



## Assessment of Body Weight Change and Exploratory Behavior in Mice Exposed to Powdered Tobacco Diet

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**Abstract:** Change in body weight and exploratory behavior were determined in mice which were randomly divided into two groups of ten mice each, weighing between 16-21g and consumed normal rat fed (0% powdered tobacco) for the control group and 1g of powdered tobacco mixed with 99g of normal rat fed, making 1% of the diet for the test group for 30 days. Food and water intake as well as body weight change were monitored. The light-dark transition box test was used to assess exploratory behavior. The result showed that the line crosses for the tobacco exposed group was significantly higher ( $P < 0.01$ ) compared to control. Similarly, the light chamber duration was also significantly higher for the tobacco exposed group ( $P < 0.001$ ) compared to the control. However, the SAP was significantly lower ( $P < 0.001$ ) compared to control. The frequency of rearing did not differ among the groups. There was an increase in Food intake, water intake and body weight in the test group ( $P < 0.01$ ) compared to control. Thus, mice exposed to powdered tobacco diet enhance exploratory behavior and increase body weight.

**Keywords:** Tobacco, light/dark box, exploration, body weight, mice.

### 1. INTRODUCTION

Tobacco is a plant that grows natively in North and South America. It is in the same family as potato, pepper and the poisonous nightshade, a very deadly plant. The seed of a tobacco plant is very small, and a 1-ounce sample contain about 300,000 seeds. It is believed that tobacco began growing in America, Africa, Europe and Asia about 6,000BC. As early as 1 BC, American/Indian began using tobacco in many different ways, such as in religions and medicinal practices. Tobacco was believed to be a cure-all. In 1760, Lorillard established a company in New York City to process tobacco, cigars and snuff. Today Lorillard is the oldest tobacco company in the U. S (1). There is some debate over where the word tobacco came from. Some believed that it originated in Mexico in the current state of Tabasco; others believe that it came from a Caribbean island named Tobago. The Mississippi tribes were probably the first to begin using tobacco in North America. In 1612, the first tobacco plantation was established in Virginia by John Rolfe (2). Throughout the 17<sup>th</sup> and 18<sup>th</sup> centuries, tobacco continued to be a cash crop of the Virginia colony as well as the Carolinas. Until 1883, tobacco excise tax accounted for one third of internal revenue collected by the United States Government (2). Over the years, more and more scientists began to understand the chemicals in tobacco as well as dangerous health effects smoking produce. In 1936, New Englander, Samuel Green stated that tobacco is an insecticide, a poison, and can kill man. In 1847, the famous Philip Morris was established, selling hand rolled Turkish cigarettes. Soon after in 1849, Liggett and Brother were established in St Louis (3). Cigarettes became popular around this time when soldiers brought it back to England from the Russian and Turkish soldiers. In 1875, Reynolds Tobacco Company was established to produce chewing tobacco. It wasn't until the 1990's that cigarettes became the major tobacco product made and sold. Still in 1902, British Philip Morris set up a New York headquarters to market its cigarettes, including a new famous Malbora brand. Along with the popularity of cigarettes, however there was a small but growing anti-tobacco campaign, with some state proposing a total ban on tobacco (4).

Phytochemically, studies have proven that tobacco plant contain tannin, flavenoid, steroids, phlobatanin, polyphenol, saponin, reducing sugar, terpenoid, glycosides and anthraquinone (5), etc. The use of *powdered tobacco* in recent time has been on the increase as a result of its speculated therapeutic effect mostly on the protein level of the body (6). This work is therefore intended to investigate the possible effects of this herb on the neurobehavior of Swiss white mice. The findings on the Swiss white mice can be extrapolated to humans (7).



**Fig1.** TOBACCO TREE

## 2. METHODS

### 2.1. Animals

Twenty-mice were bought from the University of Nigeria, weighing between 16g and 21g were used for the study. The animal were kept in well ventilated room under room temperature ( $22 \pm 1^\circ\text{C}$ ) and 12/12 hours light/dark cycle and allowed three weeks for acclimatization before the commencement of the experiment. The mice were housed singly in metabolic cages where food and water intake were monitored. They were assigned into two groups. The control group received normal rat chow, while the test group of animals received the powdered tobacco diet for a period of 30 days.

### 2.2. Procedure

Mice were carried into the test room in their home cages. Each mouse was picked by the base of its tail and placed in the center of the white compartment facing the door and allowed to explore the apparatus for 5 minutes. The mouse behavior was scored within the period and the maze cleared with a solution of 70% ethyl alcohol and then allowed to dry between tests.

Behaviors scored included the following;

- Line crossing: the number of times the animal crosses any particular line with all four limbs.
- Stretch attends posture: frequency with which the animal demonstrates forward elongation of the head and shoulders followed by retraction to original position.
- Rearing: frequency with which the animal stands on its hind legs.
- Light box duration: length of time the animal spends in the light chamber.



**Fig2.** Light and Dark Transition Box Apparatus

## 3. STATISTICAL ANALYSIS

Data Obtained from the experiments were statistically analysed using Microsoft excel, with factorial ANOVA/T-test in the statistics programme start view version for windows or Mac. Post-hoc comparison was also done using the student  $\pm$  Newman-keuls design. Values were represented as Mean  $\pm$  SEM and a “P” value less than 0.05, was considered as significant.

#### 4. RESULTS

##### Line crosses

The mean values for the control and test groups were,  $109 \pm 6.8/5\text{mins}$  and  $131.6 \pm 6.9/5\text{mins}$ . The frequency of line crosses of the tobacco exposed group of mice was statistically higher ( $P < 0.01$ ) compared to control.

##### Rearing frequency

The frequency of rearing for the mice exposed to the tobacco diet was not significantly different compared to the control. Their mean values were,  $26.3 \pm 2.1/5\text{mins}$  and  $24.5 \pm 2.2/5\text{mins}$ .

##### Stretch attend posture

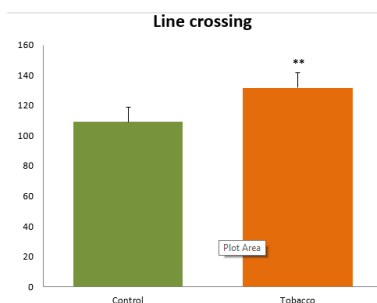
Their mean values for the control and test groups were,  $13.0 \pm 2.0$  and  $5.8 \pm 0.8/5\text{mins}$ . The frequency of SAP in the tobacco exposed group was significantly lower ( $P < 0.001$ ) compared to control.

##### Light chamber duration

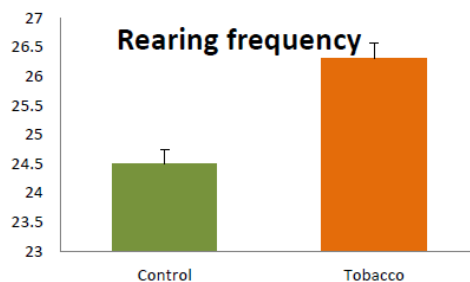
The mean values for the control and tobacco exposed group were,  $65.6 \pm 8\text{sec}$  and  $124 \pm 14.1\text{sec}$ . The mice exposed to powdered tobacco was significantly different compared to the control ( $P < 0.001$ ).

##### Body weight change

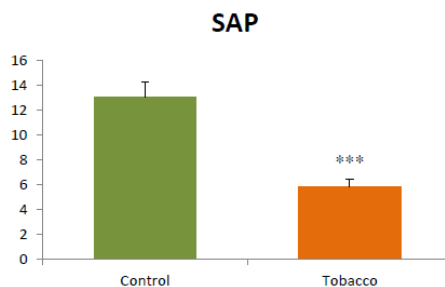
The mean values were  $7.05 \pm 0.5\text{g}$  for control and  $11 \pm 0.8\text{g}$  for the tobacco group. The group exposed to tobacco diet showed significantly higher body weight change compared to control ( $P < 0.01$ ).



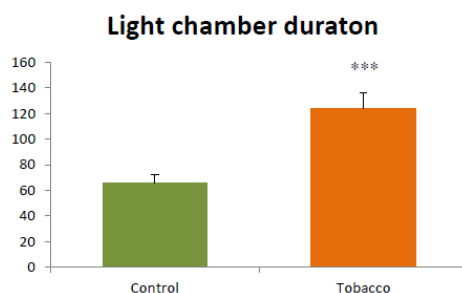
**Fig3.** Comparison of line crosses in the light-dark box test, in the control and tobacco exposed groups. Values are mean  $\pm$  SEM,  $n = 10$ . \* $P < 0.01$  vs. control.



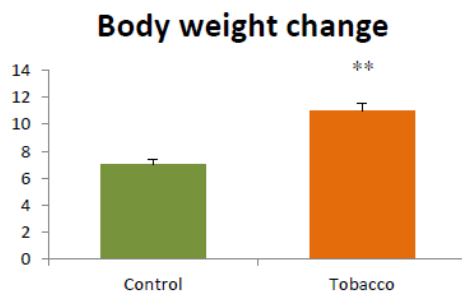
**Fig4.** Comparison of rearing frequency in the light-dark box test, in the control and tobacco exposed groups. Values are mean  $\pm$  SEM,  $n = 10$ .



**Fig5.** Comparison of Stretch attends posture in the light-dark box test, in the control and tobacco exposed groups. Values are mean  $\pm$  SEM,  $n = 10$ . \* $P < 0.001$  vs. control.



**Fig6.** Comparison of the light chamber duration in the light-dark box test, in the control and tobacco exposed groups. Values are mean  $\pm$  SEM, n = 10. \*P< 0.001 vs. control.



**Fig7.** Comparison of the body weight change between the control and tobacco exposed groups. Values are mean  $\pm$  SEM, n = 10.

\*P< 0.01 vs. control.

## 5. DISCUSSION

The light-dark box is designed to test unconditioned anxiety and exploratory behaviors. It is based on the conflict between exploring a novel environment and avoidance of bright light (8). Behaviors such as the number of line crosses and frequency of rearing are used as measures of exploration and anxiety (9). A higher frequency of these measures (line crosses, rearing) indicates increased exploratory and locomotor behavior. It was observed that the frequency of line crosses in the group of mice exposed to the tobacco diet was significantly higher compared to the control. This indicates an increased exploratory activity. This means that increase exploratory behavior in the mice exposed to the tobacco diet may be probably due to nicotine which is a major active compound in tobacco and other unknown constituents which may have a stimulatory effect on the nervous system, such as on the cerebellum, motor cortex or spinal cord (10,11). The frequency of rearing was not significantly different among the groups. Rodents are known to spent less time in well illuminated environment but this was the opposite of what the mice exposed to tobacco diet did. The light chamber duration was significantly increased in the tobacco exposed mice when compared to the control. This means that the animals spent more time exploring the illuminated surroundings and less time in the dark compartment, which implies that the test animals were less fearful. Behaviors such as frequency of stretch attend posture (SAP) in the light /dark chamber was observed to be lower in the tobacco exposed group compared to control. This means that the animals were less hesitant in moving from one place to another. It is a behavior exhibited by rodents introduced in a novel environment and it is a measure of anxiety (12). This also indicates an increase in exploratory behavior in the test group compared to the control.

The mean body weight was observed to be significantly higher in the mice exposed to tobacco diet compared to control. This could be due to the high food and water intake recorded n the study. This result is consistent with earlier studies carried out by (13).

## 6. CONCLUSION

Chronic exposure of powdered tobacco diet in mice improves exploratory behavior and increase body weight change.

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