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Research Article

The effect of olfactory exposure on the heart rate of rabbits during transport

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Summary

Rabbits can experience stress during transport. This study explores the effects of Essential Oils (EOs) on the heart rate of rabbits during transport. Rabbits were submitted to 3 different treatments: no olfactory exposure to EOs (treatment 1, control); olfactory exposure to 30 drops of *Lavandula angustifolia* EO (treatment 2); and olfactory exposure to 30 drops of a blend of 5 EOs (treatment 3, *Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, Litsea citrata* EOs) in a randomized controlled crossover study design. Treatment 1 and Treatment 2 did not induce a significant change in rabbits' heart rates, however, treatment 3 did induce a significant decrease in rabbits' heart rates after transport. These results suggest that olfactory exposure to this blend of 5 EOs decreased the rabbit's heart rate after transport. More research is needed to further evaluate the effects of olfactory enrichment with EOs in rabbits during transport.

Abstract

The study explores the effects of Essential Oils (EOs) on the heart rate of rabbits during transport. Rabbits (n = 35) were individually transported three times by car in a transport box for 5 minutes. Each rabbit was subjected to three treatments: no olfactory exposure to EOs (treatment 1, control); olfactory exposure to 30 drops of *Lavandula angustifolia* EO (treatment 2); and olfactory exposure to 30 drops of a blend of 5 EOs (treatment 3, *Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, Litsea citrata* EOs) in a randomized controlled crossover study design. Heart rate was measured before and after transport. Compared to the control, treatment 3 showed a significant decrease in HR after transport (mean = -24.33, SD = 41.77; post hoc Tukey test p = 0.007). Results suggest that the blend of EOs might help rabbits to recover homeostasis quicker after a stressful event. This suggests interesting potential field applications not only for rabbit owners but also for veterinarians. There is more research needed regarding the specific effects of EOs on rabbits.

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Introduction

Transport is considered stressful for rabbits [1,2]. During stress, the autonomic nervous system acts on various systems including the cardiovascular system by a modification of blood pressure and heart rate with tachycardia [3]. These indicators are especially interesting because the data can be collected non-invasively, avoiding the effect of the procedure. Rabbits, as a prey species, are very susceptible to stress and often disguise these signs of stress [1]. So far, the interest in travel-induced stress was specifically related to the rabbit as farm animal [2]. However, rabbits are widely accepted as pets [3] and they might accompany their owners during travel or, in case of illness, they might need to be transported to veterinary centers. Since rabbits might respond to road transport with a pronounced stress response, new treatments for travel-induced stress are still under research with specific characteristics of being cheap and not associated with adverse effects.

In literature, Essential Oils (EOs) have been already applied as a treatment for travel-induced stress in other domestic species and horses [4,5]. Particularly Lavender EO (*Lavandula angustifolia*) is recognized for its anxiolytic effects, already been studied in humans and animals [6-10].

There are other EOs that appear to be interesting as well for their proven anxiolytic effects in humans and shelter dogs [6-10]. In line with previous literature, a recent study on olfactory enrichment in shelter dogs confirmed a significant anxiolytic effect of the following 5 EOs: Ylang-ylang (*Cananga odorata*), Orange (*Citrus aurantium*), Cypress (*Cupressus sempervirens*), Lavender (*Lavandula angustifolia*) and *Litsea citrata*.

Ylang-ylang EO has shown anxiolytic effects on mice in several behavioral tests based on instinctive responses to novel environments [11]. This EO has shown anti-depressant and anxiolytic effects on humans [12]. Ylang-ylang EO furthermore induced neuropathic pain relief and ameliorated pain-associated anxiety [13].

Orange EO has gained attention for its sedative and relaxing actions in mice [14,15] and humans [16]. During the test of the elevated plus maze, anxiolytic effects have been observed in mice exposed to the scent of Orange EO [13]. Carvalho-Freitas and colleagues have demonstrated the sedative and hypnotic activity of *Citrus aurantium* on mice [15].

Cypress is known for its antibacterial [17] as well as antioxidant effects. Antioxidant therapy with Cypress is regarded as a cornerstone in the treatment of several types of neurological disorders and depression [17,18]. Lim and colleagues have also pointed out the sedative effects of Cypress EO in mice [19].

The neuropharmacological properties of *Litsea citrata* EO (also called *Litsea cubeba*) have been studied in humans [20] and its anxiolytic effects have been demonstrated [6,21]. Male mice treated orally for seven days with *Litsea cubeba* EO showed increased exploration (time spent and the number of entries) in the open arms of the elevated plus-maze test and a decrease in total distance traveled in the open arms suggesting a sedative effect of *Litsea cubeba* EO [22].

Based upon this literature, we wanted to investigate further the effect of Lavender EO, as well as the effect of a blend of the 5 EOs described above on the heart rate of rabbits during transport. Each rabbit was subjected to 3 treatments: no olfactory exposure to EO (treatment 1, control); olfactory exposure to 30 drops of Lavender EOs (treatment 2); and olfactory exposure to 30 drops of a blend of 5 EOs (treatment 3, *Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, Litsea citrata* EOs) in a randomized controlled crossover study design. The difference between treatments was measured using the heart rate.

As suggested by Galindo and colleagues [23], the interactions between compounds often result in biological activity that is greater than the activity of the isolated compounds. Therefore, we did expect a higher decrease in the heart rate of the rabbits with the blend of EOs (treatment 3) compared to the control (treatment 1) or lavender only (treatment 2).

Materials and methods

Study design

Each rabbit was subjected to each of the three treatments in a randomized controlled crossover study design. The rabbits were divided into three groups respecting the homogeneity of the rabbit population (Table 1).

The study was carried out for 4 days at the shelter 'Het Vrolijke Konijnenhol' in Oostkamp, Flanders, Belgium on February 27th (day 1, pre-test), March 5th (day 2), March 12th (day 3) and March 19th (day 4) 2020.

Animals and housing conditions

Thirty-five rabbits participated in the study, all were mixed breeds. There were seventeen females and eighteen males. The rabbits aged between ten months and seven years. The exact breed and age of most of the rabbits were difficult to establish as several rabbits have been relinquished by the owners or found as stray. Thirteen rabbits had been in the shelter for more than 6 months.

The shelter housed about 40 rabbits of varying ages (between 1 month and 7 years). The average room temperature was 10 °C during the entire study. There were no changes in the housing conditions of the rabbits due to the research. The rabbits were housed individually or with two or three conspecifics. 18 rabbits were housed in a room where three

Table 1: Schedule of the study.

Day 1 (02/27): pre-study	Day 2 (03/05): transport study	Day 3 (03/12): transport study	Day 4 (03/19): transport study
Group A (*n = 12*): individual observations	Group A (*n = 12*): treatment 1	Group C (*n = 11*): treatment 3	Group B (*n = 12*): treatment 2
Group B (*n = 12*): individual observations	Group B (*n = 12*): treatment 2	Group A (*n = 12*): treatment 1	Group C (*n = 11*): treatment 3
Group C (*n = 11*): individual observation	Group C (*n = 11*): treatment 3	Group B (*n = 12*): treatment 2	Group A (*n = 12*): treatment 1

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blocks of three cages were placed next to each other. The size of these cages ranged from 0,291 to 0,434m³ (indoor cages) and from 0,367 to 1,634m³ (outdoor cages). Each cage included one or two water bottles, one food hopper and one or two litter boxes. In the evening (so after the experiment) food and water were provided. Litter boxes were cleaned every week.

Essential oils

The EOs were obtained by steam distillation and purchased from the company Alanine Laboratory (EODIS, Belgium). The Biochemical composition and relative proportion of the constituents have been assessed by Gas Chromatograph with Mass Selective Detector for each EO (see Annex for the complete analyses reports).

The principal components of Lavender EO were linalyl acetate (34%), linalool (30%) and Lavandulyl acetate (3%).

Next to the anti-inflammatory and stress-releasing effects of linalyl acetate, Shen and colleagues [24] have discussed its utility in a stress management treatment among repeatedly stressed Ulcerative Colitis patients.

Several studies have shown the anxiolytic [25] and sedative effects of linalool [26,27]. Harada and colleagues examined the anxiolytic effects of linalool odor with light/dark box test and with elevated plus maze (EPM) and found that linalool odor has an anxiolytic effect without motor impairment in mice [25].

Lavandulyl acetate has been described for its anti-repellent effects [28], but so far, no anxiolytic effects have been reported.

The principal components of Ylang-Ylang EO were Germacrene D (21%), β-caryophyllène (17%), E,E-α-Farnesene (13%), Benzyl benzoate (6,85%) and linalool (4,42%).

Germacrenes are typically produced in a few plant species for their antimicrobial and insecticidal properties and can serve as a precursor for many other sesquiterpenes [29].

Beta-caryophyllene (BCP) has been identified as one of the best terpenes to reduce anxiety. Hwang and colleagues [30] observed that BCP improved chronic stress-related behavioral and biochemical changes in mice, concluding that BCP may be effective in treating depression and stress-related mental illnesses.

Farnesene act as a natural insect repellent [31], but its already field-reported sedative effects need still to be validated. Benzyl benzoate and linalool have shown individual anxiolytic effects in male mice [32].

The most relevant components of Orange EO were linalyl acetate (53%), linalool (21%), and α-terpinéol (6%). It is interesting to note that its 2 major components are similar to those of Lavender EO.

Litsea citrata E.O. was mainly composed of geranial (41%, geranial is considered as citral), neral (32 %), and d-limonene (12 %). While geranial and neral are mainly known for their anti-inflammatory activities [33], stress-reducing activities were reported for citral [34] and d-limonene [34,35].

Cypress EO was mainly composed of α-pinene (58%), δ-3-care (18%), and α-terpinolene (2%).

Weston-Green and colleagues reviewed the anti-anxiety and anti-depressant effects of α-pinene on humans [36].

D3-care is known to have hypnotic, anti-inflammatory, antioxidant, and anti-stress effects [37]. Terpinolene is known for its sedative effects through oral administration, as well as through nasal absorption [38].

The concentration of the 5 EOs in the blend (treatment 3) was proportionally the same, each EO was present for 20% in the blend.

Experimental set-up

The transport study was conducted by three experimenters (AH, DE and ML). The rabbit was placed in the transport box (45 L x 27 W x 30 H cm) and its heart rate was measured. The rabbit was inside the transport box for 5 minutes in the experimental analysis, while being gently transported by car by one of the investigators. The investigator drove with an average speed of 30 km/h. Immediately after the transport, the heart rate was measured in the transport box and afterward in the cage.

All controllable conditions were similar for all the rabbits during the study. Nevertheless, due to field conditions, some variables did change like time of day, weather as well as some minor noises.

Treatment

Each rabbit was subjected to three treatments: no olfactory exposure to EOs (treatment 1, control); olfactory exposure to 30 drops of *Lavandula angustifolia* EO (treatment 2); and olfactory exposure to 30 drops of a blend of 5 EOs (treatment 3, *Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, Litsea citrata* EOs).

For every treatment another but similar transport box was used so the EOs did not interfere. For each rabbit, a new piece of cloth (10 x 10cm) was used. The pieces of cloth were taped inside the transport boxes at the top.

Data collection

The parameter heart rate was used to determine the effect of olfactory exposure with EOs on this group of rabbits. The measurements of the heart rate were taken by the same experimenter to guarantee homogeneity in counting. The heart rate was measured with a standard stethoscope. All the rabbits were always carried and held by the same experimenter. Heart rate was measured at the onset of the stressful event, when the rabbit was just placed in the transport box, before getting into the car for transportation (see HR1 below) and after the stressful event when the rabbit was back in his familiar environment (its cage; see HR2 below).

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Experimenter 1 prepared the transport box for the rabbit. The transport box was disassembled, and the upper side was put upside down on the ground. Experimenter 1 taped a piece of cloth in the middle of the upper piece of the transport box and put nothing (treatment 1), 30 drops of lavender EO (treatment 2), or 30 drops of the blend of EOs (treatment 3) on the piece of cloth. The transport box was put back together.

Experimenter 2 carried the rabbit from its cage to the transport box inside the garage by holding him close to his body and supporting the hindquarters. The heart rate from the rabbit was measured in the transport box by experimenter 2 with the help of experimenter 3. Experimenter 2 held the rabbit and placed the stethoscope under the front legs. Experimenter 3 timed 30 seconds on a stopwatch.

Then experimenter 1 closed the transport box and gently carried it to the car for a 5-minute drive. During this time, experimenter 3 multiplied the counted heartbeats by two and documented this in the excel file (HR1).

Experimenter 1 came back from the transport. The heart rate was measured in the transport box by experimenter 2 while experimenter 1 timed 30 seconds on a stopwatch. The counted heartbeats were multiplied by two and documented in the excel file by experimenter 3.

Experimenter 3 took the piece of cloth from the transport box and placed it in the cage of the rabbit. The rabbit was carried to his cage in the transport box by experimenter 1 and the rabbit went by himself in his cage.

Experimenter 3 timed 30 seconds on a stopwatch while experimenter 2 counted the heart rate. The counted heartbeats were multiplied by two and experimenter 3 documented this in the excel file (HR2).

This sequence was repeated for each rabbit in each treatment.

Ethical note

An ethical note has been approved by Odisee University of Applied Sciences confirming that the rabbits were not considered laboratory animals. The animals were constantly monitored. The experiment would immediately have been stopped, should a rabbit have shown any sign of distress, impaired welfare, or clinical problem. By the IACUC, all the following measurements had been taken to ensure that the proposed research respected Animal Welfare and didn't involve any animal tests violating animal welfare.

The researchers didn't conduct any invasive procedures on the rabbits. All the rabbits were monitored accurately during the entire experiment. The researchers did not observe any negative effects of these concentrations of EOs on these rabbits (no signs of distress that could be linked to the EOs). No procedures caused any pain or distress to the animals.

Statistical analysis

A mixed linear model was valued with the response (difference in heart rate: HR2 minus HR1) and random effects. Individuals, groups and days were included in the random effects. Post hoc test evaluated for pairwise comparisons between treatments. A Tukey correction for multiple tests was applied.

Results

Two heart rate measurements were used to determine the effect of olfactory stimulation with EOs on rabbits. The first heart rate (HR1) was measured at the onset of the stressful event, when the rabbit was just placed in the transport box, before getting into the car for transportation. The second heart rate (HR2) was measured after the stressful event when the rabbit was back in his familiar environment (its cage; Table 2).

The graph shows HR2 (after) minus HR1 (before). Positive values thus signify an increase in HR, and negative values a decrease in HR. The mean heart rate increased for treatment 1 (control, no olfactory exposure to EOs), but decreased for treatment 2 (olfactory exposure to 30 drops of *Lavandula angustifolia* EO) and treatment 3 (olfactory exposure to 30 drops of the blend of EOs). Compared to treatment 1, treatment 3 showed a significant decrease in HR (Figure 1).

Discussion

Compared to treatment 1 (control, no olfactory exposure to EOs), treatment 3 (olfactory exposure to 30 drops of the blend of EOs) showed a significant decrease in HR after transport. This is in line with previous research, suggesting that a blend

Table 2: Mean and SD values of the heart rates before (HR1) and after (HR2) the stress event for each treatment. Treatment 1 (control, no olfactory exposure to EOs), Treatment 2 (olfactory exposure to 30 drops of *Lavandula angustifolia* EO) and Treatment 3 (olfactory exposure to 30 drops of the blend of EOs).

Time of measurement	Treatment	Mean (bpm)	SD
HR1 (before)	1 (control)	241.5	29.5
	2 (lavender)	236.9	47.2
	3 (blend)	245.9	36.6
HR2 (after)	1 (control)	242.7	35.1
	2 (lavender)	225.0	48.8
	3 (blend)	221.7	28.3

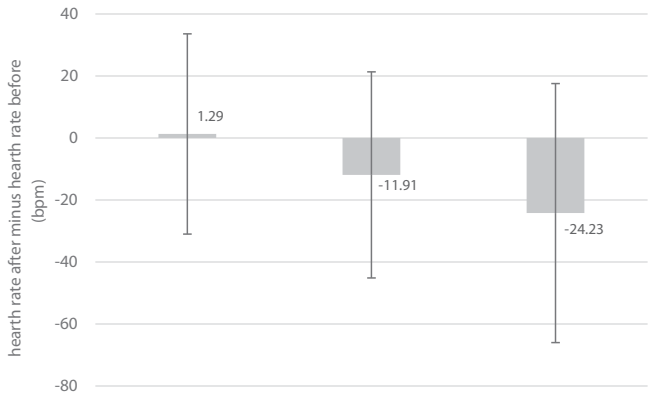

Figure 1: Intraoperative image, Complete section of the pancreas body. Histogram presenting the mean (grey) and SD (flags) values of heart rate after (HR2) minus heart rate before (HR1; bpm). Treatment 1 (control, no olfactory exposure to EOs), Treatment 2 (olfactory exposure to 30 drops of *Lavandula angustifolia* EO), and Treatment 3 (olfactory exposure to 30 drops of the blend of EOs).

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of anxiolytic EOs might have more effect than the single use of Lavender EO [8]. These results suggest that this blend of EOs might help rabbits to recover homeostasis quicker after a stressful event. This presents interesting potential field applications not only for rabbit owners but also for veterinarians. Olfactory stimulation with EOs may be useful not only during transport but also during stressful events at the shelter or at the owner's home as well as at the veterinary clinic.

Based on the literature [1,2], our premise was that transport induced stress in rabbits. In our study, results showed a slight increase in heart rate in the rabbits during transport in treatment 1 (control, not exposed to any EOs). Although the heart rate increase was not significant, we can not exclude that transport has not been stressful to them.

The study was conducted in a real-life unstandardized environment. This means that some variables varied during the experiment. The result of the study may have been affected by for example the margin of error from the heart rate measurements, the time of the day, the size of the rabbits, the weather on the days of the experiment, and some minor noise during the experiment. However, due to the repeated measures design of our experiment, we expect that the triggers mentioned above had little effect on the results.

Our results did not show a significant effect of the treatment with lavender EO on heart rate. Most studies on Lavender however pointed out its anxiolytic activity. Research conducted on humans showed that Lavender EO could decrease some agitation and emotional disorders in people suffering from dementia [39-41]. Research has shown that rodents became calmer and less agitated with the smell of lavender oil [42-44]. Gerbils exhibited less anxious behavior when exposed to the smell of lavender oil, which appeared to have a similar effect as diazepam [44]. Lavender EO was found to reduce anxiety and depression in rats. At a high concentration, a sedative effect has been induced [45]. An anticonvulsant effect of lavender EO was reported against seizures in male mice. Particularly, lavender EO inhibited the onset, shortened the duration, and reduced the intensity of seizure attacks [46]. Lavender EO appeared to decrease the incidence and severity of travel sickness in pigs but not overall levels of stress (as measured by concentrations of salivary cortisol) [47]. In dogs, lavender EO decreased barking and active behavior [9] and dogs barked less in the car when being exposed to lavender oil during transport [4]. Although its mechanism of action has not been completely determined yet, the antidepressant activities of lavender may be attributed at least in part to the NMDA receptor modulation as well as an inhibition of the SERT [48].

In contrast, some studies have shown that Lavender EO modulated motor movement and locomotion [49] and increased arousal levels [50]. It is not yet known whether the effect is concentration-dependent or individual-dependent. Following previous observations on individual coping styles [51,52], it might be interesting to investigate further if any genetic or temperamental differences might determine whether lavender EO alleviates or exacerbates anxiety in rabbits.

Our results clearly show that more research is needed to further evaluate the effects of olfactory stimulation with EO in rabbits. This research leads to other questions and pushes forward the investigation into the effects of EOs. For example (1) understanding the minimal concentration necessary to decrease anxiety in rabbits; (2) the impact of olfactory stimulation on multi-rabbit transportation, (3) different breeds and sizes of rabbits. To improve the welfare of rabbits, it is important to find tools to help them recover fast in stressful situations like for example transport, waiting at the veterinary or consultation room. In this study, we have focused on the individual transport of rabbits. It might be interesting to explore further the effects of increased transport on rabbits during commercial group transport and how EOs might support these rabbits.

Further research is needed to confirm our preliminary results, in which olfactory exposure to a blend of EOs has been more effective than olfactory exposure to one single EO. It would also be interesting to compare this blend of EOs to other blends of anxiolytic EOs. To evaluate the total effect of olfactory enrichment, future studies would benefit from a study that includes a standardized environment, behavioral observations as well as measurements of other physiological non-invasive and non-stressful parameters.

Conclusion

The primary purpose of this study was to evaluate the effect of olfactory exposure of EOs on the heart rate of shelter rabbits during transport. Results have shown that olfactory exposure to a blend of 5 EOs (*Cananga odorata, Citrus aurantium, Cupressus sempervirens, Lavandula angustifolia, and Litsea citrata EOs)* significantly decreased the rabbit's heart rate during transport. No significant decrease has been observed in the control treatment or treatment with lavender EO. EOs can be useful to help rabbits to recover homeostasis after a stressful event. There is more research needed about the specific effects of EOs on rabbits.

Author contributions

Conceptualization, AH and AS; methodology, AH and AS; software, HA; validation, AH and AS; formal analysis, HA; investigation, AH, ML, DE; resources, AH, ML, DE; data curation, ML; writing—original draft preparation, AH, ML, ML; writing—review and editing, AH and AS; visualization, AH; supervision, AH and AS; project administration, AH; funding acquisition:/ All authors have read and agreed to the published version of the manuscript.

Institutional review board statement

Ethical review and approval were waived for this study, due to IACUC.

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