Effects of Lavender Oil on Predator Odor-Induced Anxiety in Rats

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The purpose of the present study was to explore the effects of a potential treatment for anxiety due to the need for easily obtainable and inexpensive options. The study tested the anxiolytic effects of lavender oil on rats experiencing severe anxiety induced by predator odor in 12 female rats. Prior to being tested, six control rats were exposed to diffused water, while six experimental rats were exposed to diffused lavender. Anxiety was induced in the same rats using bobcat urine and was observed in the open-field and elevated plus-maze tests using behavioral measures of anxiety. The anxiety responses in the open-field test were measured as rearing, defecating, climbing walls, freezing, grooming, and spending time near walls. The elevated plus-maze measured rearing, grooming, number of closed arm entries, and time spent in the open and closed arms. Administering lavender oil did not significantly reduce anxiety-like behavior in the open-field test, but in the elevated plus-maze, experimental rats spent significantly less time in the closed arms than did the control group. Further research should explore this potential for using essential oils as treatments.

Abbreviations:  ADAA – Anxiety and Depression Association of America; APA – American Psychiatric Association; CNS – Central Nervous System; HPA – Hypothalamic-Pituitary-Adrenal

Keywords: Essential Oil; Open-Field Test; Elevated Plus-Maze; Anxiolytic; Empirical Research; Animal Behavior

Introduction

More than 69.4 million adults in the US (approximately 20.9% - 28.8% of all US adults) suffer from anxiety disorders in a given year (APA, 2021). Children have a 25.1% chance of developing an anxiety disorder between the ages of 13 and 18 years old (ADAA, 2021).

Anxiety disorders are as expensive for the United States as they are pervasive. Pharmaceutical medications are effective, but can be very costly. It is estimated that the US spends over $42.2 billion on anxiety disorders each year (Greenberg et al., 1999). Unfortunately, over 63% of those suffering from anxiety go without receiving treatment (ADAA, 2021). Since many people with anxiety disorders are not receiving treatment, less expensive and more accessible alternative treatment options for alleviating mild and severe anxiety are a priority for investigation. Aromatherapy is a therapeutic technique that has been used for centuries to reduce the perception of anxiety, but it has yet to be widely considered as a viable treatment option for anxiety (University of Maryland Medical Center, 2015).

Anxiety

Anxiety is defined as a biological and psychological response to a life-threatening stimulus (Steimer, 2002). Research supports using laboratory rats to model certain human conditions, especially anxiety, and research has found that individual components of anxiety have genetic links in rats and in humans (Steimer, 2002). Coping strategies, fear conditioning, and anxiety disorders have all been linked to specific anxiety-related gene expressions in both humans and rats (Steimer, 2002). These genetic connections are consistent with human anxiety conditions (Steimer, 2011).
Furthermore, the central nervous system (CNS) of both humans and rats has similar fear responses within the brain (Goncharova, 2013). For both humans and rats, a stimulating stressor or a fear-inducing stimulus activates the amygdala, which signals the hypothalamus. The hypothalamus then communicates to the pituitary gland to send signals to the adrenal cortex to release hormones via the Hypothalamic-Pituitary-Adrenal (HPA) Axis. When startled, the CNS in humans reacts by triggering a response resulting in the adrenal glands producing cortisol. This response parallels that seen in rats, where the CNS stimulates the adrenal glands to release the hormone corticosterone (Goncharova, 2013). Both cortisol and corticosterone produce physiological stress responses including increased heart rate and/or increased blood pressure (Goncharova, 2013). In rats, these anxiety-like behaviors are measured using the classic techniques of the open-field and elevated plus-maze tests.

The open-field test is an empty square arena enclosed by tall walls. The open nature of the chamber naturally induces anxiety-like behavior in the rats (Walsh and Cummings, 1976). The rats are measured on several different anxiety-like behaviors. Rats that spend more time near the walls and not in the center are said to be expressing anxiety-like behaviors (Walsh and Cummings, 1976). Furthermore, rats that rear on their hind legs, defecate, freeze, groom, and climb the walls are also said to be expressing anxiety-like behaviors. If these anxiety-like measures are held consistent between control and experimental groups, the open-field test has the capability to measure and compare changing levels of anxiety in the rats. Using the open-field test to compare rats’ anxiety levels between manipulated variables (e.g. aromatherapy versus water) has been previously validated (Walsh and Cummings, 1976).

The elevated plus-maze consists of a four-arm platform raised in the air; two of the arms have no walls and the other two are enclosed by tall walls. The exposed nature of the open arms induces anxiety in the rats and the closed arms reduce behaviors of anxiety (Walf and Frye, 2007). Entering and spending time in the closed arms of the maze indicates the need for security to combat or reduce anxiety (Walf and Frye, 2007). The rat’s anxiety-like behavior can also be measured by other variables in the test, such as the number of times a rat rears on its hind legs and grooms (Walf and Frye, 2007).

Both the open-field test and the elevated plus-maze are considered ethologically valid and distinct methods of measuring anxiety-like behavior in rats. For example, the elevated plus-maze can measure the anxiety of rats as a result of their unconditioned response to heights (Walf and Frye, 2007). The open-field test measures anxiety connected to exploration and open spaces (Walsh and Cummings, 1976). According to Sudakov et al. (2013), multiple tests are needed to measure an individual rat’s anxiety level since limiting a study to one test may not sufficiently and validly measure anxiety in varying circumstances or environments.

Predator Odor
A study by Sutt et al. (2008) assessed how rats (never previously exposed to cat odor) physically and behaviorally responded to cat odor. Rats exposed to a cloth soaked with cat odor exhibited anxiety-like behaviors said to result from the HPA-Axis, such as freezing, reduced exploration of a maze test, and stretching on their bellies. The study also found that adding cat odor to a maze test increased activity in the amygdala of the rats (Sutt et al., 2008). However, the elevated zero-maze, an anxiety-inducing test similar to that of the open-field and elevated plus-maze tests, did not increase the activity of the amygdala to the same degree the cat odor did. This suggested that cat odor can produce more severe anxiety responses in rats than just a maze test alone, supporting the use of predator odor to induce severe anxiety (Sutt et al., 2008).

Research has used different methods of inducing anxiety in rats. In one study, rats were exposed to two different cat collars. One cat collar contained cat odor, and the other collar contained trimethylthiazoline (a chemical found in fox feces). The cat collar with the cat odor produced more anxiety-like behaviors than the collar with trimethylthiazoline. The cat odor collar also produced significantly higher activations of brain regions associated with
anxiety, including the amygdala (Staples et al., 2008).

Ganella and Kim (2014) similarly explored the use of cat urine to induce anxiety-like responses in juvenile, preadolescent, and adolescent rats. Taken together, these studies supported the use of predator odor to produce anxiety-like responses in rats. Furthermore, instead of using just cat urine, House et al. (2011) used a cloth soaked with bobcat urine to induce the same defensive behaviors. Both studies found that rats expressed innate anxiety-like responses when exposed to various types of predator odors. These anxiety-inducing studies provided the foundation for how the current study would induce severe anxiety in rats.

Aromatherapy

Aromatherapy is the process of exposing individuals to a plant-based oil (essential oil) made by collecting the plant's extracted fragrances. Essential oils can be administered in various ways: vaporized into the air by a diffuser for inhalation, swallowed in the form of a capsule or diluted liquid, or massaged into the skin. It has been established that essential oils can decrease anxiety, as reviewed below, but the extent of their effect on severe anxiety has not been thoroughly researched.

Previous studies have found that essential oils can have calming effects on people experiencing stress by diffusing it into the air for inhalation. Inhaling diffused lavender oil has been suggested to contribute to reduced anxiety levels and blood pressures in humans (Eguchi et al., 2016). In a study by Eguchi et al. (2016), Japanese men and women experienced significantly decreased anxiety levels and blood pressures after being exposed to aroma foot massages 12 times over the course of 8 weeks. Aroma foot massages were foot massages used in combination with aromatherapy to produce anxiety-reducing effects. Since the treatment combined aromatherapy with massage, either element could have been responsible for the reduction in anxiety, but the use of aromatherapy with foot massage did reduce anxiety when compared to a no-treatment condition. Other research studies using aromatherapy alone have found it can contribute to reduced anxiety symptoms. By simply inhaling lavender aroma, patients with moderate to severe anxiety during a bone marrow biopsy experienced significantly reduced anxiety compared to patients in the control group (Abbaszadeh et al., 2020).

Rats have also been used to model the anxiolytic effects of using essential oils. For example, in a study by Rombolà et al. (2017), rats exposed to bergamot (an essential oil derived from the bergamot citrus fruit) had decreased behavioral expressions of anxiety in the open-field and elevated plus-maze tests. Another research study concluded that rats expressed markedly lower anxiety-like behaviors in the open-field and elevated plus-maze tests after oral administration of Citrus paradisi (grapefruit) when exploring its anxiolytic uses in herbal medicine and aromatherapy (Mallick and Alam, 2016). The rats froze less, increased their exploration, and spent more time in the open arms of the elevated plus-maze (Mallick and Alam, 2016). Similarly, Hritcu et al. (2012) found that rats exhibited fewer anxiety-like responses in the elevated plus-maze test after inhaling diffused lavender essential oil via a diffuser. The present study used many of the procedures outlined by these previous studies, and these studies served as the foundation for how the present study administered essential oil in combination with the anxiety-inducing stimulus.

Present Study

The purpose of this study was to explore the anxiolytic effects of lavender oil with emphasis on its potential to decrease severe anxiety induced by a perceived danger in the form of predator odor. The effect of essential oils on severe anxiety induced by predator odor has not been researched and thus the current study addressed this gap in knowledge. This study’s purpose was significant because it sought to provide evidence for the viability of treating severe anxiety induced by perceived dangers with lavender essential oils, rather than pharmacological medications being the primary treatment option.

The previous literature described in this manuscript inspired the methods of the current study. Rats were used because they can provide an ethical model. The open-field and elevated
plus-maze tests induced anxiety and measured the rats’ anxiety-like behaviors. Both tests were used to verify the results, given their different methods of inducing and measuring anxiety. Bobcat urine was added to the tests to ensure the anxiety was severe and to measure the effects of essential oils on anxiety induced by predator odor. The essential lavender oil was administered via diffusion given the previous research indicating its effectiveness at reducing anxiety (Eguchi et al, 2016). This is also a more clinically relevant route of administration, as more people are likely to administer oils via this method compared to injection or oral routes. The researchers hypothesized that rats would express decreased anxiety-like behaviors in the open-field and elevated plus-maze tests after being pre-treated with diffused lavender oil despite predator odor-induced anxiety.

Materials and Methods

Participants

Twelve adult female Sprague Dawley rats (ages 3-6 months) were housed in 5 cages (46.5 cm length x 25.5 cm width x 21.5 cm height), with three rats in two cages and two rats in three cages. The rats were kept in a room that was maintained at 23.3 °C and kept on a 12 h-12 h light-dark schedule as done by Mallick and Alam (2016). The rat cages were all supplied with free access to rat chow pellets and water (replaced every morning), as described by Rombolà et al. (2017) and Mallick and Alam (2016). The rats were handled in accordance with ethical guidelines for using laboratory animals and studies were approved by the Whitworth University Institutional Animal Care and Use Committee. Precautions were also taken to reduce excessive stress and anxiety.

The use of rats in the proposed study limited the emotional risks of inducing anxiety in human participants. All rats selected were bred into a “Swim Low” generation. Swim Low, as described by Weiss et al. (1998), refers to their swimming tendencies in the Porsolt Swim test, a test designed to assess depression-related symptoms. Swim Low rats will float for an extended period of time instead of struggling to get out of the swim tank; this is seen as a model of depression-susceptibility. These rats have been well validated as a model of depression-relevant behaviors in a number of prior studies and also exhibit reliable baseline responses in the open-field test and elevated plus-maze (Weiss et al., 1998; West and Weiss, 1998; West et al., 1999; Gutman et al., 2008; Epps et al., 2013). Although the Swim Low rats are selectively bred as models of depression, they have also been characterized for anxiety and stress-related behaviors, such as hyperlocomotion in the open-field test, elevated plus-maze, and the light-dark box (Weiss et al., 1998; S. A. Epps, personal communication). The sample size of the rats chosen for the current study was based on subject availability and prior experience with these phenotypes in Swim Low rats. Six rats were in the control group and six in the experimental group via random assignment.

Lavender Essential Oil

The independent variable for this study was lavender essential oil obtained from Aura Cacia. The lavender oil was extracted from Lavandula angustifolia. The oil was administered via diffusion from an electric essential oil diffuser prior to testing. Six drops of the oil were mixed with 1 oz of water for the diffusion, per the instructions on the bottle of oil, and as similarly done with four drops by Hritcu et al. (2012). The rats in the experimental group were exposed to lavender oil that was diffused in a confined area over the course of 60 min before being tested, as was done by Hritcu et al. (2012). Control rats were similarly exposed to diffused water for 60 min before assessment.

Open-Field Test Responses

The open-field test induced and measured anxiety responses. The open-field test was a 91.44 cm wide x 91.44 cm long x 40.64 cm high chamber of clear Plexiglas, with a 15.24 cm x 15.24 cm square grid marked on the floor with tape. The anxiety responses were scored by two viewers, one recording the time spent in the center of the arena with a stopwatch and the other viewer recording the number of behavioral occurrences. The open-field was
wiped down with Lysol and paper towel after each test. Approximately 5 to 10 minutes passed between subsequent tests to ensure the field was dry and free of any scent.

Multiple anxiety-like behaviors, or behavioral expressions of fear in response to a perceived danger, were quantitatively recorded as dependent variables. While in the open-field test, the number of times the rats reared on their hind legs, groomed their bodies, climbed the walls of the open-field, defecated (feces only), or suddenly froze were recorded as integers as described by Walsh and Cummings (1976). The time spent near the walls and time spent away from the walls were recorded in seconds. Spending time near the walls was defined as the rats having all four paws within the squares directly adjacent to the walls of the arena.

**Elevated Plus-Maze Test Responses**

The elevated plus-maze was composed of four arms, each measuring 50.80 cm long x 10.16 cm wide. There were two open arms that had edges 1.27 cm high to prevent jumping and there were two closed arms with 30.48 cm high walls of black Plexiglas. The anxiety responses were scored by the same two viewers, one recording the time spent in the closed arms with a stopwatch and the other viewer recording the number of behavioral expressions. The maze was cleaned between tests in the same manner as the open-field.

For the elevated plus-maze, the number of times the rats reared on their hind legs, groomed their bodies, and entered the closed arms (labeled: “closed arm entries”) were all recorded as integers as described by Walf and Frye (2007). The time spent in the closed arms and time spent in the open arms were recorded in seconds. Spending time in the open arms was defined as the rats having all four paws outside of the confines of the closed arms.

As previously discussed, the open-field test and elevated plus-maze were treated as distinct methods for measuring anxiety-like behaviors. Therefore, the researchers did not use the tests to replicate each other, but to test the anxiety-like variables differently. The measurements that set the two tests apart are the time spent away from and near the walls in the open-field test, and the closed arm entries and the time spent in the open/closed arms in the elevated plus-maze. These are the variables that are typically measured for these two tests (O’Leary et al., 2013). Grooming and rearing were included with the elevated plus-maze to gain insight into whether behavioral variables in the elevated plus-maze would differ from the results of the open-field test.

**Predator Odor**

Both the control and the experimental groups entered the open-field and elevated plus-maze tests with bobcat urine-soaked gauze pads hanging above the arenas by tape. Bobcat urine contains 2-phenylethylamine, which triggers the olfactory system to produce severe anxiety-related behavior in rats (Ferrero et al., 2011). The bobcat urine was obtained online from The Pmart and it was applied to gauze pads as similarly done with cloth in the study by Sutt et al. (2008). The urine was added to five 5.08 cm x 5.08 cm gauze pads for the open-field test and to four gauze pads for the elevated plus-maze test. Fifteen drops of urine were added by pipette to each pad to properly saturate them and they were attached to pieces of tape that spanned the top of the open-field and elevated plus-maze tests in two directions. The amount of gauze pads and urine was chosen to ensure the predator odor could be smelled by the rats throughout the entire space of the two tests. One piece of tape ran along the middle of each test (north to south) and the other piece ran along the middle (east to west). The urine-soaked gauze pads were pinched inside the pieces of tape spanning the top of the tests in five different locations for the open-field test and four different locations for the elevated plus-maze. In the open-field test, gauze pads were hung in the center, right of the center, left of the center, above the center, and below the center. In the elevated plus-maze test, gauze pads were hung in the middle of all four arms. A gauze pad was not placed in the center of the test to prevent overcrowding of predator odor in the closed arms. All pads were placed in a 30.48 cm radius so that the odor would not be too close to the walls, thereby influencing the rats to avoid the walls of the open-field.
Procedure and Design

The rats were randomly assigned to a control group and an experimental group. Six rats in the control group were individually removed from their cages one-by-one during separate testing days until all rats were tested. When a control rat was selected, they were placed into an isolation chamber for 60 min in a separate room where water was diffused by an essential-oil diffuser. After 60 min, the researchers removed the rat from the chamber by hand and then placed it into the center of the open-field test where the five urine-soaked gauze pads hung above it. Each rat spent 20 min inside the open-field test while being assessed by researchers on their anxiety-like behaviors as described above and in the studies by Rombolà et al. (2017) and Mallick and Alam (2016). The researchers also recorded the amount of time, in seconds, the rats spent near the walls of the test (squares adjacent to the walls) versus away from the walls, as described by Rombolà et al. (2017). After 20 min expired, the researchers returned the control rat to their designated cage, cleaned the field with Lysol, allowed the field to dry for five to ten minutes, and prepared for the experimental group. The tape with the gauze pads was disposed of, and new tape with new predator odor-soaked gauze pads was placed on the open-field test for the experimental group.

Similar to the control group, each experimental rat was taken from its cage and placed in an isolation chamber in a separate room for diffusion. In contrast, the experimental group was treated with the lavender essential oil (from Aura Cacia) instead of water. Six drops of the lavender essential oil were placed in the diffuser with 1 oz of water for each experimental rat. During diffusion, each rat was exposed to the diffused oil for 60 min before being placed in the open-field test for 20 min and being measured as previously described. After the 20 min, the rats were removed from the test and placed back into their cage.

Six control rats were also tested in the elevated plus-maze using similar methods as previously described for the open-field test. After the 60 min of water diffusion, each rat was placed into the center of the test where four urine-soaked gauze pads hung above the test. The rats spent 10 min in the elevated plus-maze to be measured for anxiety-like behaviors as described above and in Rombolà et al. (2017). The amount of time, in seconds, the rats spent in the open arms of the test and the closed arms of the test were also recorded. After the 10 min in the test, each rat was returned to its cage. The same cleaning procedure and replacement of gauze pads was completed as described.

Six experimental rats were similarly tested after 60 min of diffusion of lavender oil. The rats were each placed into the center of the elevated plus-maze. The measurements were taken again for the experimental rats. After 10 min, the rats were returned to their cages.

The results were statistically analyzed as a between-groups design. The hypothesis assumed anxiety responses would occur significantly less on average in the experimental group as compared to the control group. The hypothesis was tested using the MANOVA procedure in the statistical analysis software SPSS. A Type I error rate of 0.05 (i.e., \( \alpha = 0.05 \)) was used for hypothesis testing.

Results

The present study hypothesized that rats would express decreased anxiety-like behaviors in the open-field and elevated plus-maze tests after being pre-treated with diffused lavender oil despite predator odor-induced anxiety.

Open-Field Test Results

The means and standard deviations of the anxiety-like behaviors for the control and experimental groups are shown in Table 1 for the open-field test. The table shows the means and standard deviations for the number of times the rats climbed the walls, reared on hind paws, defecated, froze, and groomed. In Table 2, the average time and standard deviations for time spent near or away from the walls are shown for the open-field test. The inferential results of the statistical analysis for the open-field test are shown in Table 3. No statistically significant differences were shown for any open-field test measure. The hypothesis that the experimental group would exhibit fewer instances of climbing walls, rearing, defecating, freezing, grooming,
and spending time near walls in the open-field test was not supported.

**Elevated Plus-Maze Test Results**

For the elevated plus-maze test, Table 4 shows the means and standard deviations of the average number of times the control and experimental rats reared, groomed, and entered the closed arms. There were no statistically significant differences in occurrences of rearing, grooming, or closed arm entries. The hypothesis that the experimental group would rear, groom, and enter the closed arms less often than the control group was not supported in the elevated plus-maze.

The average time and standard deviations for time spent in the closed or open arms of the elevated plus-maze are shown in Table 5. There was a statistically significant difference between the experimental and the control group for time spent in the closed arms of the test, $F(1, 10) = 6.091, p = 0.033, \eta^2_p = 0.379$. Table 6 shows the inferential results of the statistical analysis for the elevated plus-maze. The hypothesis that the experimental group would spend less time in the closed arms was supported.

**Table 1.** Open-field test scores of anxiety by behavioral occurrence in terms of averages and variability from sum occurrences (M(SD)).

<table>
<thead>
<tr>
<th>Anxiety-like behavioral occurrences</th>
<th>Control (n = 6)</th>
<th>Experimental (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climbing walls</td>
<td>79.2 (12.2)</td>
<td>80.2 (9.8)</td>
</tr>
<tr>
<td>Rearing</td>
<td>43.5 (19.9)</td>
<td>33.0 (18.9)</td>
</tr>
<tr>
<td>Defecating</td>
<td>0.0 (0.0)</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>Freezing</td>
<td>7.3 (5.3)</td>
<td>6.0 (4.5)</td>
</tr>
<tr>
<td>Grooming</td>
<td>16.8 (7.8)</td>
<td>9.5 (5.4)</td>
</tr>
</tbody>
</table>

**Table 2.** Open-field test scores of anxiety by time spent in terms of averages and variability from the total time (M(SD)).

<table>
<thead>
<tr>
<th>Anxiety-like measures of time (seconds)</th>
<th>Control (n = 6)</th>
<th>Experimental (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climbing walls</td>
<td>1085.0 (61.4)</td>
<td>1032.3 (84.1)</td>
</tr>
<tr>
<td>Time spent away from walls</td>
<td>115.0 (61.4)</td>
<td>167.7 (84.1)</td>
</tr>
</tbody>
</table>

**Table 3.** Inferential results of anxiety responses from Table 1 and Table 2 on open-field test.

<table>
<thead>
<tr>
<th>Anxiety measures</th>
<th>n</th>
<th>df numerator</th>
<th>df denominator</th>
<th>F</th>
<th>p-value</th>
<th>$\eta^2_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climbing walls</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.024</td>
<td>0.879</td>
<td>0.002</td>
</tr>
<tr>
<td>Rearing</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.917</td>
<td>0.361</td>
<td>0.084</td>
</tr>
<tr>
<td>Defecating</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>1.00</td>
<td>0.341</td>
<td>0.091</td>
</tr>
<tr>
<td>Freezing</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.243</td>
<td>0.571</td>
<td>0.033</td>
</tr>
<tr>
<td>Grooming</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>3.599</td>
<td>0.087</td>
<td>0.265</td>
</tr>
<tr>
<td>Time spent</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>1.536</td>
<td>0.244</td>
<td>0.133</td>
</tr>
</tbody>
</table>

**Table 4.** Elevated plus-maze scores of anxiety by behavioral occurrence in terms of averages and variability from sum occurrences (M(SD)).

<table>
<thead>
<tr>
<th>Anxiety-like behavioral occurrences</th>
<th>Control (n = 6)</th>
<th>Experimental (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climbing walls</td>
<td>27.0 (10.3)</td>
<td>29.7 (9.5)</td>
</tr>
<tr>
<td>Grooming</td>
<td>13.1 (1.8)</td>
<td>1.5 (1.4)</td>
</tr>
<tr>
<td>Closed arm entries</td>
<td>10.2 (5.0)</td>
<td>11.3 (3.3)</td>
</tr>
</tbody>
</table>

**Table 5.** Elevated plus-maze scores of anxiety by time spent in terms of averages and variability from the total time (M(SD)).

<table>
<thead>
<tr>
<th>Anxiety-like measures of time (seconds)</th>
<th>Control (n = 6)</th>
<th>Experimental (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time spent in closed arms</td>
<td>336.0 (51.9)</td>
<td>462.3 (51.5)</td>
</tr>
<tr>
<td>Time spent in open arms</td>
<td>64 (51.9)</td>
<td>137.7 (51.5)</td>
</tr>
</tbody>
</table>
Table 6. Inferential results of anxiety responses from Table 4 and Table 5 on elevated plus-maze.

<table>
<thead>
<tr>
<th>Anxiety measures</th>
<th>N</th>
<th>df between</th>
<th>df error</th>
<th>F</th>
<th>p-value</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.356</td>
<td>0.546</td>
<td>0.034</td>
</tr>
<tr>
<td>Grooming</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.034</td>
<td>0.858</td>
<td>0.003</td>
</tr>
<tr>
<td>Closed arm entries</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>0.229</td>
<td>0.642</td>
<td>0.022</td>
</tr>
<tr>
<td>Time spent in closed arms</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>6.091</td>
<td>0.033*</td>
<td>0.379</td>
</tr>
</tbody>
</table>

*p<0.05

Discussion

The purpose of this research study was to investigate the effects of lavender oil on severe anxiety in rats induced by bobcat odor. For the dependent variable of anxiety-like behaviors in the open-field test, the experimental group did not exhibit a significant difference in anxiety-like behaviors compared to the control group (as defined by climbing walls, rearing, defecating, grooming, and staying near the walls). For the dependent variables in the elevated plus-maze test, the experimental group did not exhibit significantly less anxiety-like behaviors (as defined by rearing, grooming, and closed arm entries). However, the time the experimental group spent in the closed arms was significantly lower than the control group. The findings from the open-field test did not support the hypothesis that diffused lavender oil significantly reduces anxiety-like behaviors in the experimental group as compared to the control group; however, the findings from the elevated plus-maze did support the hypothesis in terms of the experimental group’s decreased time spent in the closed arms of the test.

The findings of the present study are mostly inconsistent with previous research, which studied the anxiolytic effects of lavender oil on anxiety induced by the open-field and elevated plus-maze tests alone. It has been found previously that lavender oil has significant anxiolytic effects on rats in the elevated plus-maze and open-field test when anxiety was induced by the tests themselves. The studies by Rombolà et al. (2017), Mallick and Alam (2016), and Hritcu et al. (2012) found that treating rats with essential oil reduced behavioral responses to anxiety in both the elevated plus-maze and the open-field test. The present study found different anxiolytic-like results in both tests. It was found that administering diffused lavender oil did not significantly reduce the occurrence of anxiety-related behavior in the open-field test; however, grooming did have a p-value of 0.087, suggestive of a non-significant trend toward significance. For the elevated plus-maze test, one anxiety-like behavior measure was found to be statistically significant, which could indicate moderate support for the hypothesis. The time the experimental rats spent in the closed arms of the test was significantly less than that of the control rats, corroborating the results of previous research like Rombolà et al. (2017).

The present study treated the open-field test and the elevated plus-maze as unique tests for measuring different anxiety-like behaviors. It was intentional that the behaviors measured in the open-field test would not also be measured in the elevated plus-maze to avoid a duplicate study; however, grooming and rearing were measured in both to track potential differences between the two tests. While there was a possible trend toward decreased grooming by lavender-exposed rats in the open-field test, this was not replicated in the elevated plus-maze. Both the open-field and elevated plus-maze tests were relatively consistent, with the majority of behavioral measures of anxiety not producing statistical significance. Thus, without both tests of anxiety indicating any other significant differences in anxiety-like behavior, the overall hypothesis of the present study was not supported, with only moderate support for limited parameters of anxiety-like behavior.

The present study did have significant differences from the above studies referenced. For example, the present study induced anxiety with bobcat odor in addition to the tests’ natural anxiety-inducing factors, as similarly done with just bobcat odor in the study by House et al. (2011). The bobcat urine was used to produce a more severe anxiety than previous research to test the extent to which the lavender oil can reduce anxiety. The anxiety induced by bobcat urine may have been too intense and may have exceeded the “threshold” for the lavender oil to show any effect in the behavioral measurements.
Research from Sutt et al. (2008) and Staples et al. (2008) found significant anxiety-like responses from predator odor in their studies on rats in the open-field and elevated plus-maze tests. The findings from these two studies are consistent with the idea that the anxiety-inducing effects of predator odor were possibly too great for diffused lavender oil to overcome.

Additionally, the oil was administered via diffusion in the current study, as similarly done in the study by Hritcu et al. (2012), but was not administered orally or as an injection, such as in the research by Mallick and Alam (2016) and Rombolà et al. (2017) respectively. The sample size did not match the number of rats the other studies used, and the rats in this study were all bred into a Swim Low generation, a selectively bred line based on depression-related behaviors on the Porsolt Swim Test. These rats have genetic differences from standard laboratory rats due to years of selective breeding, and this may affect their responses to anxiety-inducing factors, including exacerbation of stress-related behaviors (Weiss et al., 1998; S. A. Epps, personal communication). Further research should address the differences between Swim Low and other rats in terms of anxiety-like responses to better understand how the results from Swim Low rats may differ from those of other studies. Additionally, more research is needed to understand why the rats’ behaviors in the elevated plus-maze produced different results than the open-field test.

Limitations

There were several limitations to the present study. The handling of rats before and after diffusion, the diffusion process, and the time of day could have contributed to the anxiety levels of the rats prior to entering the open-field and elevated plus-maze tests. The rats were picked up by hand and carried to the tests in a separate room. Some rats may have perceived the process of diffusion as a dangerous threat warranting an anxiety response. The level of anxiety prior to being tested may have varied since the rats were measured at varying times of day and levels of human cortisol and rat corticosterone are known to vary throughout the day based on diurnal cycles (Wust et al., 2000; Mohawk et al., 2007).

The range of times spread between 2:30 pm to 8:00 pm with rats tested earlier potentially having higher anxiety-like responses because they were tested earlier in the day. All of these potential limitations could have contributed to the rats having more or less anxiety prior to the test measurements. Thus, the measurements of anxiety in the open-field test and elevated plus-maze could have fluctuated too much to show the effects of lavender oil.

Additionally, the present study used only female rats and tested a small sample size. Biological sex differences and small sample sizes could have produced varying results in comparison to using a large sample size and both male and female rats, or only male rats. For examples, Mallick and Alam (2016) and Hritcu et al. (2012) used only male rats in their studies with 5 to 6 groups of 10 rats. The female rats in the current study may have exhibited a different perception of danger or altered anxiety responses than male rats, masking potential effects of lavender oil. In relation to humans, Wust et al. (2000) found that females have a similar increase in cortisol in the morning as compared to men; however, there is a significant delay in the decrease of cortisol for females, implying a sex difference in cortisol levels. Similarly, a study by Marcondes et al. (2001) discussed how female Wistar rats demonstrated anxiety-like behaviors differently throughout the estrous cycle, with rats in proestrus having lower anxiety levels than rats in diestrus when being tested in the elevated plus-maze. Thus, further research should investigate these differences in sex, phases of estrous cycles, and whether anxiety responses are altered as a result.

Another limitation was that two different diffuser types were used: one for diffusing water and one for diffusing lavender oil. The amount of diffusion could have varied between the two diffusers due to diffuser type, rate, and dispersion (bursts every thirty seconds for experimental versus continuous diffusion for the control) making the amount of exposure to each liquid different. Both groups of rats remained in the diffuser chamber with the diffused liquid for the full hour and thus were still exposed to the independent variable for the same amount of time; however, the concentration of diffused liquid at any given
time may have been less in the experimental group than in the control group.

**Future Research**

The hypothesis that rats would express decreased anxiety-like behaviors in the open-field and elevated plus-maze tests after being pre-treated with diffused lavender oil despite predator odor-induced anxiety was only partially supported by the data. However, given the significant results of previous research and the significant increase in the amount of time that the experimental rats spent in the open arms of the elevated plus-maze in the current study, further research should continue exploring the potential for treating anxiety with essential oils. Follow-up studies could address the variables of sex, estrous cycle, or varying levels of anxiety to validate the results of the research. It is also possible that there are other essential oils that have anxiolytic effects, such as bergamot and citrus paradisi.

The present study used predator odor in combination with the open-field test and the elevated plus-maze, which had not been done in previous research when measuring the efficacy of essential oils. Lavender oil may have anxiolytic effects, but these effects might not be enough to reduce anxiety induced by predator odor. This should be researched further with lavender and other essential oils, as measuring the extent of their anxiolytic effects could have numerous benefits for understanding their use as treatments for people with anxiety.

Finally, the present researchers propose that future research should study what brain mechanisms essential oils affect when reducing general anxiety in rats, so that the information could be applied to the efficacy of using essential oils to reduce severe anxiety in humans. If more information could be provided on the inner workings of anxiolytic effects, treatment options would have a more grounded basis for their pursuits.

**Conclusion**

The current study investigated the efficacy of using lavender essential oil to reduce predator odor-induced anxiety-like behaviors in rats. Bobcat urine was combined with the open-field test and elevated plus-maze to induce severe anxiety. Treating rats with lavender oil did not significantly decrease the occurrence of all anxiety-related behaviors in the open-field test and elevated plus-maze. The anxiety-like behavior of spending time in the closed arms of the elevated plus-maze was significantly reduced for the experimental group that received the lavender oil treatment. These results do not clearly support or discourage the use of lavender essential oil, but can provide inspiration to explore the benefits, drawbacks, and limits of essential oils. With more than 69.4 million people today experiencing the negative effects of anxiety, alternative treatment options need to be explored (APA, 2021). Anxiety disorders are widespread, debilitating, and expensive. There should be more treatments outside of medication that are inexpensive and easily obtainable by the public. Essential oils could be one such treatment and further research should investigate their anxiolytic effects. If essential oils can reduce severe anxiety states, then there is the potential that they could be used clinically, or as a means of treatment for people who cannot afford medication or who do not have access to healthcare. With children in the United States having a 25.1% chance of developing an anxiety disorder between the ages of 13 and 18 years old (ADAA, 2017), there is no time to waste in researching any potential treatments that can reduce that chance, and essential oils could be a solution to the problem.

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