken as an index of anxiolytic action when a dark compartment is simultaneously available. Anxiolytic activity is characterized by increased activity in bright section of the test box. Apparatus comprised of a wooden open top box, some portion (66X34X58 cms) of it painted black and illuminated under a dim light (20W). Black portion was partioned from the rest of the portion painted white (66X48X58 cms) and brightly illuminated with a light (100W) source located 20-cm above the box. Floor area was marked into 9-cm squares. Access between these two areas was provided by means of 20X16 cm opening located at the floor level in the middle of partition. Rats placed individually at the centre of the white area were observed over a period of 5 min for the number of line crossings in bright and dark areas and time spent in dark area[12].

Two-choiceethanol test

To test whether MPH stimulates rewarding centre and cause addictive behaviour, the two choice ethanol test was performed.The rats were given a choice between water and 10% ethanol. The bottles are measured (refilled if necessary) every 2 days for 10 two-dayperiods (i.e,over 20 days). Data were expressed in ml/day/kg weight. The body weight of the rats were recorded at least once a week [13].

Histomorphologicalstudies of prefrontal cortex

Perfusion

Rats were deeply anesthetized with ether and secured on a dissection board, and its chest cavity is opened to expose the heart. About 15mL of 0.9% saline was perfused through the left ventricle at the rate of 1mL/min. This was followed by perfusion of 10% formalin at the same flow rate. The animal was decapitated and the brain was removed and kept in 10% formalin for 48h (post fixation). Paraffin blocks were made in an embedding bath. Coronal sections of 5µm thickness were cut in the prefrontal region using a rotary microtome (Jung Biocutt 2035, Leica, Germany). Twenty sections from each animal were mounted serially on air dried gelatinized slides.

Staining

The sections were stained with cresyl violet stain. One hundred milligrams of cresyl violet was dissolved in 100 ml of distilled water. To this 0.5 ml of10% acetic acid was added to give a pH of 3.5-3.8. The stain was filtered before use [14].

Scoring

The slides are screened using a Nikon trinocular microscope (H600L) under 40X magnification.In each, prefrontal region was selected (400 X 400µm area) using imaging software NIS Elements Br version 4.30.

Numbers of viable neurons were counted. Ten sections from each rat were considered. Slides from different groups of rats were decoded to avoid manual bias while counting the cells [14].

Statistical analysis

The data were expressed as mean ±SD. The significance of differences among the groups were assessed using one-way analysis of Variance (ANOVA) test followed by Bonferroni's multiple comparison post hoc test. *P* values <0.05 were considered as significant.

RESULTS

Open field activity

The number of peripheral square crossings has not differed between control and MPH treated rats indicating that MPH has not affected the locomotor and exploratory activity in general.Methylphenidate at both the doses increased the number of central square crossings which indicates that MPH has not produced any anxiety like behavior.Time spent in central square area has also not differed ($p>0.05$) between control and MPH treated rats. This also indicates that MPH has not resulted in any anxiety like behavior (Fig.1). Rearing activities were significantly ($p<0.001$) increased in MPH treated rats.Grooming activity was also significantly increased ($p<0.01$) in rats treated with 2mg/kg dose of MPH. These are indications of increased emotional activity.

Dark/Bright arena test

The rats who received 5mg/kg dose of MPH has spent significantly ($p<0.001$) more time in dark section of the test box when compared to control as well as 2mg/kg dose of MPH treated rats. Rats who received 2mg/kg dose did not spent more time in dark chamber when compared to control. There was a significant difference ($p<0.001$) between the two doses of MPH treatment as far as time spent in dark area was concerned (Fig.2). This indicates that MPH at 5mg/kg dose has produced an anxiety like behavior

The number of line crossings in bright area did not differed significantly ($p>0.05$) between control and MPH treated rats. Rats who have treated with 5mg/kg dose of MPH has spent significantly ($p<0.001$) less time in bright section of the test box compared to control, but not those who treated with 2mg/kg dose. There is also a significant ($p<0.001$) difference between the two doses of MPH tested.

Two choice ethanol test

Comparison of mean of water or ethanol intake on day one of the experiment did not show any statistically significant ($p>0.05$) difference between the control and MPH treated rats.Comparison of mean of water or ethanol intake on day eight of the experiment did not show any statistically significant ($p>0.05$) difference between the control and MPH treated rats.The mean volume of ethanol consumption by rats treated with MPH was high on day 9 of the experiment. At 2mg/kg dose the significance of difference was less ($p<0.01$), but at 5mg/kg dose the significance was more ($p<0.001$). There was no statistically significant difference between the two doses of MPH treatment.On day 10 of the experiment the mean ethanol consumption was significantly more in both the doses of MPH treated rats compared to control. The significance was 0.01 for 2mg/kg dose of MPH treatment and 0.001 for 5 mg/kg dose of MPH treatment. There was no significant ($p>0.05$) difference between two doses of MPH treatment with respect to ethanol consumption on day 10 of the experiment (Fig.3).

Histomorphological study of prefrontal cortex

There was a significant ($P<0.001$) loss of neurons in the medial prefrontal cortex of the rats treated with both the doses of MPH. There was also a significant difference between the two doses of MPH treatment, with severe loss of neurons observed at 5mg/kg dose of MPH treatment (Fig.4,5 & 6).

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Figure 1: Open field activities by rats treated with different doses of MPH. Values are expressed as mean and error bar indicates mean ± SD. Comparison between Control Vs MPH, * = p<0.05, ** = p<0.01, *** = p<0.001

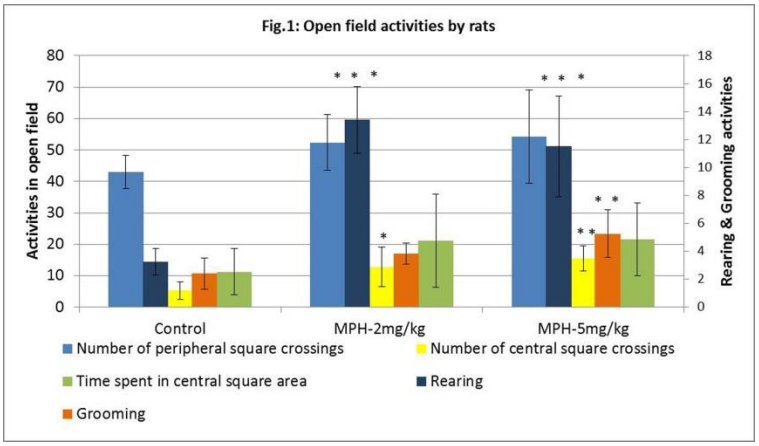

Figure 2: Observation of dark/bright arena test performed by rats treated with MPH. Values are expressed as mean and error bar indicates mean ± SD. Comparison between Control Vs MPH, *** = p<0.001

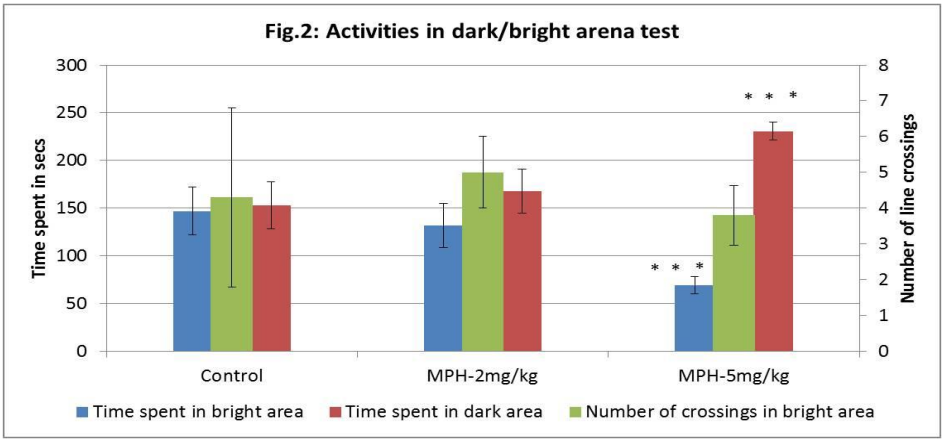

Figure 3: Observation of two choice test by rats treated with MPH. Values are expressed as mean and error bar indicates mean ± SD. Comparison between Control Vs MPH, ** = p<0.01, *** = p<0.001

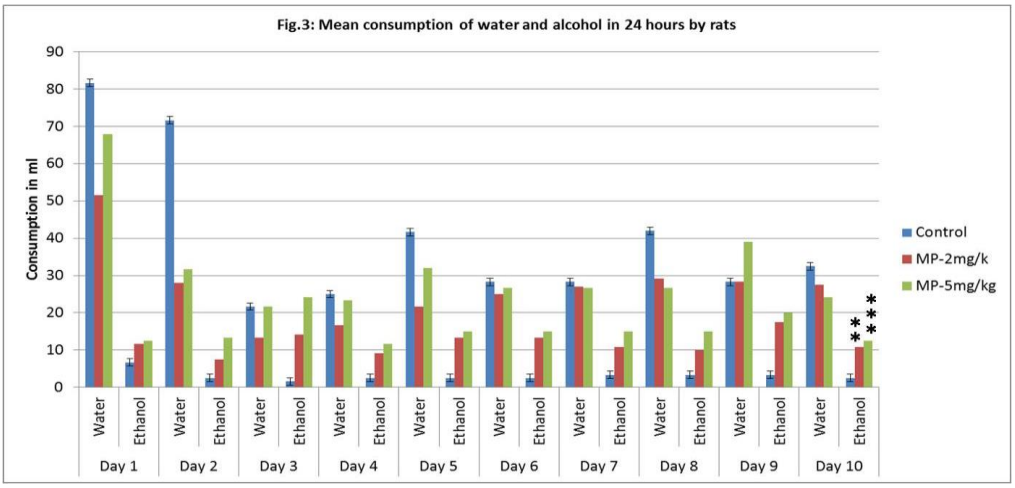

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Figure 4: Mean number of neurons in medial prefrontal cortex of rats treated with MPH. Values are expressed as mean and error bar indicates mean ± SD. Comparison between Control Vs MPH, *** = p<0.001

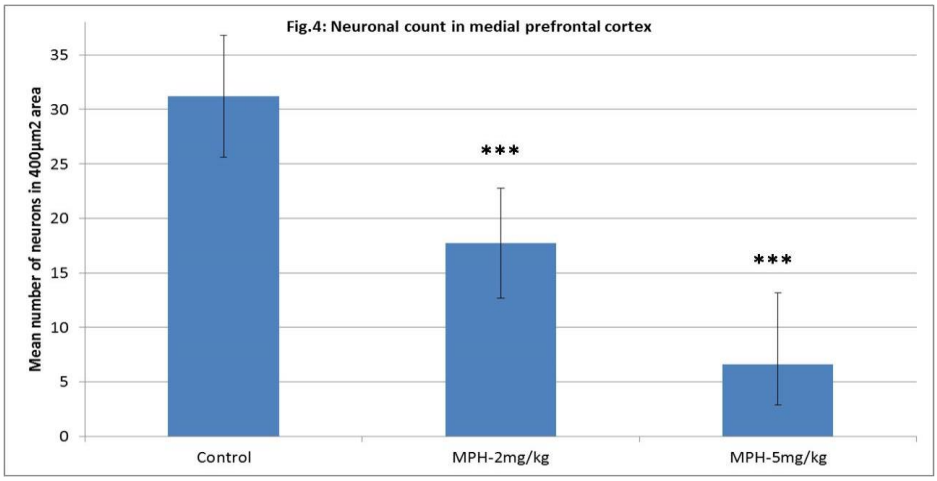


Figure 5: Photomicrograph showing prefrontal cortex of control rats stained with cresyl violet under 40X magnification. Scale bar indicates 100µm length & square area represents 400x400µm area.

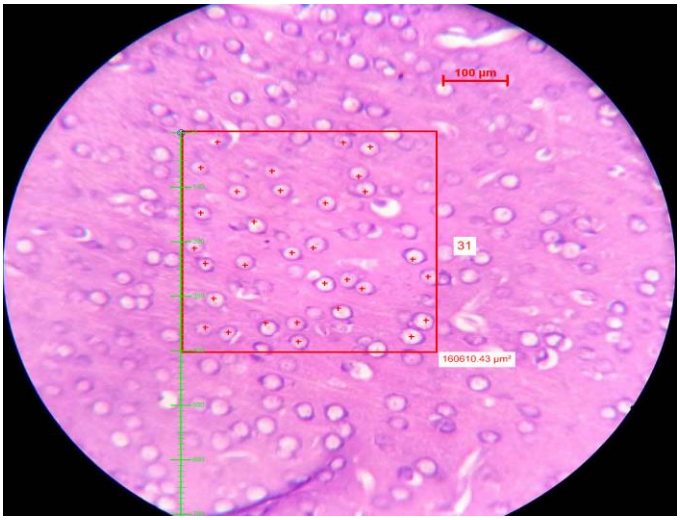
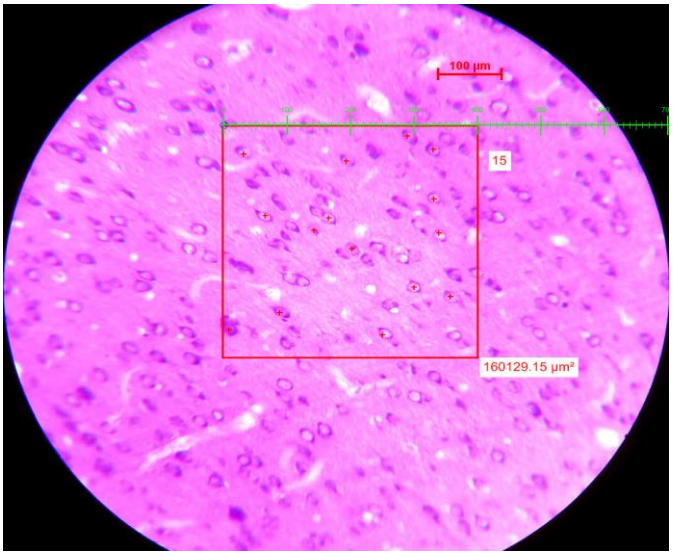


Figure 6: Photomicrograph showing medial frontal cortex of rats treated with 2mg/kg dose of MPH, stained with cresyl violet under 40X magnification. Scale bar indicates 100µm length & square area represents 400x400µm area.



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DISCUSSION

The objective of the study was to find whether juvenile MPH treatment cause altered and or addictive behavior in rats during early adulthood. To summarize, the present study demonstrates an anxious and addictive behavior in adult rats who were exposed to MPH during early developmental stage.

The results of the open field test demonstrate that MPH has not affected locomotor and exploratory activity. But emotional activities like rearing and grooming were increased.An enhanced locomotor response was observed in rats after 5mg/kg dose of MPH treatment in an earlier study by [15].The dark/bright arena test was performed to see whether MPH causes anxiety like behavior or anxiolytic action.An increased activities in dark area or time spent in dark area with correspondingly a decrease in this activity in bright area is considered as anxiety like behavior. The results of this study demonstrate that MPH at 5mg/kg dose has produced an anxiety like behavior in rats. An increased activity in the bright section is an indication of anxiolytic action and in our study number of line crossings or time spent in bright section has not increased which indicates that MPH has not caused any anxiolytic action. It is also interesting that the number of line crossings in central area of the open field test box and also time spent in central area has not affected by MPH. A decreased activity in the central area is often considered as anxiety like behavior. The different behavior expressed in two different study models may be due to different sensitivity of the rats. In general open field activity is mainly used to evaluate locomotor and exploratory activities while dark/bright arena test for anxiety like behavior or anxiolytic activity.Though our results were not consistent with study by Vendruscolo et al., [13] but the anxiety like behavior was demonstrated in dark/bright arena test. In their study anxiety like behavior was not demonstrated in open arms of elevated plus maze. This could be due to methodological differences and also different animal strains used. It is likely that the different tests used may assess partially distinct types of emotionality [16]. Anxiety like behavior in adult rats after MPH exposure during early development was also reported by Bolanos et al., [17] and Britton et al., [18].

The two choice ethanol test was used to study the addictive behavior after MPH treatment. Rats were given free access to both water and ethanol simultaneously after overnight fasting. The mean intake of both water and ethanol was calculated for 10 consecutive days.There was no consistent pattern (either increase or decrease) with respect to both water and ethanol intake during 10 days of experiment. However mean volume of ethanol consumption was more in MPH treated rats compared to their control counterparts. Statistical comparison of ethanol consumption on day 9 and 10 reveals that juvenile treatment of MPH in rats (both doses) has resulted in a significant increase in ethanol consumption during early adulthood. This is certainly an addictive behavior. However it is also to be remembered that increase in ethanol consumption may be due to sensitivity of rats to pharmacological effects of ethanol rather than MPH effect. But it is also interesting that the control rats who have not received MPH did not show any significant increase in ethanol consumption on day 9 or 10. Hence it can be fairly considered that MPH treatment showed addictive behavior towards ethanol. Considering saccharine or cocaine as a stimulus for addictive behavior and also more sensitive test models to demonstrate addictive behavior would be the idealistic approach in future. Although stimulant medicationssuch as methylphenidate improve behavioral and neurocognitive deficits of ADHD [19], disagreements remain regarding the use of stimulant medicationsand whether they make individuals more vulnerable or lessvulnerable to later addiction to stimulant drugs, such as cocaine[20].On the basis of extensive meta-analysis[7], it is quite clear that when stimulant treatment for ADHD is initiated during childhood, there is a decreasedrisk for developing a substance use disorder during adulthood.

The histomorphological study of medial prefrontal cortex showed considerable loss of neurons after juvenile treatment of MPH. MPH is known to enhance the Dopamine and Norepinephrine concentration in the specific areas of the brain including prefrontal cortex [11]. Neuronal loss could be due to ongoing apoptosis process in the developing rat brain or due to necrotic cell death. From the results of the present study the significant neuronal loss after MPH treatment compared to control is suggesting more of necrotic cell death.However increased dopaminergic and adrenergic activity with neuronal loss after MPH treatment was not reported earlier. Another possible reason for the loss of neurons could be the alcohol. The rats were given free access to water as well as ethanol for 10 consecutive days. Since same rats were tested for histomorphological studies, the neuronal loss could be due to ethanol consumption. Though the amount of neuronal loss was too high even for 10 days ethanol consumption, it is necessary to study the neuronal quantification without ethanol consumption. Further histomorpholgical evaluation in areas of the brain concerned with addiction would throw more light in this regard.

To summarize the present study demonstrates an anxiety like behavior which is associated with addictive behavior in rats during early adulthood after juvenile MPH treatment. The benefit with respect to learning and memory abilities after MPH treatment in ADHD and possible altered and addictive behavior in adulthood (as shown in the present study) has to be carefully assessed. MPH is been extensively used children with ADHD and also in adolescent group the addictive behavior is of concern. This study opens many avenues to take forward the research in understanding the MPH induced addictive behavior in adulthood.

CONCLUSIONS

MPH treatment to juvenile rats resulted in anxiety like behavior and addictive behavior during early adulthood. These effects were associated with loss of neurons in prefrontal cortex.

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