RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 9: 1481-1486 (September 2025)

Revealing the Behavioral Effects of Hair Dye Exposure in Rats by Sociability, Anxiety and Recognition Assessments

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10.18805/IJAR.BF-2014

ABSTRACT

Background: Most people use hair dye to change their hair color. The aim of this study was to investigate how hair dye affects rat behavioral tests.

Methods: This study examines the effects of hair dye exposure on the Three-Chambered Sociability, open field, elevated plus maze and novel object recognition tests. Group 1 (n=8) was dyed twice, Group 2 (n=8) was dyed four times, Group 3 (n=8) was dyed eight times and Group 4 (n=8) was water-treated and dried eight times.

Result: The control group (Group 4) sniffed certain objects more in the first occupied cage. The groups exposed to less dye explored for longer periods ($p<0.05$ higher in Groups 1 and 2 than in the control group). In the open field test, Group 2 showed more scratching, hunger and urination. The control group showed more cage interaction than the other groups ($p<0.05$). In the elevated plus maze test, no difference in time was found between the groups. In conclusion, hair dye chemicals may affect rat behavior, but neurotoxicity and oxidative stress tests are required to confirm this.

Key words: Elevated plus-maze test, Hair dye, Novel object recognition test, Open field test, Three-chamber sociability test.

INTRODUCTION

Hair dye is a widely used cosmetic product that changes the color of human hair. Hair dyes contain various chemicals such as hydrogen peroxide and P-Phenylene-diamine (PPD), ethanolamine, pheny lenediamine, monoethanolamine and aminophenol and can cross the skin barrier and enter the bloodstream (Da França *et al*., 2015). Hair dye use is increasing among young people. (Sharma and Singh, 2011). Hydrogen peroxide and monoethanolamine are reported as the main causes of hair dye-induced dermatitis and hair loss (Seo *et al*., 2012). The potential harms of chemicals in hair dyes can also indirectly contribute to stress (Abdul-Razzaq, 2023). Hair dye chemicals can cause irritation, allergies and stress on the skin and can also have a toxic effect on the nervous system by passing through the skin barrier. Ammonia and peroxide can weaken the structure of the hair (Pereira-Silva *et al*., 2022). High levels of the stress hormone cortisol, which regulates the stress response (Orkun Erkılıç *et al*., 2024; Ozcan *et al*., 2024; Orkun Erkılıç and Bayraktar, 2025a; 2025b), can negatively affect hair growth and accelerate hair loss (Haque *et al*., 2024).

Behavioral experiments are particularly common in the study of anxiety and depression. This study examined the effects of hair dyes on anxiety with these tests. The Three-chamber sociability test is used to assess sociability and social recognition in rodents (Kaidanovich-beilin *et al*., 2011). The open field test assesses exploration and anxiety in rodents, while the elevated plus maze test measures anxiety based on rodents' preference for indoor/ outdoor spaces. General cognitive functions and memory are linked to BDNF (Bayraktar, 2019). Negativities such as allergies, irritation or shedding caused by hair dye can lead to stress and chronic stress is known to contribute to memory problems by reducing BDNF levels.The novel object recognition test is used to assess recognition memory and cognitive function in rodents. (Dagli Gul *et al*., 2024). The precise mechanisms underlying the effects of hair dyes on behavior in rats are not fully understood. With the hypothesis that hair dye chemicals may interact through neurobiological pathways, this study aimed to investigate the effects of hair dye usage frequency on behavior and anxiety in rats. Despite the limitations of current methodology, this study addresses previously unexamined effects of hair dye on animal behavior and highlights the need for more sensitive behavioral testing methods.

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How to cite this article: Ozcan, G.B., Bozok, U.G., Korkusuz, B.B., Demirboga, B., Rabetian, S. and Erkilic, T.O. (2025). Revealing the Behavioral Effects of Hair Dye Exposure in Rats by Sociability, Anxiety and Recognition Assessments. *Indian Journal of Animal Research*. **59(9):** 1481-1486. doi: 10.18805/IJAR.BF-2014.

Submitted: 26-04-2025 **Accepted:** 06-06-2025 **Online:** 15-07-2025

MATERIALS AND METHODS

Three-month-old male Wistar albino rats (n=8/group) obtained from Ankara Experimental Animal Center were kept at 21±2°C and 12-hour light/dark cycle. Rats were given water and pellet food freely (Abhijeet *et al*., 2021). In the study approved by Ankara Medipol University local ethics committee (22.11.2023-HDY1/1), rats were divided into four groups: groups dyed 2, 4 and 8 times in 21 days and control group which was watered 8 times and dried. On the 22nd day of the experiment, open field, novel object recognition, Three-Chambered Sociality and elevated plus maze tests were applied. For dyeing, 1 cc dye and 1 cc oxidant were mixed and applied to the neck of short-haired rats (2×3 cm) for 30 minutes. Dye absorption was tested on the fur. The entire experimental protocol was carried out with this timing and dosage.

Behavior tests

No abnormal stress behavior was observed in the cages during the experiment. All rats were kept in standard conditions and closely monitored before behavioral assessments. Before painting, all animals, including the control group, underwent similar procedures to reduce stress. Both the experimental and control groups were kept in a stress-free environment before the test.

Novel object recognition test (NORT)

In this test, which evaluates learning and memory in rats, the rat first got used to an empty space for 5 minutes. Then, it explored two similarly shaped objects for 10 minutes. The number of touches, sniffs and the time spent close to the object were recorded. In the second 5 minutes, a second object was introduced and the rat's interaction with this new object was observed.

Open field test (OFT)

This is a test in which the rat is placed in a new environment and its behaviors (time spent in the corner/center, standing up, scratching, defecating, urinating) are recorded for 5 minutes.

Three-chamber sociability test

This is a test in which the rat first gets used to an empty space, then an empty cage is placed on one side and a cage containing a rat on the other side. It is observed for 10 minutes which cage the rat shows more interest in. Then, the cage containing a new rat is replaced with the empty cage and it is observed for another 10 minutes whether the rat shows more interest in the familiar or the new rat. Total test time is 25 minutes.

Elevated plus maze (EPM) test

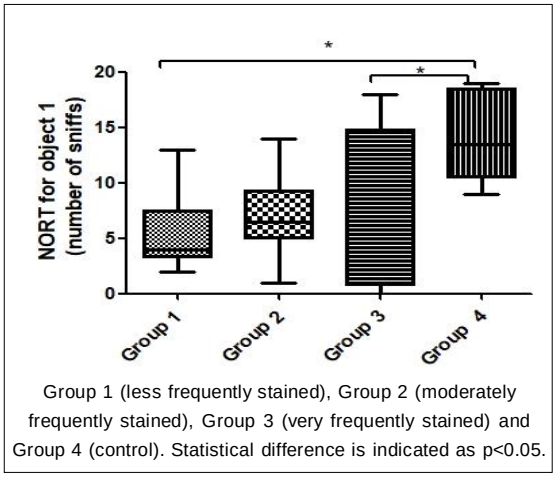
The rat is observed to move from a closed arm to an open area. If it does not exit within 300 seconds, the experiment is stopped and three trials are performed. The average time to exit the closed arm is taken to evaluate the avoidance response.

Statistical analysis

In this study, ANOVA was used when parametric assumptions were met and Kruskal-Wallis test was used when they were not met. Tukey post hoc tests were applied for parametric data and Dunn-Bonferroni post hoc tests were applied for non-parametric data for groups found to be different. Statistical significance level was accepted as $p<0.05$ and analyzes were performed with SPSS IBM v22.

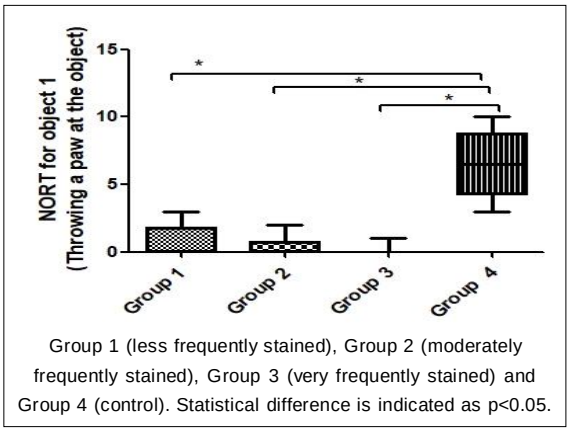
RESULTS AND DISCUSSION

Hair dyes contain aromatic amines such as PPD, which are associated with neurotoxicity and oxidative stress (Singh *et al*., 2015). These compounds can interfere with neurotransmitter systems and trigger oxidative stress, leading to behavioral changes (Syeda and Cannon, 2022). In the novel object recognition test, the control group (Group 4) showed significantly higher values than the other groups in the duration of sniffing ($p=0.010$) and scratching



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control). Statistical difference is indicated as $p<0.05$.

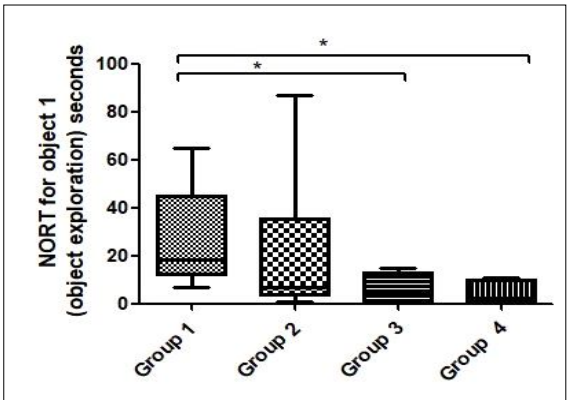
Fig 1: Differences between groups according to the number of times they sniffed the first object according to the novel object recognition test.



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control). Statistical difference is indicated as $p<0.05$.

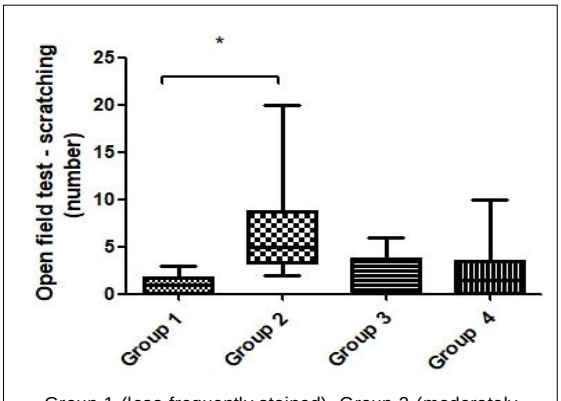
Fig 2: Pawing at the first object as a new object recognition behavior.

(p<0.001) the first object. The duration of exploring the first object was significantly longer in Group 1 (p=0.011). There was no significant difference between the groups in the duration of sniffing (p=0.093), scratching (p=0.084) and exploring (p=0.466) the second object (Fig 1,2). These results suggest that hair dye exposure may cause differences in some behavioral responses, with the control group showing greater interest in the first object. However, this effect is not consistent across all behavioral responses.Our study showed that there was a significant increase in the exploration time in rats exposed to hair dye, especially in Groups 1 and 2, compared to the control group (p<0.05). However, there were no significant differences in other behaviors. This finding is consistent with previous studies showing that ethanolamine and other compounds can affect rat behavior and supports the low acute toxicity of hair dyes. The findings that ethanolamine increases recognition memory support our observation of behavioral changes associated with chemical exposure (Table 1, Fig 3). Open field test results showed that there were frequency-dependent changes in locomotor activity and exploratory behavior and that scratching and urination were significantly more frequent in Group 2 (p<0.05). In rats exposed to hair dye (especially Groups 1 and 2), a significant increase in exploration time was observed compared to the control group (p<0.05), but there were no differences in other behaviors directed towards the secondary object. These results are consistent with previous studies indicating low to moderate acute toxicity of hair dyes and findings that ethanolamine increases recognition memory (Nasrin *et al*., 2024). In the open field test, frequency-dependent changes in locomotor activity and exploratory behavior were detected and in Group 2, significant increases in itching and urination (p<0.05). These findings are consistent with the literature reporting that chemical exposure can lead to behavioral changes (Al-Shaikh *et al*., 2018 and Sinha *et al*., 2023) (Fig 4,5).



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control). Statistical difference is indicated as p<0.05.

Fig 3: Comparison of novel object recognition time between groups.



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control). Statistical difference is indicated as p<0.05.

Fig 4. Number of scratching behaviors in the open field test.

In the open field test, no significant difference was found between the groups in terms of time spent in corners (p=0.306), center (p=0.306), standing up (p=0.435) and defecation (p=0.754) behaviors. However, the frequency of itching (p=0.010) and urination (p=0.014) was significantly higher in Group 2 than in the other groups (p<0.05). These results suggest that hair dye exposure may affect certain behavioral responses, increasing itching and urination behaviors in Group 2. The lack of difference in time spent in corners and centers and standing up and defecating behaviors suggests that this effect is not the same for all behavioral responses (Table 2).

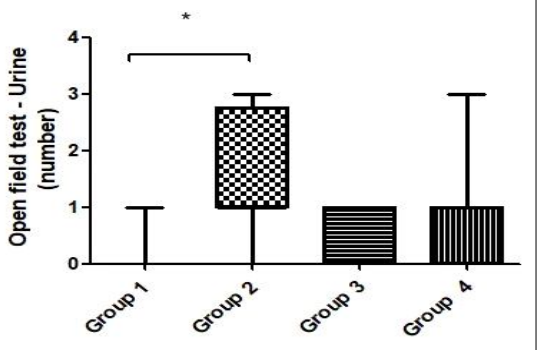
In the Three-chamber sociability test, there was no significant difference between the groups in scratching the empty and first occupied cages, but a significant difference was found in scratching the second occupied cage (p=0.022). Similarly, there was no difference in sniffing the empty and second occupied cages, but a significant

Table 1: Analyzing exposure to hair dye with new object recognition test.

	Group 1	Group 2	Group 3	Group 4	p[a]
Smelling the second object	2.5 (6-0)	0.5 (5-0)	0 (5-0)	5.5 (11-0)	0.093
Pawing at the second object	0 (1-0)	0 (0-0)	0 (0-0)	0 (1-0)	0.084
Discovering the first object (sec)	18.5 (65-7)	7 (87-1)	4 (15-0)	2.5 (11-1)	0.0110
Discovering the second object (sec)	37.5 (100-0)	0.5 (158-0)	0 (132-0)	0.59 (3.2-0)	0.466

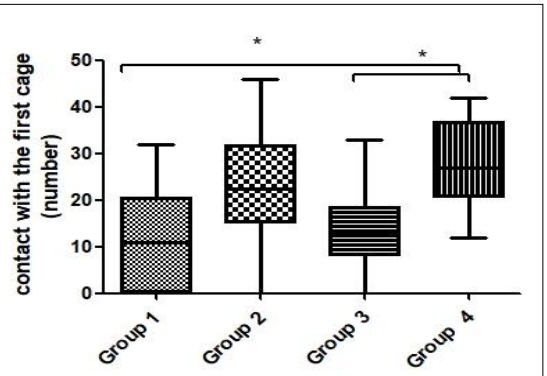
a: Kruskal-wallis test; Median (min-max).

difference was found in sniffing the first occupied cage (p=0.033). In Group 4, cage contact in the first region was significantly higher than in Groups 1 and 3 and second cage contact at the 15th minute was significantly higher than in the other groups (p<0.05). These results show that hair dye exposure may affect social interaction behaviors, especially creating significant differences in scratching the second occupied cage and sniffing the first occupied cage. (Fig 6, Table 3).



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control).

Fig 5: Number of urination behaviors in the open field test.



Group 1 (less frequently stained), Group 2 (moderately frequently stained), Group 3 (very frequently stained) and Group 4 (control). Statistical difference is indicated as p<0.05.

Fig 6: Number of first contacts with the cage in the Three-chamber sociability test.

In the elevated plus maze test, no significant difference was found between the groups in terms of the time spent in the open arm (p=0.428) and closed arm (p=0.428) (Group 1: Open 105.63±51.99 sec, Closed 194.38±51.99 sec; Group 2: Open 77.88±29.74 sec, Closed 222.13±29.74 sec; Group 3: Open 115.50±48.60 sec, Closed 184.50±48.60 sec; Group 4: Open 98.13±50.76 sec, Closed 201.88±50.76 sec). In addition, the ratio of closed arm entries to open arm entries did not show any significant difference between the groups. These results suggest that exposure to hair dye had no significant effect on the anxiety levels of the rats (Table 4).

Stress can negatively affect behavior (Kirbaþ *et al*., 2024) and increased dyeing frequency can increase stress levels. Hydrogen peroxide exposure has been observed to increase activity in rats (Bouayed and Soulimani, 2019). Aminophenols in hair dyes can complicate behavioral outcomes by causing allergic reactions (Beiki *et al*., 2018). The elevated plus maze test revealed that the control group (Group 4) interacted with the cages more frequently than the other groups (p<0.05), indicating a change in social behavior. Neurotoxic compounds in hair dyes are a concern for their long-term effects on social interaction and anxiety behaviors. This study showed that hair dye application in rats led to significant changes in novel object memory, locomotion, social interaction and anxiety-like behaviors. These findings are consistent with existing literature that emphasizes the effects of hair dye components with neurotoxic potential, especially PPD (Alajaji, 2024). Stress is a physical or emotional response of an individual to challenging situations (Orkun Erkılıç and Bayraktar, 2025c; 2025d). The lack of significant changes in certain behavioral measures may be due to adaptation, individual stress thresholds, or insufficient study duration. The endocrine

Table 2: Analyzing exposure to hair dye with open field test.

	Group 1	Group 2	Group 3	Group 4	p[a]
Corner (sec)	279 (288-237)	280 (295-239)	264.5 (297-234)	273.5 (300-234)	0.306
Centre (sec)	21 (63-12)	20 (61-5)	35.5 (66-3)	25 (66-0)	0.306
Standing up	22.5 (51-4)	15.5 (32-11)	19 (32-5)	14.5 (28-7)	0.435
Defecation	1 (3-0)	1 (5-0)	2 (7-0)	1 (5-0)	0.754

a: Kruskal-wallis test; Median (min-max).

Table 3: Analyzing exposure to hair dye with three-chamber sociability test.

	Group 1	Group 2	Group 3	Group 4	p[a]
Scratching the empty cage	0 (0-0)	0 (1-0)	0 (1-0)	0 (2-0)	0.460
Scratching the first full cage	0 (1-0)	1.5 (10-0)	0.5 (8-0)	0 (4-0)	0.155
Sniffing the empty cage	3 (13-0)	6 (15-0)	5 (12-0)	7 (12-0)	0.312
Sniffing the second filled cage	6.5 (24-0)	7 (14-0)	8 (22-0)	11 (15-1)	0.626

a: Kruskal-wallis test; Median (min-max).

Table 4: Analyzing exposure to hair dye with elevated plus maze test.

	Group 1	Group 2	Group 3	Group 4	p[b]
Open arm (sec)	105.63±51.994	77.88±29.739	115.50±48.603	98.13±50.758	0.428
Closed arm (sec)	194.38±51.994	222.13±29.739	184.50±48.603	201.88±50.758	0.428

b: Analysis of Variance; Mean±Standard deviation.

system regulates body functions with hormones and hair health is also under the control of hormones (Bayraktar, 2020). Hair dyes may contain harmful chemicals such as the endocrine disruptor resorcinol and neurotoxic lead acetate (Nwose *et al*., 2022). Future research should investigate the long-term effects of hair dye exposure, mechanisms of neurotoxicity and should be confirmed with human studies. Research on natural plant-based hair dyes is increasing with the search for safe and sustainable alternatives in the cosmetic industry (Cui *et al*., 2022). These innovations provide solutions to health problems while also supporting environmentally friendly practices.

CONCLUSION

In conclusion, this study provides significant evidence that hair dye compounds can markedly influence rat behavior, including object recognition, physical activity, social interactions and anxiety-related behaviors. The findings highlight the necessity of further investigation into the neurotoxic effects of hair dye exposure, particularly through assessments of oxidative stress and neurotoxicity. These findings emphasize the neurotoxic potential of specific hair color components and highlight the necessity for additional research to understand their implications for human health.

Disclaimers

The opinions and conclusions in this article belong to the authors and do not bind their institutions. Authors are responsible for the accuracy of the information, but are not responsible for any losses arising from its use.

Informed consent

The research experiment was approved by the local ethics committee for animal experimentation.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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