Journal of Ethnopharmacology 215 (2018) 34–41

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Inhaled *Lavandula angustifolia* essential oil inhibits consolidation of contextual- but not tone-fear conditioning in rats

Laura Segismundo Coelho[a], Nelson Francisco Correa-Netto[a], Marcia Yuriko Masukawa[a], Ariadiny Caetano Lima[a], Samia Maluf[b], Alessandra Linardi[a], Jair Guilherme Santos-Junior[a,*]

[a] Department of Physiological Sciences, Santa Casa of São Paulo Medical School, São Paulo, SP, Brazil
[b] Samia Maluf Aromatherapy Institute, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Anxiety
Aromatherapy
Lavender
Memory

ABSTRACT

Although the current treatment for anxiety is effective, it promotes a number of adverse reactions and medical interactions. Inhaled essential oils have a prominent action on the central nervous system, with minimal systemic effects, primarily because of reduced systemic bioavailability. The effects of drugs on the consolidation of fear conditioning reflects its clinical efficacy in preventing a vicious cycle of anticipatory anxiety leading to fearful cognition and anxiety symptoms. In this study, we investigated the effects of inhaled *Lavandula angustifolia* essential oil on the consolidation of aversive memories and its influence on c-Fos expression. Adult male Wistar rats were subjected to a fear conditioning protocol. Immediately after the training session, the rats were exposed to vaporized water or essential oil (1%, 2.5% and 5% solutions) for 4 h. The next day, the rats underwent contextual- or tone-fear tests and 90 min after the test they were euthanized and their brains processed for c-Fos immunohistochemistry. In the contextual-fear test, essential oil at 2.5% and 5% (but not 1%) reduced the freezing response and its respective c-Fos expression in the ventral hippocampus and amygdala. In the tone-fear test, essential oil did not reduce the freezing response during tone presentation. However, rats that inhaled essential oil at 2.5% and 5% (but not 1%) showed decreased freezing in the three minutes after tone presentation, as well as reduced c-Fos expression in the prefrontal cortex and amygdala. These results show that the inhalation of *L. angustifolia* essential oil inhibited the consolidation of contextual- but not tone-fear conditioning and had an anxiolytic effect in a conditioned animal model of anxiety.

1. Introduction

According to the guidelines for anxiety disorders published by the World Federation of Societies of Biological Psychiatry (Bandelow et al., 2012) there is strong evidence for the clinical efficacy of selective serotonin reuptake inhibitors in the treatment of anxiety disorders. However, the therapeutic effects occur only after chronic treatment and, at the beginning of treatment, there are adverse effects, including an increase in anxiety, insomnia, nausea, sexual dysfunction, cardiac arrhythmia and arterial pressure (Nutt, 2000; Ravindran and Stein, 2010; Baldwin and Polkinghorn, 2005), as well as pharmacokinetic interactions with other drugs (Muscatello et al., 2012).

The c-Fos protooncogene has been particularly useful for examining anxiety and associated brain structures. Because c-Fos expression is quickly induced following activation (Sharp et al., 1993), c-Fos immunoreactivity has been extensively used to assess neuronal activation in animal models of anxiety (Duncan et al., 1996) and aversive memories (Radulovic et al., 1998). Furthermore, anxiolytic drugs have been shown to modulate c-Fos expression in various areas of the brain (Bechtholt et al., 2008).

Aromatherapy is a treatment that uses plant essential oils. The aromatic volatile molecules contained in the essential oil interact with receptors in the olfactory epithelium leading to stimulation of the central nervous system (Perry and Perry, 2010). This action explains the significant effects of aromatherapy on emotional responses, although there are few systemic effects because of the reduced systemic bioavailability of inhaled drugs (Bäckman et al., 2014; Forbes et al., 2011).

Lavender (*Lavandula angustifolia*) essential oil has a long history of use in emotional disorders (Cavanagh and Wilkinson, 2002) and its anxiolytic activity via the serotonergic system has been described in clinical (Kasper et al., 2015; Baldinger et al., 2014) and experimental (Takahashi et al., 2014; Chioca et al., 2013) studies. Linalool is the major pharmacologically active constituent involved in the anti-anxiety effect of lavender oil (Umezu et al., 2006; Souto-Maior et al., 2011;

* Correspondence to: Department of Physiological Science, Santa Casa of São Paulo Medical School, Rua Cesário Motta Junior, 61, Vila Buarque, São Paulo, SP 01221-020, Brazil.
E-mail address: guilherme.stos.jr@gmail.com (J.G. Santos-Junior).

https://doi.org/10.1016/j.jep.2017.12.038
Received 30 June 2017; Received in revised form 5 December 2017; Accepted 26 December 2017
Available online 27 December 2017
0378-8741/

Linck et al., 2010). However, Takahashi et al. (2011) suggested that a synergistic effect of linalyl acetate and linalool is essential for the whole oil to work as an inhaled anti-anxiety agent.

From a clinical perspective, the effects of drugs in the consolidation of fear conditioning can be interpreted as their clinical efficacy in preventing a vicious cycle of anticipatory anxiety leading to fearful cognition and anxiety symptoms in aversive situations (Inoue et al., 2011). Anxious individuals show increased arousal by cues signaling danger and are more likely to interpret emotionally ambiguous stimuli in a threat-related manner. These cognitive biases play a pivotal role in the maintenance and possibly the etiology of anxiety (MacLeod and Mathews, 2012). In this study, we investigated the effect of inhaled *L. angustifolia* essential oil in the consolidation of aversive memories and the corresponding changes in c-Fos expression.

2. Material and methods

2.1. Subjects

Male Wistar rats (N = 144, 12 weeks old, 180–210 g) from Animal House of the Department of Physiological Sciences were housed in standard cages (40 × 34 × 17 cm, n = 4–5 per cage) with a wood chip bedding at 20–22 °C and 50% humidity on a 12 h light/dark cycle, with lights on at 7:00 a.m. The rats had free access to rat chow pellets and tap water, except during the tests, and were acclimatized to the housing conditions for at least seven days prior to the beginning of the experiments. The animal experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/Santa Casa, protocol no. 04/15) and animal care was based on the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No.8023, revised 1978).

2.2. Behavioral procedures

All of the animal manipulations were done between 10:00 a.m. and 4:00 p.m. The conditioning procedure was done in an active avoidance apparatus (Ugo Basile, Italy). Each rat was individually confined in the black compartment of the apparatus. After 2 min, the conditioned stimulus (tone, 70 dB, 5 s) and, in the last second, an unconditioned stimulus (foot-shock, 1 mA, 1 s) were delivered. The tone-shock pairing was presented five times, 30 s apart, and the rat was removed from the apparatus 30 s after the last pairing. In the unconditioned groups, the unconditioned stimulus (foot shock) was not delivered.

One day after conditioning, the rats underwent a contextual or tone fear test. In the contextual fear test (Fig. 1), each rat was placed in the

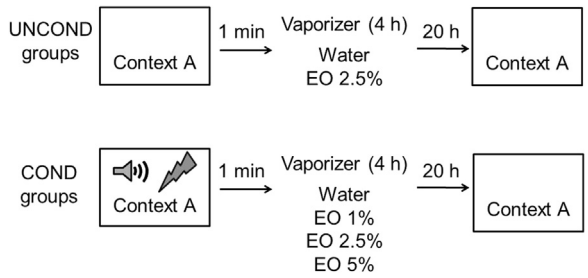

Fig. 1. The experimental design used for contextual fear conditioning. Top: /in the unconditioned groups (UNCOND), rats were confined in Context A for 5 min, without foot-shock (unconditioned stimulus). Thereafter, in another room, the rats were exposed to vaporized water or essential oil for 4 h. The next day, the rats were confined in Context A for 5 min to record freezing behavior. Bottom: In the conditioned groups (COND), the rats were confined in Context A and, after 2 min, a sequence of five unconditioned stimuli (foot-shocks, 1 mA, 1 s) were delivered 30 s apart. Thirty seconds after the last stimulus, the rats were removed from Context A and placed in another home where they were exposed to vaporized water or essential oil for 4 h. The next day, the rats were confined in Context A for 5 min to record freezing behavior.

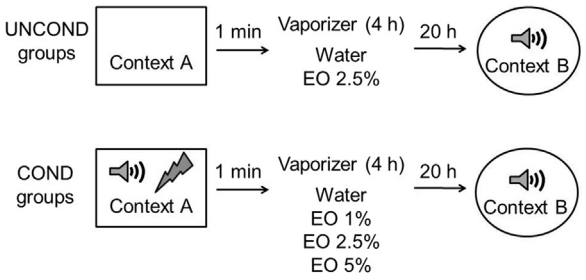

Fig. 2. The experimental design used for auditory fear conditioning. Top: In the unconditioned groups (UNCOND), rats were confined in Context A for 5 min, without foot-shock (unconditioned stimulus) or tone (conditioned stimulus). Subsequently, in another room, the rats were exposed to vaporized water or essential oil for 4 h. The next day, the rats were confined in Context B for 8 min. The freezing behavior was recorded during the first 3 min (before the tone), in the subsequent 3 min (under the tone – 70 dB, 4 s) and then in the last 2 min (after the tone). The tone was presented five times, 30 s apart. Bottom: In the conditioned groups (COND), rats were confined in Context A and, after 2 min, the conditioned stimulus (tone, 70 dB, 5 s) and, in the last second, an unconditioned stimulus (foot-shock, 1 mA, 1 s) were delivered. The tone-shock pairing was presented five times, 30 s apart. Thirty seconds after the last pair of stimuli, the rats were removed from Context A and placed in another home where they were exposed to vaporized water or essential oil for 4 h. The next day, rats were confined in Context B for 8 min and the freezing behavior was recorded during the first 3 min (before the tone), in the subsequent 3 min (under the tone – 70 dB, 4 s) and then in the last 2 min (after the tone). The tone was presented five times, 30 s apart.

conditioning environment, where it remained for 5 min. Unconditioned and conditioned stimuli were not delivered. The time spent in freezing behavior during the test was recorded. In the tone fear test (Fig. 2), each rat was placed in a cylindrical chamber (new environment), where it remained for 8 min. The chamber was placed in a room different from that used for conditioning so as to avoid spatial cues. The conditioned stimulus (tone), but not the unconditioned stimulus, was delivered 5 times at 30 s intervals, beginning at the end of the third minute. The time spent in freezing behavior during the test was recorded during the first 3 min (before the tone), in the subsequent 2 min (under the tone) and then in the last 3 min (after the tone). The first stage represents animals basal emotional level. The second represents fear related to emotional memory. The third represents the ability of the animals to return to the basal emotional level. Finally, the total time spent in freezing behavior during the 8 min of the test was used to determine the correlation between freezing and c-Fos expression.

2.3. Essential oil exposure and experimental groups

Lavandula angustifolia essential oil was provided by By Samia® (São Paulo, SP, Brazil), together with a report of its GC-MS analysis. The chromatographic analysis identified l-linalool (34.88%) and linalyl acetate (42.94%) as the main compounds.

The rats were exposed to vaporization for 4 h, a period sufficient for activation of the neurochemical processes involved in memory consolidation (Bourtchouladze et al., 1998; Igaz et al., 2002). The essential oil was diluted in water to final concentrations of 1%, 2.5% and 5% prior to placing the solution in the vaporizing chamber. These concentrations were chosen based on a previous study (Chioca et al., 2013). Control groups were exposed only to water vapor. The rats were exposed to the essential oil immediately after the conditioning procedure by placing them in a room (6 m^2) that had previously been saturated with essential oil by vaporization for 15 min.

Each experiment (contextual or tone fear) consisted of five experimental groups (N = 12 per group): UNCOND-Water (rats subjected to the conditioning session without shock delivery and then immediately exposed to water vapor), UNCOND-EO 2.5% (rats subjected to the conditioning session without shock delivery and then immediately exposed to essential oil at 2.5%), COND-Water (rats subjected to the conditioning session with both conditioned and unconditioned stimuli

and then immediately exposed to water vapor), COND-EO 1%, COND-EO 2.5% and COND-EO 5% (rats subjected to the conditioning session with both conditioned and unconditioned stimuli and then immediately exposed to essential oil at 1%, 2.5% and 5%, respectively).

2.4. Immunohistochemistry

Ninety minutes after the test, the rats were deeply anesthetized with an overdose of thionembutal (100 mg/kg, i.p.) and perfused transcardially with 300 ml of 0.1 M phosphate-buffered saline (PBS) followed by 300 ml of 4% paraformaldehyde. The brains were removed, stored in paraformaldehyde for 24 h and then kept in a 30% sucrose/PBS solution for 48 h. The brains were then frozen with dimethyl butane and stored at −80 °C. When required, serial coronal sections (30 μm) were cut with a freezing microtome and stored in anti-freezing solution for subsequent immunohistochemistry by free-floating staining.

A conventional avidin-biotin-immunoperoxidase technique was used in six rats per group. This number of rats is considered sufficient for immunohistochemical studies. The rat brains used in the histological analysis were randomly chosen from the 12 rats in each group. Free-floating sections were pre-treated with hydrogen peroxidase for 10 min followed by PBS for 30 min. Thereafter, sections were incubated overnight with a primary antibody (rabbit anti-c-Fos 1:5000, Cell Signaling, USA) in PBS-T solution (30 ml PBS, 30 μl Triton X-100). Subsequently, the sections were incubated for 2 h with a secondary antibody (goat anti-rabbit IgG, 1:600, Vector, USA) at room temperature. The sections were then treated with avidin-biotin complex for 2 h and then incubated with nickel-intensified DAB. The sections were rinsed in PBS (pH 7.4) between each step and were kept on a rotator between each incubation and rinse step. The sections were mounted on gelatin-coated slides, dried, dehydrated and cover-slipped. The nomenclature and nuclear boundaries were based on the atlas of Paxinos and Watson (2007) and the planes of the sections used for cell counting were matched as closely as possible to known landmarks. The encephalic regions considered in here were the prefrontal cortex [infralimbic (IL), prelimbic (PrL) and cingulated anterior (Cg1)], dorsal hippocampus [Cornus Ammonis 1 (dCA1), Cornus Ammonis 3 (dCA3), dentate gyrus (dDG)], ventral hippocampus (vCA1 and vDG) and amygdala [basolateral (BlA) and central (CeA) nucleus].

A Nikon Eclipse E600 microscope connected to a computer was used to capture images from each section. Micrographs were generated for PrL, IL and Cg1 (+3.00 to 3.70 AP), dDG, dCA1, dCA3, BlA and CeA (−2.00 to −3.00 AP), and (vCA3 and vDG −4.80 to −5.80 AP). Immunoreactive cells were counted bilaterally in four consecutive sections using the software package Image J (NIH Image, USA). The data were expressed as the density of cells (number of c-Fos labeled cells/mm^2), calculated by dividing the number of c-Fos-positive neurons by the total area of each region.

2.5. Statistical analysis

The results were expressed as the mean ± SEM. The behavioral and Fos results was analyzed by one-way ANOVA followed by Newman Keuls *post hoc* when necessary. The Pearson correlation test was used to examine the correlation between the behavioral results and c-Fos expression. The level of significance was set at $p < 0.05$.

3. Results

3.1. Inhaled essential oil of L. angustifolia inhibits the consolidation of contextual fear conditioning

One-way ANOVA detected a significant difference in the percentage of freezing among the experimental groups during the 5 min test [$F_{(5,65)} = 54.39$, $p < 0.01$]. As expected, COND-Water (rats subjected to shock during training and then exposed to vapor) showed significantly more freezing behavior then the UNCOND groups (rats not subjected to shock during training and then exposed to vapor or essential oil at 2.5%) (Fig. 3). This finding indicated that the protocol used here was able to induce contextual fear memory.

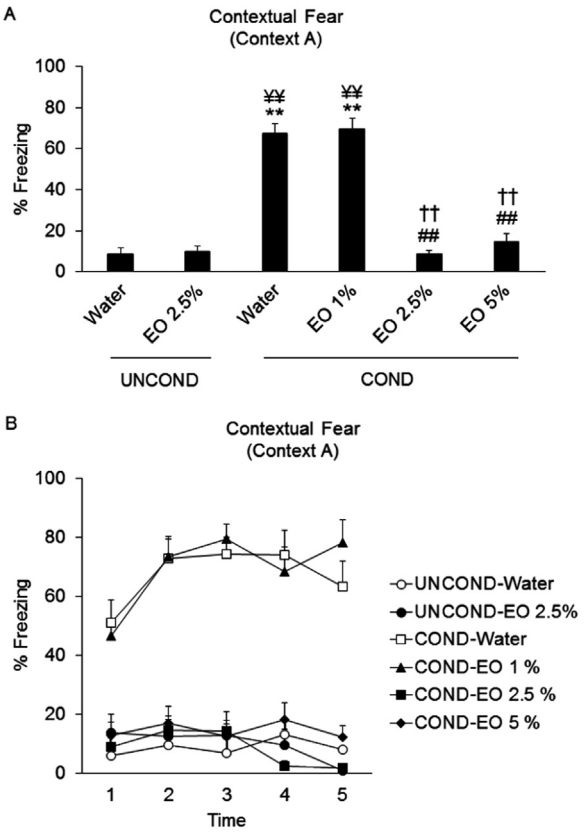

Fig. 3. Percentage of freezing behavior during the 5 min of the contextual fear test. The columns or points are the mean ± SEM (N = 12/group). $^{**}p < 0.01$ compared to UNCOND-Water. $^{¥¥}p < 0.01$ compared to UNCOND-EO 2.5%. $^{\#\#}p < 0.01$ compared to COND-Water. $^{††}p < 0.01$ compared to COND-EO 1% (N = 12/group).

Rats subjected to shock and exposed to essential oil at 2.5% and 5% (COND-EO 2.5% and COND-EO 5%, respectively) showed similar freezing when compared to the UNCOND groups, while the group exposed to essential oil at 1% (COND-EO 1%) did not differ from COND-Water group (Fig. 3). Retrieval of contextual fear memory in the COND-Water group increased c-Fos expression in the vDG [$F_{(5,29)} = 2.86$, $p < 0.05$], vCA3 [$F_{(5,29)} = 8.22$, $p < 0.01$], BlA [$F_{(5,29)} = 39.68$, $p < 0.01$] and CeA [$F_{(5,29)} = 5.63$, $p < 0.01$]. Interestingly, c-Fos expression in these nuclei in the COND groups exposed to essential oil at 2.5% and 5% (but not 1%) was similar to that in the UNCOND groups (Table 1 and Fig. 4).

There was significant correlation between the percentage of freezing behavior and c-Fos expression in the vDG ($r = 0.47$; $p < 0.01$), vCA3 ($r = 0.75$; $p < 0.01$), BlA ($r = 0.89$; $p < 0.01$) and CeA ($r = 0.57$; $p < 0.01$).

3.2. Inhaled essential oil of L. angustifolia did not change the consolidation of tone fear conditioning

One-way ANOVA revealed a significant difference among experimental groups in the freezing behavior prior tone exposure (pre-tone fear) [$F_{(5,65)} = 3.66$, $p < 0.01$]. the Newman-Keuls *post hoc* test showed that rats subjected to shock and then exposed to vapor or essential oil at 1% (COND-EO 1%) showed more freezing behavior than non-shocked rats (UNCOND groups) ($p < 0.05$). Rats that were subjected to shock and then exposed to essential oil at 2.5% and 5% (COND-EO 2.5% and COND-EO 5%, respectively) did not differ from the other experimental

Table 1
c-Fos immunoreactivity in rats subjected to contextual fear conditioning.

Nucleus	UNCOND- Water	UNCOND- EO 2.5%	COND- Water	COND-EO 1%	COND-EO 2.5%	COND-EO 5%	ANOVA
IL	129 ± 12	141 ± 11	148 ± 15	167 ± 5	129 ± 13	97 ± 14 $^{\#\dagger\dagger}$	$F_{(5,29)} = 3.61$; $p < 0.05$
PrL	151 ± 9	159 ± 8	168 ± 17	153 ± 3	143 ± 8	99 ± 13 $^{**\#\#¥¥\dagger\dagger\ddagger}$	$F_{(5,29)} = 5.43$; $p < 0.01$
Cg1	126 ± 18	117 ± 13	128 ± 17	137 ± 9	134 ± 8	101 ± 23	$F_{(5,29)} = 0.69$; $p = 0.63$
dDG	95 ± 14	142 ± 24	140 ± 12	145 ± 9	107 ± 14	88 ± 12	$F_{(5,29)} = 2.66$; $p < 0.05$
dCA1	254 ± 29	297 ± 29	368 ± 33	335 ± 14	297 ± 10	158 ± 57 $^{\#\#¥\dagger\dagger}$	$F_{(5,29)} = 4.38$; $p < 0.01$
dCA3	144 ± 24	183 ± 19	183 ± 53	197 ± 14	139 ± 19	115 ± 19	$F_{(5,29)} = 1.32$; $p = 0.28$
vDG	64 ± 8	82 ± 10	116 ± 10 *	106 ± 10 *	67 ± 10	90 ± 13	$F_{(5,29)} = 2.86$; $p < 0.05$
vCA3	115 ± 9	115 ± 4	212 ± 12 $^{**¥¥}$	170 ± 12 $^{*¥}$	120 ± 18 $^{\#\#}$	115 ± 16 $^{\#\#}$	$F_{(5,29)} = 8.22$; $p < 0.01$
BlA	68 ± 5	83 ± 7	130 ± 2 $^{**¥¥}$	141 ± 7 $^{**¥¥}$	65 ± 4 $^{\#\#\dagger\dagger}$	68 ± 2 $^{\#\#\dagger\dagger}$	$F_{(5,29)} = 39.68$; $p < 0.01$
CeA	95 ± 9	112 ± 13	161 ± 33 *	130 ± 5	60 ± 7 $^{*\#\#\dagger\dagger}$	86 ± 4 $^{\#\dagger}$	$F_{(5,29)} = 5.63$; $p < 0.01$

The data are expressed as the mean ± S.E.M. (n = 6/group) and represent the density of c-Fos immunoreactivity (number of c-Fos labeled cells/mm^2). $^{*}p < 0.05$ and $^{**}p < 0.01$ in relation to UNCOND-Water. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ in relation to COND-Water. $^{¥}p < 0.05$ and $^{¥¥}p < 0.01$ in relation to UNCOND-EO 2.5%. $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ in relation to COND-EO 1%. $^{\ddagger}p < 0.05$ and $^{\ddagger\ddagger}p < 0.01$ in relation to COND-EO 2.5%. COND – conditioned, EO – essential oil, UNCOND – unconditioned, IL – infralimbic cortex, PrL – prelimbic cortex, Cg1 – cingulated anterior cortex, dCA1 – dorsal Cornus Ammonis 1, dCA3 – dorsal Cornus Ammonis 3, dDG – dorsal dentate gyrus, vCA1 – ventral Cornus Ammonis 1, vDG – ventral dentate gyrus, BlA – basolateral nucleus of amygdala and CeA – central nucleus of amygdala.

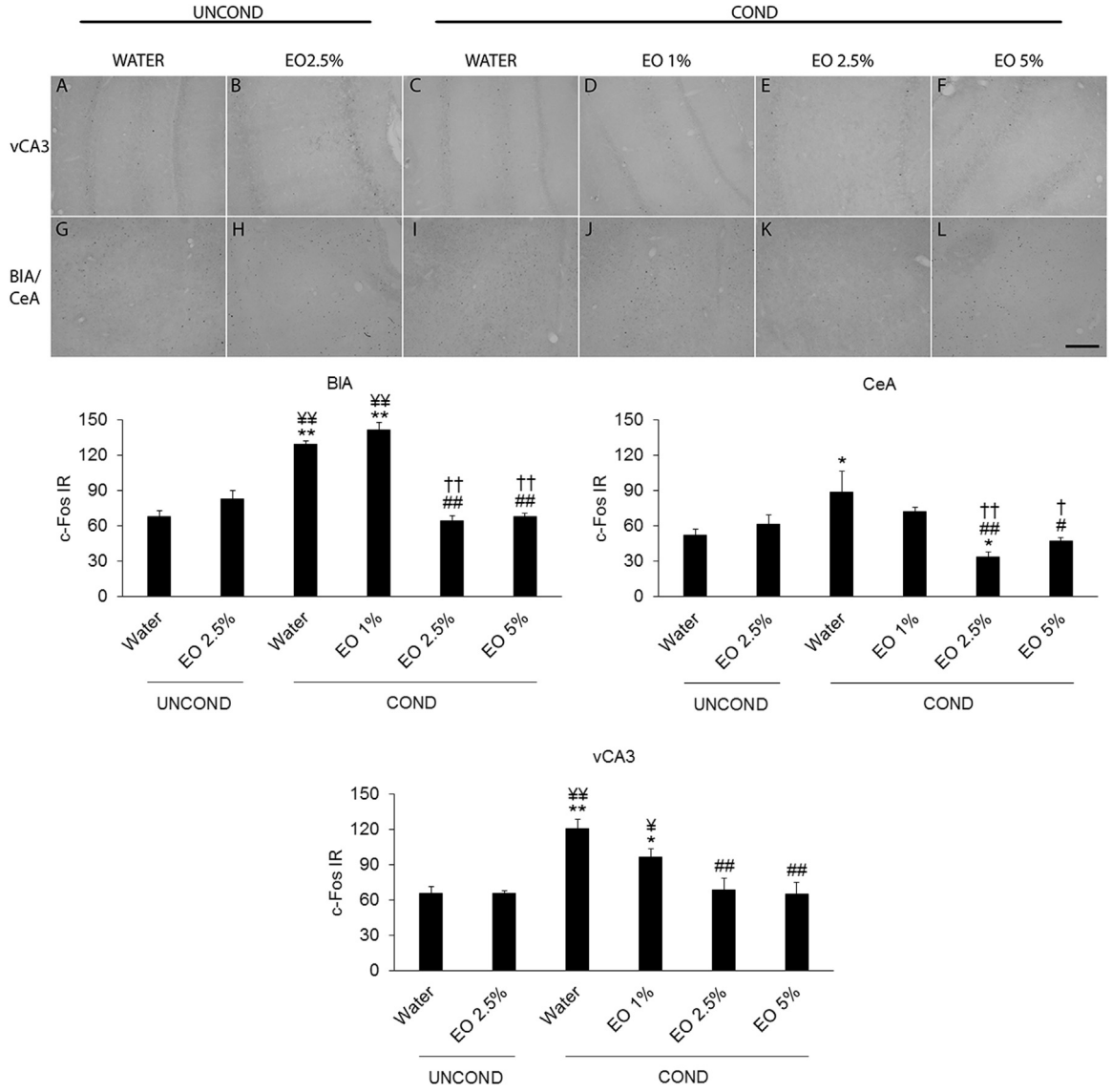

Fig. 4. Photomicrograph of c-Fos immunoreactivity in the encephalic nuclei of rats subjected to the contextual fear test. vCA3 = ventral Cornus Ammonis 3; BlA = basolateral nucleus of amygdala; CeA = central nucleus of amygdala. Scale bar = 250 μm. The graphs represent the density of c-Fos immunoreactivity (number of c-Fos labeled cells/mm^2) and the columns represent the mean ± SEM (N = 6/group). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to UNCOND-Water. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to COND-Water. $^{¥}p < 0.0$ and $^{¥¥}p < 0.01$ compared to UNCOND-EO 2.5%. $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ compared to COND-EO 1%. $^{\ddagger}p < 0.05$ and $^{\ddagger\ddagger}p < 0.01$ compared to COND-EO 2.5%.

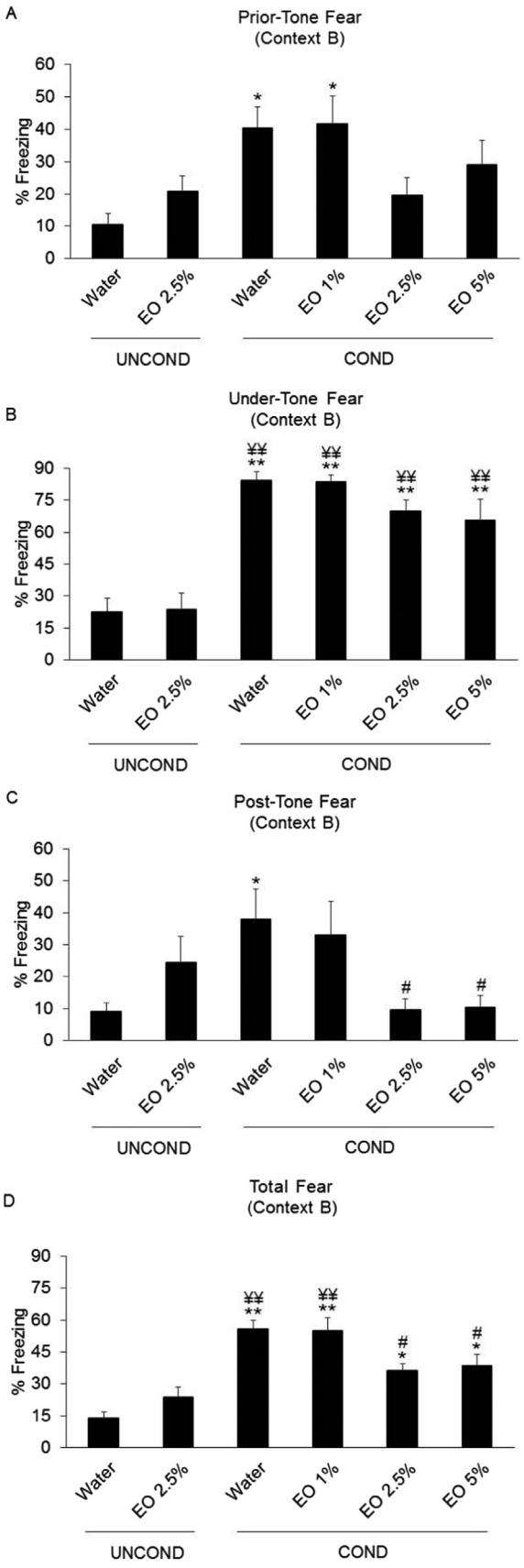

Fig. 5. Percentage of freezing behavior in the tone fear test. (A) Percentage of freezing behavior during the 3 min before the period of tone presentation. (B) Percentage of freezing behavior during the 3 min of tone presentation. (C) Percentage of freezing behavior during the 2 min after the period of tone presentation. (D) Percentage of freezing behavior during the 8 min of test. The columns represent the mean ± SEM (N = 12/group). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to UNCOND-Water. $^{¥¥}p < 0.01$ compared to UNCOND-EO 2.5%. $^{\#}p < 0.05$ compared to COND-Water.

groups (Fig. 5A).

In relation to freezing behavior under exposure to tone (tone fear), one-way ANOVA revealed a significant difference among experimental groups [$F_{(5,65)} = 16.68$, $p < 0.01$]. The Newman-Keuls *post hoc* test showed that rats subjected to shock and exposed to vapor (COND-Water) had more freezing behavior than non-shocked rats (UNCOND groups) ($p < 0.01$). Essential oil alone at the three concentrations tested (COND-EO 1%, COND-EO 2.5% and COND-EO 5%) did not affect the freezing response (Fig. 5B).

There was a significant difference among groups in relation to the percentage of freezing behavior post exposure to tone (post-tone fear) [$F_{(5,65)} = 3.46$, $p < 0.01$; one-way ANOVA]. The Newman-Keuls *post hoc* showed that rats subjected to shock and then exposed to vapor or essential oil at 1% (COND-Water and COND-EO 1%) had more freezing behavior than non-shocked rats ($p < 0.05$ and $p < 0.01$, respectively). Rats subjected to shock and then exposed to essential oil at 2.5% and 5% (COND-EO 2.5% and COND-EO 5%) showed a significant decrease in freezing behavior when compared to rats exposed to vapor alone (COND-Water) (Fig. 5C).

In relation to freezing behavior during the 8 min of test, one-way ANOVA revealed a significant difference among experimental groups [$F_{(5,65)} = 10.17$, $p < 0.001$]. The Newman-Keuls *post hoc* showed that rats subjected to shock and then exposed to vapor or essential oil at 1% (COND-Water and COND-EO 1%) had more freezing behavior than non-shocked rats ($p < 0.001$). The rats subjected to shock and then exposed to essential oil at 2.5% and 5% (COND-EO 2.5% and COND-EO 5%) showed a significant decrease in freezing behavior when compared to rats exposed to vapor alone (COND-Water) and exposed to essential oil at 1% (COND-EO 1%) ($p < 0.05$), although they also differed from non-shocked rats exposed to vapor ($p < 0.05$) (Fig. 5). The retrieval of tone fear memory in the COND-Water group increased c-Fos expression in the IL [$F_{(5,65)} = 4.33$, $p < 0.01$], PrL [$F_{(5,65)} = 7.47$, $p < 0.01$], BlA [$F_{(5,65)} = 12.72$, $p < 0.01$] and CeA [$F_{(5,65)} = 35.14$, $p < 0.01$]. Rats subjected to shock and then exposed to essential oil at 2.5% and 5% (COND-EO 2.5% and COND-EO 5%, respectively) showed a decrease in c-Fos in the BlA and CeA when compared to the corresponding vapor control group. The highest concentration of essential oil (COND-EO 5%) also decreased c-Fos expression in the IL and PrL when compared to the corresponding vapor control (Table 2 and Fig. 6).

There was a significant correlation between the percentage of freezing behavior during the total 8 min of the test and c-Fos expression in the IL ($r = 0.43$; $p < 0.01$), PrL ($r = 0.44$; $p < 0.01$) and BlA ($r = 0.59$; $p < 0.01$). Although there was a weak but significant correlation for CeA ($r = 0.28$; $p = 0.01$).

4. Discussion

The results of this study show that inhaled lavender essential oil inhibits the consolidation of contextual fear memory. Although the exposure to essential oil did not change the consolidation of tone fear memory, rats that underwent aromatherapy showed a reduction in the freezing response when compared to the vapor-treated group at the stage after tone presentation and during the 8 min of test. These behavioral results suggest an anxiolytic-like action of lavender essential oil. Interesting, those results were confirmed by the pattern of c-Fos expression in several limbic structures related to aversive memory and anxiety. Our results agree with previous clinical (Kasper et al., 2015; Baldinger et al., 2014) and experimental (Takahashi et al., 2014, 2011;

Table 2
c-Fos expression in rats subjected to tone fear conditioning.

Nucleus	UNCOND- Water	UNCOND- EO 2.5%	COND- Water	COND-EO 1%	COND-EO 2.5%	COND-EO 5%	ANOVA
IL	103 ± 12	94 ± 11	151 ± 7 $^{*¥}$	129 ± 12	134 ± 11	91 ± 14 $^{\#††}$	$F_{(5,29)} = 4.33$; $p < 0.01$
PrL	139 ± 14	108 ± 13	188 ± 10 $^{*¥¥}$	175 ± 11 $^{¥¥}$	154 ± 6 $^{¥}$	108 ± 15 $^{\#\#†††‡}$	$F_{(5,29)} = 7.47$; $p < 0.01$
Cg1	113 ± 10	83 ± 10	122 ± 6 $^{¥}$	156 ± 12 $^{*\#¥¥}$	98 ± 8 ††	81 ± 13 $^{\#††}$	$F_{(5,29)} = 7.72$; $p < 0.01$
dDG	107 ± 12	118 ± 17	142 ± 7	145 ± 12	135 ± 9	135 ± 14	$F_{(5,29)} = 1.22$; $p = 0.32$
dCA1	268 ± 24	278 ± 43	325 ± 33	541 ± 57 $^{**\#\#¥¥}$	268 ± 24	359 ± 24	$F_{(5,29)} = 7.42$; $p < 0.01$
dCA3	130 ± 10	120 ± 24	154 ± 14	188 ± 29	125 ± 5	135 ± 29	$F_{(5,29)} = 1.14$; $p = 0.36$
vDG	82 ± 10	75 ± 15	103 ± 5	93 ± 18	98 ± 5	103 ± 18	$F_{(5,29)} = 0.67$; $p = 0.64$
vCA3	101 ± 11	78 ± 9	138 ± 12 $^{¥}$	145 ± 4 $^{¥}$	120 ± 19	122 ± 7	$F_{(5,29)} = 4.14$; $p < 0.01$
BlA	61 ± 1	54 ± 6	116 ± 12 $^{**¥¥}$	112 ± 11 $^{**¥¥}$	64 ± 5 $^{\#\#††}$	68 ± 2 $^{\#\#††}$	$F_{(5,29)} = 12.72$; $p < 0.01$
CeA	68 ± 5	97 ± 7 **	126 ± 4 $^{**¥¥}$	99 ± 2 $^{**\#\#}$	53 ± 2 $^{\#\#¥¥††}$	53 ± 2 $^{\#\#¥¥††}$	$F_{(5,29)} = 35.14$; $p < 0.01$

The data are expressed as the mean ± S.E.M. (n = 6/group) and represent the number of c-Fos labeled cells per 2.5 × 10^3 μm^2. $^{*}p < 0.05$ and $^{**}p < 0.01$ in relation to UNCOND-Water. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ in relation to COND-Water. $^{¥}p < 0.05$ and $^{¥¥}p < 0.01$ in relation to UNCOND-EO 2.5%. $^{†}p < 0.05$ and $^{††}p < 0.01$ in relation to COND-EO 1%. $^{‡}p < 0.05$ and $^{‡‡}p < 0.01$ in relation to COND-EO 2.5%. COND – conditioned, EO – essential oil, UNCOND – unconditioned. IL – infralimbic cortex, PrL – prelimbic cortex, Cg1 – cingulated anterior cortex, dCA1 – dorsal Cornus Ammonis 1, dCA3 – dorsal Cornus Ammonis 3, dDG – dorsal dentate gyrus, vCA1 – ventral Cornus Ammonis 1, vDG – ventral dentate gyrus, BlA – basolateral nucleus of amygdala and CeA – central nucleus of amygdala.

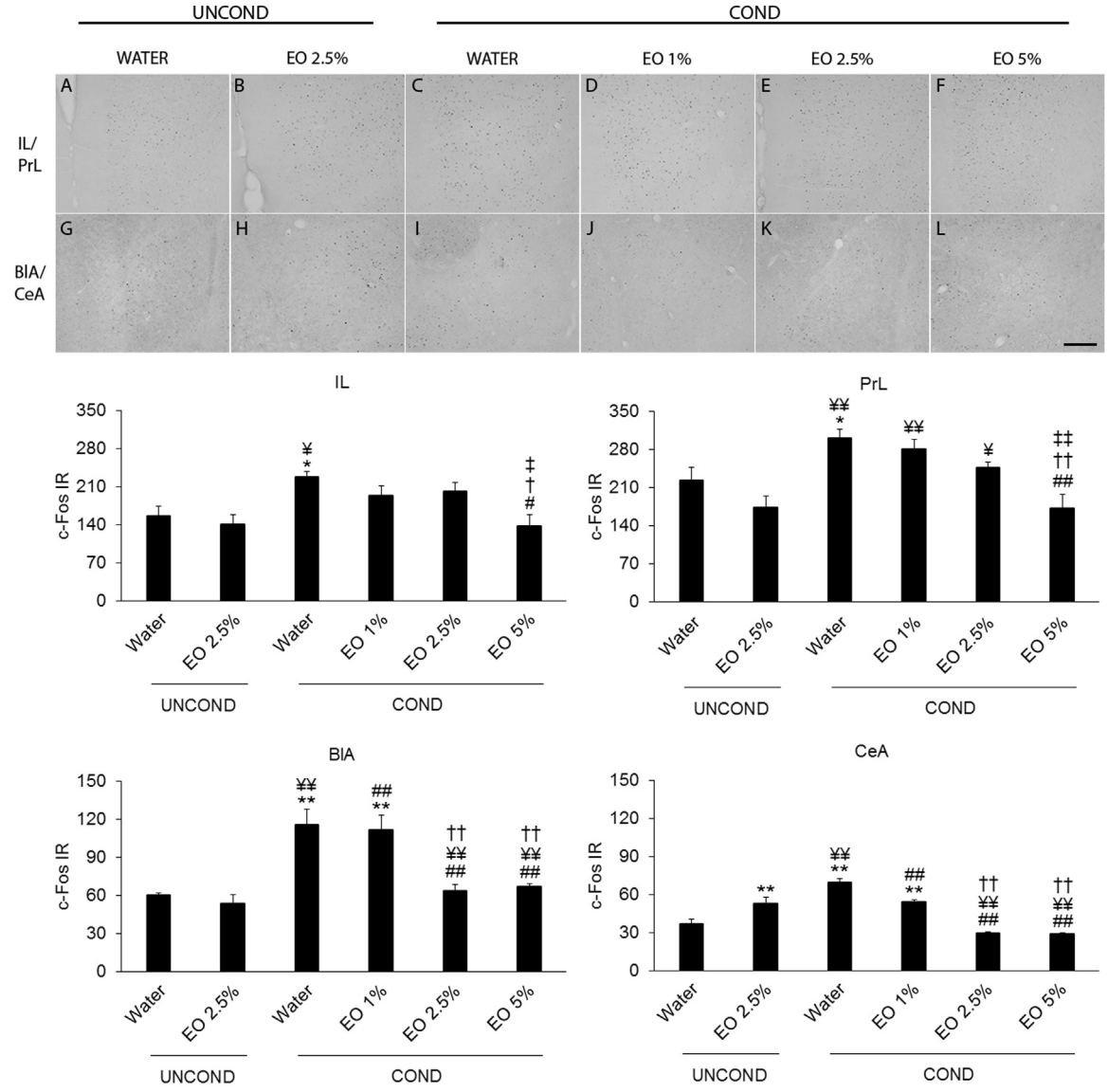

Fig. 6. Photomicrograph of c-Fos immunoreactivity in the encephalic nuclei of rats subjected to the tone fear test. IL = infralimbic cortex; PrL = prelimbic cortex; BlA = basolateral nucleus of amygdala; CeA = central nucleus of amygdala. Scale bar = 250 μm. The graphs represent the density of c-Fos immunoreactivity (number of c-Fos labeled cells/mm^2) and the columns represent the mean ± SEM (N = 6/group). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to UNCOND-Water. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to COND-Water. $^{¥}p < 0.05$ and $^{¥¥}p < 0.01$ compared to UNCOND-EO 2.5%. $^{†}p < 0.05$ and $^{††}p < 0.01$ compared to COND-EO 1%. $^{‡}p < 0.05$ and $^{‡‡}p < 0.01$ compared to COND-EO 2.5%.

Chioca et al., 2013; Umezu et al., 2006; Shaw et al., 2011) studies that reported an anxiolytic effect of lavender essential oil. Finally, Takahashi et al. (2014) showed that anxiolytic effect of inhaled lavender was similar in both normosmic and anosmic mice. Therefore, the anxiolytic effect of lavender depends on the interaction of its volatile compounds to the central nervous system targets.

Animal models of anxiety can be grouped into conditioned or unconditioned paradigms based on the behavioral response. The first paradigm involves conditioned responses to stressful stimuli while the second includes ethologically- based paradigms involving non-learned spontaneous reactions (Cryan and Sweeney, 2011; Steimer, 2011).

A study using the unconditioned paradigm showed that the anxiolytic effect of lavender essential oil was accompanied by an increase in striatal and hippocampal levels of serotonin and a decrease in the turnover rate in the elevated plus-maze test (Takahashi et al., 2014). Moreover, in the open field test, the anxiolytic effect reduced the expression of c-Fos expression in the hypothalamic nucleus and central amygdala (Shaw et al., 2011). Chioca et al. (2013) showed that pretreatment with the serotonin 5-HT_{1A} receptor antagonist WAY100635 inhibited the anxiolytic effect of lavender in the marble burying test, while a combination of ineffective doses of the 5-HT_{1A} receptor agonist 8-OH-DPAT with lavender essential oil reduced the number of marbles. The influence of the 5-HT_{1A} receptor on the anxiolytic effect of lavender essential oil was confirmed in a clinical study that reported a reduction in 5-HT_{1A} receptor binding in the temporal and fusiform gyrus, hippocampus, insula and cingulated cortex following the oral intake of Silexan® (lavender essential oil) compared with placebo (Baldinger et al., 2014).

Important advances in the neurobiology of anxiety have come from unconditioned animal models. However, the predictive value of these models, such as the plus-maze and light-dark transition tests or stress-induced hyperthermia, appears to be limited to benzodiazepine-related drugs (Shaw et al., 2011). In addition, cognitive biases play a pivotal role in the maintenance, and possibly etiology, of anxiety (MacLeod and Mathews, 2012). In view of these two important issues and the participation of the serotoninergic system in the anxiolytic effects of lavender essential oil, in this study we investigated the effects of inhaled lavender essential oil on the conditioned fear paradigm.

Conditioned fear is based on Pavlovian conditioning in that a neutral stimulus (context or tone) is presented together with an aversive stimulus (foot shock). With repeated pairing of the neutral stimulus per se elicited the expression of conditioned fear. Interestingly, the efficiency of drugs in affecting the consolidation of fear conditioning could be viewed as an indicator of their clinical efficacy in preventing a vicious cycle of anticipatory anxiety (Inoue et al., 2011). As shown here, inhaled lavender essential oil impaired contextual but not tone fear conditioning. Although the relationship between the serotonergic system and fear conditioning is not straightforward, in a recent review Bauer (2015) suggested that 5-HT_{1A} agonists are anxiolytic and impair both cued- and contextual-fear conditioning (Bauer, 2015). However, Avanzi et al. (2003) found no systemic effect of a 5-HT_{1A} receptor agonist on cue-elicited freezing (Avanzi et al., 2003). Consequently, it is possible that the effects of inhaled lavender essential oil seen here were partly attributable to the well-established 5-HT_{1A} agonist properties of lavender. Although no effect was seen in tone fear conditioning under the stage of tone presentation, the groups treated with essential oil (2.5% and 5%) had a lower freezing response when compared to the vapor group in the stage after tone presentation. This decreased freezing behavior suggested that treated rats were less anxious than non-treated animals and they are more resilient to return to their emotional basal level.

Although the role of 5-HT1A receptor is well established in the anxiolytic effects of lavender, there are other pharmacological mechanisms which could explain the results obtained in the present study. Lopez et al. (2017) showed that lavender essential oil displaced ^{3}H-citalopram from binding to the serotonin reuptake transport (SERT) in rat cortex suspension, in a dose dependent manner. This effect is also detected for linalool, but not for linalyl acetate. Interesting, selective serotonin reuptake inhibitors (SSRIs) are the first line treatment for anxiety disorders (Reinhold et al., 2011). Moreover, a review article suggested that SSRIs impair contextual fear, but enhance tone fear (Burghardt and Bauer, 2013).

Besides serotoninergic system, NMDA signaling is also involved in anxiety and learning and memory (Nandhra et al., 2013; Amaral and Roesler, 2008). Lavender essential oil (Lopez et al., 2017), linalool (Lopez et al., 2017; Elisabetsky et al., 1995, 1999) and linalyl acetate (Lopez et al., 2017) interact with glutamatergic NMDA receptor. Therefore, the results of the present study could be explained as a pharmacological antagonism of NMDA receptors. However, there is evidence showing that subcutaneous injection of MK-801, an antagonist of NMDA receptor, did not impair memory consolidation in both tone and contextual fear paradigm (Gould et al., 2002).c-Fos immunoreactivity has been extensively used as a tool to assess neuronal activation in animal models of anxiety (Duncan et al., 1996) and aversive memories (Radulovic et al., 1998). Two studies have evaluated the anxiolytic effect of inhaled essential oil and changes in c-Fos expression in unconditioned animal models of anxiety (Shaw et al., 2011; Saiyudthong et al., 2015). In the first study, the anxiolytic effect of vetiver essential oil in the elevated plus-maze test was accompanied by a decrease in c-Fos expression in the central nucleus of the amygdala (Shaw et al., 2011). In the other study, the anxiolytic effects of lavender essential oil in the open field test was associated with a decrease in c-Fos expression in the paraventricular and dorsomedial nuclei of the hypothalamus (Saiyudthong et al., 2015). As shown here, contextual fear conditioning increased c-Fos expression in the amygdala (basolateral and central nuclei) and in the ventral hippocampus (dentate gyrus and CA3). These c-Fos changes were abolished by inhaled lavender essential oil. In relation to tone fear conditioning, there was increased c-Fos expression in the prefrontal cortex (infralimbic and prelimbic portions) and in the amygdala (basolateral and central nuclei). Again, inhaled lavender essential oil inhibited these c-Fos changes. Interestingly, a triad of encephalic nuclei, including the prelimbic portion of the prefrontal cortex, ventral hippocampus and amygdala, represents a major brain circuit involved in the expression of fear conditioning (Sierra-Mercado et al., 2011; Bolles, 1970).

In the present investigation, the rats were exposed to essential oil immediately after the training session, i.e., one day before the test. Hence, it is unlikely that the pattern of c-Fos expression seen in the lavender groups was attributable to the presence of volatile compounds in the central nervous system during the test session. Since inhaled lavender essential oil abolished freezing behavior in the test session of contextual fear conditioning, we suggest that lavender blocks the consolidation of contextual aversive memory. As a result, there was no aversive information to retrieve, which explains the pattern of c-Fos expression seen in the corresponding experiment. Although lavender did not inhibit the consolidation of tone fear memory, it significantly reduced the freezing behavior throughout the test. Freezing behavior is a rodent's natural defensive reaction to fear-eliciting stimuli (Bolles, 1970) and results from a complex interaction among the prefrontal cortex, amygdala and periaqueductal gray (Giustino and Maren, 2015; Jhou, 2005; Tovote et al., 2016; Canteras et al., 2010). Based on our findings, we suggest that the mice exposed to lavender essential oil were less anxious during the test session, which would explain the patterns of c-Fos expression seen in the tone fear conditioning experiment.

Overall, the results of this study indicate that inhaled lavender essential oil exerts an anxiolytic effect in a conditioned animal model of anxiety, a finding in agreement with previous evidence showing an anxiolytic effect of lavender in unconditioned animal models of anxiety. These data suggest that aromatherapy with lavender essential oil could be a clinically effective way of preventing the vicious cycle of anticipatory anxiety that leads to fearful cognition and the symptoms of

anxiety in aversive situations.

Acknowledgements

L.S.C. and N.F.C-N. were CAPES PhD fellowship and M.Y.M. was CAPES master fellowship.

References

Amaral, O.B., Roesler, R., 2008. Targeting the NMDA receptor for fear-related disorders. Recent Pat. CNS Drug Discov. 3, 166–178.

Avanzi, V., Silva, R.C., Macedo, C.E., Brandão, M.L., 2003. 5-HT mechanisms of median raphe nucleus in the conditioned freezing caused by light/foot-shock association. Physiol. Behav. 78, 471–477.

Bäckman, P., Adelmann, H., Petersson, G., Jones, C.B., 2014. Advances in inhaled technologies: understanding the therapeutic challenge, predicting clinical performance, and designing the optimal inhaled product. Clin. Pharmacol. Ther. http://dx.doi.org/10.1038/clpt.2014.27.

Baldinger, P., Höflich, A.S., Mitterhauser, M., Hahn, A., Rami-Mark, C., Spies, M., Wadsak, W., Lanzenberger, R., Kasper, S., 2014. Effects of Silexan on the serotonin-1A receptor and microstructure of the human brain: a randomized, placebo-controlled, double-blind, cross-over study with molecular and structural neuroimaging. Int. J. Neuropsychopharmacol. http://dx.doi.org/10.1093/ijnp/pyu063.

Baldwin, D.S., Polkinghorn, C., 2005. Evidence-based pharmacotherapy of generalized anxiety disorder. Int. J. Neuropsychopharmacol. 293–302. http://dx.doi.org/10.1017/S1461145704004870.

Bandelow, B., Sher, L., Bunevicius, R., Hollander, E., Kasper, S., Zohar, J., Möller, H.J., 2012. Guidelines for the pharmacological treatment of anxiety disorders, obsessive-compulsive disorder and posttraumatic stress disorder in primary care. Int. J. Psychiatry Clin. Pract. http://dx.doi.org/10.3109/13651501.2012.667114.

Bauer, E.P., 2015. Serotonin in fear conditioning processes. Behav. Brain Res. http://dx.doi.org/10.1016/j.bbr.2014.07.028.

Bechtholt, A.J., Valentino, R.J., Lucki, I., 2008. Overlapping and distinct brain regions associated with the anxiolytic effects of chlordiazepoxide and chronic fluoxetine. Neuropsychopharmacology. http://dx.doi.org/10.1038/sj.npp.1301616.

Bolles, R.C., 1970. Species-specific defensive reactions and avoidance learning. Psychol. Rev. 71, 32–48.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., Kandel, E.R., 1998. Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn Mem. 5, 365–374.

Burghardt, N.S., Bauer, E.P., 2013. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: implications for underlying fear circuits. Neuroscience. http://dx.doi.org/10.1016/j.neuroscience.2013.05.050.

Canteras, N.S., Resstel, L.B., Bertoglio, L.J., Carobrez, A., de, P., Guimarães, F.S., 2010. Neuroanatomy of anxiety. Curr. Top. Behav. Neurosci. 2, 77–96.

Cavanagh, H.M., Wilkinson, J.M., 2002. Biological activities of lavender essential oil. Phytother. Res. http://dx.doi.org/10.1002/ptr.1103.

Chioca, L.R., Ferro, M.M., Baretta, I.P., Oliveira, S.M., Silva, C.R., Ferreira, J., Losso, E.M., Andreatini, R., 2013. Anxiolytic-like effect of lavender essential oil inhalation in mice: participation of serotonergic but not GABAA/benzodiazepine neurotransmission. J. Ethnopharmacol. http://dx.doi.org/10.1016/j.jep.2013.03.028.

Cryan, J.F., Sweeney, F.F., 2011. The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br. J. Pharmacol. http://dx.doi.org/10.1111/j.1476-5381.2011.01362.x.

Duncan, G.E., Knapp, D.J., Breese, G.R., 1996. Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res. 〈http://dx.doi.org/10/1016/0006-8993(95)01486-1〉.

Elisabetsky, E., Marschner, J., Souza, D.O., 1995. Effects of linalool on glutamatergic system in the rat cerebral cortex. Neurochem Res. http://dx.doi.org/10.1007/BF00973103.

Elisabetsky, E., Brun, L.F., Souza, D.O., 1999. Anticonvulsant properties of linalool in glutamate-related seizure models. Phytomedicine. http://dx.doi.org/10.1016/S0944-7113(99)80044-0.

Forbes, B., Asgharian, B., Dailey, L.A., Ferguson, D., Gerde, P., Gumbleton, M., Gustavsson, L., Hardy, C., Hassall, D., Jones, R., Lock, R., Maas, J., McGovern, T., Pitcairn, G.R., Somers, G., Wolff, R.K., 2011. Challenges in inhaled product development and opportunities for open innovation. Adv. Drug Deliv. Rev. http://dx.doi.org/10.1016/j.addr.2010.11.004.

Giustino, T.F., Maren, S., 2015. The role of the medial prefrontal cortex in the conditioning and extinction of fear. Front. Behav. Neurosci. http://dx.doi.org/10.3389/fnbeh.2015.00298.

Gould, T.J., McCarthy, M.M., Keith, R.A., 2002. MK-801 disrupts acquisition of contextual fear conditioning but enhances memory consolidation of cued fear conditioning. Behav. Pharmacol. 13, 287–294.

Igaz, L.M., Vianna, M.R., Medina, J.H., Izquierdo, I., 2002. Two time periods of hippocampal mrna synthesis are required for memory consolidation of fear-motivated learning. J. Neurosci. 22, 6781–6789.

Inoue, T., Kitaichi, Y., Koyama, T., 2011. SSRIs and conditioned fear. Prog. Neuropsychopharmacol. Biol. Psychiatry. http://dx.doi.org/10.1016/j.pnpbp.2011.09.002.

Jhou, T., 2005. Neural mechanisms of freezing and passive aversive behaviors. J. Comp. Neurol. 493, 111–114.

Kasper, S., Anghelescu, I., Dienel, A., 2015. Effficacy of orally administered Silexan in patients with anxiety-related restlessness and disturbed sleep – a randomized, placebo-controlled trial. Eur. Neuropsychopharmacol. http://dx.doi.org/10.1016/j.euroneuro.2015.07.024.

Linck, V.M., da Silva, A.L., Figueiró, M., Caramão, E.B., Moreno, P.R., Elisabetsky, E., 2010. Effects of inhaled linalool in anxiety, social interaction and aggressive behavior in mice. Phytomedicine. http://dx.doi.org/10.1016/j.phymed.2009.10.002.

Lopez, V., Nielsen, B., Solas, M., Ramirez, M.J., Jager, A.K., 2017. Exploring pharmacological mechanisms of lavender (Lavandula angustifolia) essential oil on central nervous system targets. Front. Pharmacol. http://dx.doi.org/10.3389/fphar.2017.00280.

MacLeod, C., Mathews, A., 2012. Cognitive bias modification approaches to anxiety. Annu Rev. Clin. Psychol. http://dx.doi.org/10.1146/annurev-clinpsy-032511-143052.

Muscatello, M.R., Spina, E., Bandelow, B., Baldwin, D.S., 2012. Clinically relevant drug interactions in anxiety disorders. Hum. Psychopharmacol. http://dx.doi.org/10.1002/hup.2217.

Nandhra, H.S., Murphy, C.L., Sule, A., 2013. Novel pharmacological agents targeting memory and cognition in the treatment of anxiety disorders. Hum. Psychopharmacol. 28, 538–543.

Nutt, D., 2000. Treatment of depression and concomitant anxiety. Eur. Neuropsychopharmacol. 〈http://dx.doi.org/10;1016/S0924-977X(00)00110-3〉.

Perry, N., Perry, E., 2010. Aromatherapy in the management of psychiatric disorders: clinical and neuropharmacological perspectives. CNS Drugs 20, 257–280.

Radulovic, J., Kammermeier, J., Spiess, J., 1998. Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. J. Neurosci. 18, 7452–7461.

Ravindran, L.N., Stein, M.B., 2010. The pharmacologic treatment of anxiety disorders: a review of progress. J. Clin. Psychiatry. http://dx.doi.org/10.4088/JCP.10r06218blu.

Reinhold, J.A., Mandos, L.A., Rickels, K., Lohoff, F.W., 2011. Pharmacological treatment of generalized anxiety disorder. Expert Opin. Pharmacother. http://dx.doi.org/10.1517/14656566.2011.618496.

Saiyudthong, S., Pongmayteegul, S., Marsden, C.A., Phansuwan-Pujito, P., 2015. Anxiety-lie behavior and c-fos expression in rats that inhaled vetiver essential oil. Nat. Prod. Res. http://dx.doi.org/10.1080/14786419.2014.992342.

Sharp, F.R., Sagar, S.M., Swanson, R.A., 1993. Metabolic mapping with cellular resolution: c-fos vs. 2-deoxyglucose. Crit. Rev. Neurobiol. 7, 205–228.

Shaw, D., Norwood, K., Leslie, J.C., 2011. Chlordiazepoxide and lavender oil alter unconditioned anxiety-induced c-fos expression in the rat brain. Behav. Brain Res. http://dx.doi.org/10.1016/j.bbr.2011.05.014.

Sierra-Mercado, D., Padilla-Coreano, N., Quirk, G.J., 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. http://dx.doi.org/10.1038/npp.2010.184.

Souto-Maior, F.N., de Carvalho, F.L., de Morais, L.C., Netto, S.M., de Sousa, D.P., de Almeida, R.N., 2011. Anxiolytic-like effects of inhaled linalool oxide in experimental mouse anxiety models. Pharmacol. Biochem. Behav. http://dx.doi.org/10.1016/j.pbb.2011.08.029.

Steimer, T., 2011. Animal models of anxiety disorders in rats and mice: some conceptual issues. Dialog-. Clin. Neurosci. 13, 495–506.

Takahashi, M., Satou, T., Ohashi, M., Hayashi, S., Sadamoto, K., Koike, K., 2011. Interspecies comparison of chemical composition and anxiolytic-like effects of lavender oils upon inhalation. Nat. Prod. Commun. 6, 1769–1774.

Takahashi, M., Yamanaka, A., Asanuma, C., Asano, H., Satou, T., Koike, K., 2014. Anxiolytic-like effect of inhalation of essential oil from *Lavandula officinalis*: investigation of changes in 5-HT turnover and involvement of olfactory stimulation. Nat. Prod. Commun. 9, 1023–1026.

Tovote, P., Esposito, M.S., Botta, P., Chaudun, F., Fadok, J.P., Markovic, M., Wolff, S.B., Ramakrishnan, C., Fenno, L., Deisseroth, K., Herry, C., Arber, S., Lüthi, A., 2016. Midbrain circuits for defensive behaviour. Nature. http://dx.doi.org/10.1038/nature17996.

Umezu, T., Nagano, K., Ito, H., Kosakai, K., Sakaniwa, M., Morita, M., 2006. Anticonflict effects of lavender oil and identification of its active constituents. Pharmacol. Biochem. Behav. http://dx.doi.org/10.1016/j.pbb.2006.10.026.