Behavioral responses to emotional challenges in female rats living in a seminatural environment: The role of estrogen receptors

Olivia Le Moëne and Anders Ågmo

Department of Psychology, University of Tromsø, Norway

Corresponding author: olivia.s.moene@uit.no

Abstract:

Estrogen receptors (ERs) are involved in sexual as well as non-sexual behaviors. In the present study we assessed the effects of stimuli inducing positive or negative affect on sociosexual, exploratory and fear-related behaviors of female rats housed in groups (4 females, 3 males) in a seminatural environment. Ovariectomized females were treated with oil, 17β-estradiol benzoate (EB, 18 µg/kg), the ERα agonist propylpyrazoletriol (PPT), or the ERβ agonist diarylpropionitrile (DPN) (both 2 x 10 mg/rat). On the test day, the females were exposed to a sequence of events consisting of lavender odor, Mozart's Sonata for Two Pianos K448, chocolate pellets, white noise and fox odor (2,3,5-Trimethyl-3-thiazoline, TMT). All these events are known to induce positive or negative affect. Behavior was carefully observed from the video record. White noise suppressed sexual behaviors and reduced the time spent in the open area of the environment. TMT had no consistent effect whereas exposure to music caused avoidance of the open area. Exposure to chocolate increased exploratory and social behavior. Lavender odor enhanced exploratory behavior. PPT and EB stimulated sexual behaviors, whereas DPN was ineffective. Co-occurrence analyses of the sequence of behavioral patterns revealed that PPT and EB consistently belonged to clusters different from oil and DPN, whereas DPN was separate from oil only under fear-inducing experimental conditions. These data, from a procedure with external validity, confirm that the ERα is crucial for sexual behaviors, that these behaviors are reduced under stressful conditions, and that the ERβ may have some role in fear-related behaviors.

Key words: seminatural environment, sexual behavior, social behavior, fear, positive emotion, co-occurrence analysis, propylpyrazoletriol, diarylpropionitrile

1. Introduction

Female rodents only express sexual behavior when their brain is exposed to appropriate concentrations of ovarian hormones. Estrogens and progesterone normally act synergistically, but high doses of estrogens can activate all aspects of female sexual behavior in the absence of progesterone, whereas progesterone is ineffective in the absence of estrogens regardless of dose (e.g. Södersten & Eneroth, 1982). It is known that all female sexual behaviors, including being attractive for males and being attracted to males (reviewed in Le Moëne & Ågmo, 2017) as well as the display of paracopulatory behaviors and lordosis (e.g. Ogawa et al., 1998; Rissman et al., 1997), are dependent on the estrogen receptor α (ERα). The estrogen receptor β (ERβ) does not contribute to these behaviors, since female mice lacking this receptor show perfectly normal sexual behaviors (Ogawa et al., 1999; Walf et al., 2008; Antal et al., 2012) and since ERβ agonists are unable to activate these behaviors in ovariectomized female rats (Mazzucco et al., 2008).

In addition to being necessary for the display of sexual behaviors, estrogens have several behavioral effects, some of which may be relevant for the sex behaviors. Among those, modifications of fear and anxiety responses might be particularly important. There are also data suggesting that estrogens may alter the response to events inducing positive affect, for example the ingestion of tasty foods like sucrose or chocolate (e.g. Clarke & Ossenkopp, 1998; Reynaert et al., 2016). However, the effects of fear-inducing stimuli or situations or of stimuli causing positive affect on sexual behavior in female rats have not been studied. In fact, available data are limited to studies of the effects of stress on subsequent sexual behavior. It can be argued that fear stimuli activate stress responses, and studies of stress could therefore provide some information about the potential effects of fear on sexual behavior. Acute stress in the form of short restraint reduces the display of female copulatory behavior in females rendered sexually receptive with estradiol alone, whereas no effect was observed in females given estradiol + progesterone (Truitt et al., 2003). When females can

pace sexual interaction, restraint reduces the time spent with the male as well as the number of mounts received regardless of the presence or absence of progesterone. Receptivity was not modified, though (Uphouse et al., 2005). Interestingly, restraint stress had no effect in a test for sexual incentive motivation (Uphouse et al., 2008). It appears, then, that acute stress has minor or no consequences for female sexual behavior. Chronic stress, however, has consistently been found to facilitate the display of lordosis and paracopulatory behavior and to reduce rejections (Brotto et al., 1999; Williams et al., 1992).

A common feature of all these studies is that they have evaluated the effects of prior, but not present, stress. Thus, the immediate consequences of fear-inducing stimuli for sexual behavior remains unknown. There is, however, abundant evidence for a role of estrogens in non-sexual, anxiety-like responses. Treatment of ovariectomized mice and rats with estradiol has been reported to enhance the time spent on the open arms of an elevated plus maze (e.g. Nomikos & Spyraki, 1988), or in the center of an open field (e.g. Walf & Frye, 2007), and to reduce a passive avoidance response (Díaz-Véliz et al., 1997). All these effects are usually interpreted as suggesting reduced fear or anxiety. Other studies have failed to find an effect of estradiol in anxiety tests (e.g. Walf & Frye, 2008; Martínez-Mota et al., 2000), and still others found anxiogenic effects (Mora et al., 1996). One hypothesis proposed to account for these contradictory observations is that estrogens are anxiogenic in threatening environments and anxiolytic in safe environments (Morgan & Pfaff, 2001). Such an effect could conceivably be useful for assuring that another ERα dependent behavior, copulation, occurs more easily in safe than in dangerous environments (see, e.g. Frye et al., 2006, for an elaboration of this argument). Direct experimental evidence for this conjecture is lacking, though.

The anxiolytic-like effects of estrogens are often attributed to actions at the ERβ. Mice without a functional ERβ are more fearful than the wildtype (Krezel et al., 2001), and treatment with a selective ERβ agonist reduces fear in female rats (Kudwa et al., 2014) and

mice (Krezel et al., 2001; Oyola et al., 2012; Walf et al., 2008b), whereas selective ERα agonists are ineffective. It has also been reported that ERα knock-out mice are not different from the wildtype in several of the anxiety procedures (Krezel et al., 2001). However, anxiogenic effects of a selective ERα agonist in fear-inducing environments (elevated plus maze and novel open field) have been reported (Lund et al., 2005). It has also been found that the ERα is anxiogenic in the light/dark box and in a brightly lit open field (Spiteri et al., 2010b; Spiteri et al., 2012). Thus, it can be proposed that ERα and ERβ agonists might have opposite effects in fear-inducing contexts.

One of the purposes of the present study was to determine whether fear-inducing stimuli actually inhibit female sexual behavior, and if agonists selective for the ERα and ERβ would have different effects on the nonsexual responses to these stimuli. To that end, ovariectomized females were given either estradiol or selective ER agonists. Fear was induced by exposing the females to a 90 dB white noise or to synthetic fox odor. Loud noise as well as 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) produce strong fear responses in rats (e.g. Endres et al., 2005; Fendt et al, 2005; Homiack et al., 2017; Weyers et al., 1994).

Another purpose of this study was to evaluate the effects of stimuli inducing positive affect rather than fear, and the potential role of the ERs for responses to such stimuli. Estrogen-modulation of responses to attractive, non-sexual stimuli have only been systematically studied with regard to food ingestion. It is well known that estrogens reduce food intake (e.g. Butera, 2010). It appears that the ERα is responsible for the effects of estrogens, since ERα knockout mice do not reduce food intake in response to hormone treatment (Geary et al., 2001). Moreover, a specific ERα agonist does reduce food intake whereas an ERβ agonist is ineffective (Shen et al., 2017). It has been suggested that post-ingestive factors rather than reduced hedonic impact of tastants underlie the reduced food intake (Hrupka et al., 1997; Flanagan-Cato et al., 2001). This proposal is reinforced by the

fact that estrogens enhance the hedonic response to and consumption of tasty foods, such as sucrose (Clarke & Ossenkopp, 1998) or chocolate (Reynaert et al., 2016; Boswell et al., 2006; Lampert et al., 2013).

A less known effect of estrogens is to increase the fear-reducing effect of music. It has repeatedly been reported that music has anxiolytic activity in several of the standard procedures (Li et al., 2010; Cruz et al., 2015). When the sonata for 2 pianos (Köchel number 448) by W.A. Mozart is played to ovariectomized female rats, the anxiolytic effect is reduced compared to that found in proestrus females, but it can be restored by treatment with estradiol (Escribano et al., 2014). In the same study, it was found that the anxiogenic effect of loud white noise was not altered by ovariectomy or estrogen treatment. In mice, the effects of music seem less dependent on ovarian hormones, although both estradiol and progesterone had some effects (Chikahisa et al., 2007). The potential role of the different ERs has not been explored.

Another stimulus with anxiolytic effects in several rodent procedures is the odor of lavender oil (Umezu et al., 2006; Shaw et al., 2007; Tsang & Ho, 2010; Tsang et al., 2013; Linck et al., 2010). There are also observations suggesting that this oil activates positive affect in rodents and humans (Frasnelli et al., 2015). To our knowledge, there are no data concerning possible modifications of the effects of lavender oil by ovarian hormones.

An additional purpose of the present study was to evaluate the effects of the positive stimuli lavender oil, music, and chocolate on estradiol + progesterone-activated sexual behavior in ovariectomized rats and to determine if and how non-sexual responses were modified by ER ligands.

We have previously argued that an understanding of the behavioral consequences of the central nervous actions of the ovarian hormones is best achieved in experimental setups with external validity (Chu & Ågmo, 2014; Chu & Ågmo, 2015b; Chu & Ågmo, 2016). This

means that the setup should include as many as possible of the elements found in the natural context in which the behavior normally is shown. In the case of sexual behaviors in rats, an important feature is that it occurs in multi-male, multi-female groups, and in a physical environment making it possible for the rats to temporarily escape from other group members.

In view of these considerations, we administered estradiol as well as the selective ERα agonist propylpyrazoletriol (PPT) and the selective ERβ agonist diarylpropionitrile (DPN) to ovariectomized female rats housed in a seminatural environment in groups consisting of 4 females and 3 males. During the period in which the agonists could be expected to have their maximal effect, we introduced the events mentioned earlier into the environment. This made it possible not only to determine the role of the ERs in social and sexual interactions in a group of rats, but also how they affected the response to these events, and how the positive and negative events themselves affected behavior. These data would provide us with a better understanding of how the ERs control sexual behavior and responses to emotion-inducing events in a procedure with external validity.

2. Material and Methods

2.1 Subjects

Wistar rats (females, 250 g and males 300 g upon arrival) were obtained from Charles River WIGA (Sulzfeld, Germany). The rats were housed in same-sex pairs in standard Macrolon® IV cages prior to the beginning of the experiment. Commercial rat pellets (RM1, Special Diets Services, Witham, UK) and tap water were available *ad libitum*. The animal rooms were maintained at 21 ± 1°C and humidity was 55 ± 10%. Lights were set on a reversed 12:12 h cycle, being on between 23:00 and 11:00 h. Females were ovariectomized 14 days prior to the

introduction into the seminatural environment under isofluorane anesthesia. For a detailed description of the surgical procedure see Ågmo (1997).

All experimental procedures employed in the present experiment were approved by the Norwegian Food Safety Authority and were in agreement with the European Union council directive 2010/63/EU.

2.2 Apparatus

The seminatural environment has previously been used in a number of studies and has been described in detail elsewhere (Chu & Ågmo, 2014; Chu & Ågmo, 2015b). Briefly, it consisted of a complex burrow system and a large open area (Fig. 1). The burrow system included four nest boxes provided with nest material and was maintained in complete darkness for the whole experiment. Infrared (850 nm) lamps provided the light necessary for video recording. The open area (1.2 * 2 m) contained 12 wooden sticks and three small shelters made of transparent red plastic. The open field was submitted to a reversed light/dark cycle (12D:12L) with a 180 lx light from 23:00 to 11:00 and a 30 lx light from 11:00 to 23:00. Artificial dusks and dawns were provided by slowly changing light intensity from night to day and day to night during a 30 min period preceding and following the light period. Video cameras were fixed to the ceiling about 2 m above the burrow and the open area, respectively. They were connected to digital video recorders.

The ventilation system in the animal facility produced an ambient noise of about 40 dB.

2.3 Hormones and selective estrogen receptor ligands

Estradiol benzoate (EB) and progesterone (P) (both from Sigma Aldrich, St Louis, MO) were dissolved in peanut oil (Den norske Eterfabrikk, Norway) and administered

subcutaneously (SC) in a dose of 18 μg/kg and 1 mg/rat, respectively. The injection volume was 1 ml/kg for EB and 0.2 ml/rat for progesterone. EB was administered 48 h before P.

The estrogen receptor agonists propylpyrazoletriol (PPT) and diarylpropionitrile (DPN) were obtained from Tocris Bioscience, Bristol, UK. Both PPT and DPN were dissolved in undiluted dimethylsulfoxide (Sigma Aldrich) right before SC injection. Both were administered at a dose of 2 * 10 mg/kg body weight in a volume of 1 ml/kg. There was an interval of 24 h between injections. PPT is selective to ERα with a 410-fold preference compared to ERβ, and with a relative binding affinity of 50% compared to estradiol (Stauffer et al., 2000). DPN is selective to the ERβ with a 72-fold preference compared to ERα with a relative binding affinity of 18% (Meyers et al., 2001). PPT and DPN reach their maximum serum concentration about 30 min after SC injection and have a half-life of 6.0 ± 0.03 h and 8.2 ± 1.7 h, respectively (Sepehr et al., 2012).

The doses of EB and P employed here have been used successfully in several earlier studies (e.g. Chu et al., 2017; Snoeren et al., 2015). They produce close to maximal receptivity and high intensity of paracopulatory behaviors (Spiteri & Ågmo, 2006). The dose of PPT was based on earlier studies. One showed that 2 * 10 mg/kg of PPT given 48 and 24 h before test produced a high lordosis quotient, similar to that of 2 * 2 μg/rat of EB. A dose of 2 mg/kg was inactive (Miller et al., 2005). Another study showed that 2 * 5 mg/rat, 48 h and 24h before test, produced a lordosis quotient of about 0.8 when combined with progesterone 0.5 mg/rat (Mazzucco et al., 2008). Thus, a dose of 2 * 10 mg/kg of PPT was used in order to assure clear behavioral effects. Concerning DPN, the dose was chosen somewhat arbitrarily. The ERβ does not participate in the activation of sexual behavior, so we needed to find another basis for determining the appropriate dose. Since many studies comparing the effects of PPT and DPN employ the same dose of both compounds (e.g. Pisani et al., 2016; Walf & Frye, 2005) we simply decided to do the same.

2.4 Experimental conditions

Since the aims of this study include an analysis of behavior in situations producing positive and negative affect, it was essential to introduce events into the seminatural environment that reasonably could be expected to produce diverse emotional reactions. Five such events, all mentioned in the Introduction, were used.

1. Lavender odor stemmed from an essential oil extracted from *Lavandula angustifolia* (AromaBio, Lyon, France). Immediately before the beginning of the experimental session, 1.5 ml of this oil was put on a cotton pad in an airtight jar. An air stream could be made to flow through the jar whenever desired with the help of an air pump and a system of solenoid valves (Olfactory Stimulus Package, Medical associates, Georgia, Vt). Odorless plastic tubing (I.D. 3/16" , O.D. 5/16" , wall thickness 1/16"; TYGON® Inert, Saint-Gobain Verneret, Charny, France) connected the pump with the valves and eventually with a nozzle in the wall of one of the tunnels in the burrow section of the seminatural environment, as well as with another nozzle in the wall of the open area. The airflow (around 3 l/min) could be directed through one of two jars, or made to bypass the jars and consist of unscented room air instead. Room air was blown through the nozzles during the entire experimental session, except when it was replaced by an olfactory stimulus. Lavender odor was introduced into the seminatural environment for 30 min with the help of this system. The amount of lavender oil used here has been found to alter behavior even with short exposure times (e.g. Shaw et al., 2007).

2. Classical music (Mozart's sonata for two pianos K448), played by Murray Perahia and Radu Lupu, recorded at Snape Maltings Concert Hall, Suffolk, England (CD from Sony Music Entertainment). The piece lasts 24 min and 18 s. The music file was uploaded on a computer and played through A60 stereo speakers from Creative (Clas Ohlson, Norway) at a sound intensity of 55-60 dB as measured at floor level.

3. Thirty-five one g chocolate pellets (Bioserve, *Flemington, NJ)* were placed on a Petri dish (diameter 100 mm) which was put in the middle of the open area. After 30 min, the dish was removed. At that time, most pellets had been removed by the rats.

4. A 90 dB white noise was produced by a noise generator (Lafayette instruments, Lafayette, IN) connected to two loudspeakers (Scan-Speak Discovery 10F/8414G10, Hifi Kit Electronic, Stockholm), one suspended about 2 m above the burrow, and another at the same height above the open area. The noise was turned on for a period of 15 min.

5. Fox odor was produced by making air flow through a jar containing 35 µl of 2,5-dihydro-2,4,5-trimethylthiazoline (TMT; Contech, Delta, BC, Canada) on a cotton pad. The odor system described above was used. Short exposure to odor from this amount of TMT has been found to alter behavior in a large open field (Morrow et al., 2002). The odor exposure lasted for 30 min.

Music and chocolate may not be standard parts of rats' natural habitat, and these stimuli might therefore reduce external validity. We used them because they are among the few, non-sexual stimuli, known to cause positive affect in non-deprived rats. Sucrose might have been used instead of chocolate pellets, but the latter have the advantage of allowing for for quantification of each individual's consumption. This would not have been possible with a sucrose solution.

2.5 Procedure

Prior to introducing each experimental group into the seminatural environment, the floor of the entire environment was covered with a three cm thick layer of aspen wood chips (Tapvei, Harjumaa, Estonia). Four 0.5 l water bottles and about 5 kg of standard food pellets were located in a corner of the open area. After each experimental session, the bedding was removed and the entire environment was cleaned and disinfected.

The rats were released in the seminatural environment on day 0 at 13:00. About four hours before, they had been weighed and marked in order to be identifiable on the video record.

The rats were left undisturbed for the first 5 days in the seminatural environment. On days five and six the females were captured and injected with the appropriate compound. On day 7, all females received P. Four hours later, the sequence of experimental conditions started. All experimental conditions were separated by a 50-minutes rest period (Table 1). The experiment was terminated after the last experimental condition, and the females were again weighed.

[Insert table 1 here]

The order of presentation of the experimental conditions was fixed throughout the whole experiment. Four hours after the P injection, lavender odor was presented, followed by music, chocolate, white noise and the fox odor.

This order of events was based on several considerations. Predator odor has been reported to alter behavior for several hours (Fendt et al., 2005). Consequently, exposure to fox odor needed to be the last condition. The duration of potential effects of lavender oil is not known, but anxiolytic effects are normally obtained immediately or within few minutes after the end of exposure (e.g. Tsang et al., 2013). The ventilation system in the room housing the seminatural environment assures 15 air changes per hour, leading to a fast decline in the concentration of odorant. We found it reasonable to suppose that the 50 min interval before the next condition would be sufficient both for the odor and for its possible effects to dissipate. In fact, the data confirmed that supposition. Concerning music, the reported effects were usually obtained during exposure (e.g. Escribano et al., 2014). There are no data as to the duration of effect, but again we supposed that it should be less than 50 min after the end of the piece. The duration of the positive affect produced by chocolate eating or of the fear

reaction produced by white noise has not been determined. It may be pointed out, though, that the chocolate-induced positive affect is present already during consumption (La Mela et al., 2010; Reynaert et al., 2016). With regard to white noise, pilot data revealed that the behavioral effects of the noise were most evident at the onset, and that behavior began to normalize already during the last few minutes of noise exposure. Therefore, the 50 min interval was probably sufficient for any noise effects to dissipate.

2.6 Design

Each group in the seminatural environment consisted of seven rats, four females and three males. The group members came from different cages, meaning that they were unknown to each other before the introduction into the environment. Ten such groups were used in this experiment.

[insert Figure 1 here]

In all groups, each of the four females received a different treatment. 1. Oil on days 5 and 6, P on day 7. 2. EB on day 5, oil on day 6, and P on day 7. 3. PPT on days 5 and 6, P on day 7. 4. DPN on days 5 and 6, P on day 7. This means that all treatments were present in all housing groups.

2.7 Behavioral observations

Based on extensive observation of the video record, we established an ethogram for the scoring of the rats' behavior (Table 2). Scoring was made with the Observer XT 12.5 software (Noldus, Wageningen, the Netherlands). Pilot data showed that a 15 min observation period was sufficient to detect behavioral differences between conditions and treatments. Thus, the last 15 min of the lavender, music, and fox odor exposure were observed, whereas we recorded behaviors for the first 15 min of chocolate availability. This allowed us to

determine the latency to approach and grab the chocolate. The entire 15 min period of exposure to white noise was observed. Behavior during the 15 min preceding the lavender odor was recorded as a baseline. The frequency and/or duration of the occurrences of the behavior patterns were recorded, specifying the individual initiating the behavior, the individual to whom it was directed, and the location of the behavior. We also calculated the number of transitions between zones (see Figure 1), as well as the number of visits to and the time spent in the open area. In the case of latencies, subjects not displaying the behavior were assigned a latency of 900 s, the duration of the observation. The lordosis quotient (LQ, number of lordoses / number of mounts) was also calculated. Please note that some females displayed one or more lordoses in the absence of male mounting, usually in response to tactile stimulation of posterior body parts. The LQ may, consequently, be larger than 1.

[insert Table 2 here]

2.8 Statistical analysis

Whenever possible, data were analyzed with two-factor ANOVA for repeated measures on one factor. The between-groups factor was treatment and the within-groups factor was experimental condition. After significant main effect, the Tukey HSD test was used for *a posteriori* comparisons. We calculated the effect size η^2 for the effect of treatment and the partial effect size η_p^2 for effect of experimental condition and for the interaction. The effect size for Tukey's HSD was expressed as Cohen's d ($d = (x_1-x_2)/\sigma$).

When the data deviated from the normal distribution according to Shapiro-Wilk's test, or the error variances were non-homogenous according to Hartley's F_{max} test, we used nonparametric tests. The effect of treatment was evaluated with the Kruskal-Wallis test whereas the effect of experimental condition was analyzed with Friedman's ANOVA. In case of significance, *post hoc* analyses were made as recommended by Conover (Conover, 1999).

The effect size was calculated as eta squared (η_H^2) for the Kruskal-Wallis tests and as Kendall's *W* for the Friedman test (Tomczak & Tomczak, 2014). Cliff's δ was used for the non-parametric *post hoc* comparisons (Cliff, 1996). Some data were analyzed with the χ^2 test, and/or the Fisher exact test. Effects sizes for these tests were calculated with Cramer's V and Cohen's d, respectively.

Significance level was $p < 0.05$. Data in text and figures are expressed as mean + SEM. The IBM SPSS Statistics, version 23 was used for parametric tests and the free software R, version 3.4.3 with base, PMCMRplus, effsize and lsr packages for non-parametric tests.

2.9 Co-occurrence analysis

The seminatural environment allows the subjects to express a substantial part of their natural behavioral repertoire. In fact, the continuous flow of behavior patterns is recorded. This makes it possible to determine treatment- or condition-induced modifications of that flow. In other words, how the experimental manipulations might have altered the structure of behavior. Analyses of the frequency or duration of particular behavioral items cannot reveal this kind of effects. Thus, in order to fully exploit the data obtained, we subjected the behavioral record to an analysis of co-occurrence. Since the behavior patterns were recorded in chronological order, this is easily made. We used a moving window of four behavior patterns, and determined how often one behavior pattern occurred together with another in the same window. This is defined as a co-occurrence. The window moved, by steps of one behavior pattern, over the entire individual record. The frequency of co-occurrences was entered in a matrix with the behavior patterns in rows and columns, the co-occurrence frequency appearing at the intersections. Treatments and experimental conditions were also included in the matrix. These were the raw data for the analysis. Descending hierarchical

classification was used in order to find clusters of related behavior (Reinert, 1983; Reinert, 1990; Valax et al., 1990, see also LePape et al., 1997). The descending hierarchical classification is based on the probability for an item to be proportionally more present in a cluster than it is in the entire data set, as evaluated by χ^2 analysis. Each item is permutated from one cluster to the other to test the robustness of the classification, until statistically independent profiles of items appear (Marchand & Ratinaud, 2012). Communities can therefore be interpreted as groups of individuals and behaviors significantly more co-occurring together than with items of another community.

The criterion for including elements in their respective classes is a higher frequency compared to the average occurrence, as well as an association with the class determined by χ^2 values equal to or higher than 3.84. This gives an error margin of 0.05 when df = 1 (de Oliveira Andrade Jr. & de Oliveira Andrade, 2016).

Finally, co-occurrence networks were established and visualized using the Fruchterman-Reingold algorithm. Calculations were performed with the free software IRAMUTEQ (Interface de R pour les Analyses Multidimensionnelles de Textes et de Questionnaires; available at http://www.iramuteq.org/).

3. Results

The pattern of effects of experimental condition and of treatment were similar for the frequency of recorded behaviors and the total duration as well as the mean duration of each behavioral episode, whenever these could be calculated. Therefore, we only present frequency data. These have the advantage of being available for all behaviors.

3.1 Effects of experimental conditions

3.1.1 Female sexual behaviors

The sex behavior data did not satisfy the criteria for ANOVA. The distribution greatly deviated from normality according to the Shapiro-Wilk test and Hartley's F_{max} test showed error variances to be non-homogenous. Therefore, these data were analyzed using non-parametric statistics. Moreover, since the aim of these analyses was to determine how experimental conditions affected sexual behaviors, we limited the analyses to females treated with EB or PPT. The females treated with oil or DPN expressed an extremely low level, or none at all, of these behaviors (see 3.2.1). Thus, these females could not contribute with any useful data to an analysis of the effects of experimental condition on sexual behaviors, since no such behaviors were displayed in any condition.

The lordosis frequency in the collapsed EB and PPT groups differed between conditions ($\chi^2_{DF=5} = 12.67$, $p = 0.027$, W = 0.12). It was lower during exposure to white noise ($p = 0.023$, $\delta = 0.30$) than at baseline (Fig. 2 A). The LQ also differed between experimental conditions ($\chi^2_{DF=5} = 15.56$, $p = 0.008$, W = 0.16), being lower during exposure to white noise ($p = 0.002$, $\delta = 0.45$) than at baseline (Fig. 2 B). Likewise, the frequency of paracopulatory behaviors differed between conditions ($\chi^2_{DF=5} = 15.57$, $p = 0.008$, W = 0.16). It was lower during exposure to white noise ($p = 0.004$, $\delta = 0.37$) than at baseline (Fig. 2 C). The frequency of rejection did not vary between the experimental conditions ($\chi^2_{DF=5} = 1.72$, $p = 0.887$, W = 0.02; Fig. 2 D),

3.1.2 Female attractivity to males

Behaviors indicative of female attractiveness were analyzed using non-parametric statistics due to lack of normality. Here, all treatments were included since also the females treated with Oil or DPN were somewhat attractive to the males. The number of mounts received by the females was affected by the experimental conditions ($\chi^2_{DF=5} = 12.20$, $p = 0.032$, $W = 0.32$), but none of the conditions differed from baseline (ps > 0.092; data not

shown). The frequency of male pursuit of the females also differed between the experimental conditions ($\chi^2_{DF=5} = 19.07$, $p = 0.002$, $W = 0.50$). The pursuit frequency was lower during exposure to fox odor than at baseline ($p = 0.043$, $\delta = 0.17$). The other conditions had no effect on the frequency of pursuit (all ps > 0.270; Fig. 3 A).

The frequency of male anogenital sniffing of the females did not vary between the experimental conditions ($\chi^2_{DF=5} = 8.27$, $p = 0.142$, $W = 0.05$) and there was no meaningful effect on male resting with females (data not shown). To the contrary, the frequency of male sniffing of the females differed between the experimental conditions ($\chi^2_{DF=5} = 16.85$, $p = 0.005$, $W = 0.09$). Males sniffed females more often during exposure to chocolate than at baseline ($p = 0.019$, $\delta = 0.34$; Fig. 3 B).

3.1.3 Exploratory behavior

Ambulatory activity, expressed as the frequency of transition between zones in the entire seminatural environment, differed between the experimental conditions ($F_{5,170} = 10.59$, $p < 0.001$, $\eta_p^2 = 0.24$). Lavender ($p < 0.05$, d = 0.62), chocolate ($p < 0.05$, d = 0.92) and white noise ($p < 0.001$, d = 0.80) enhanced activity, whereas exposure to music and TMT had no effect (ps > 0.06; Fig. 4 A).

The transitions between zones in the open area also differed between experimental conditions ($F_{5,170} = 10.17$, $p < 0.001$, $\eta_p^2 = 0.23$). Exposure to lavender odor ($p < 0.05$, d = 0.67) and to chocolate ($p < 0.05$, d = 0.63) increased activity. The other conditions had no effect (ps > 0.166; Fig. 4 B). The number of transitions between zones in the burrow was also affected by experimental condition ($F_{5,170} = 17.34$, $p < 0.001$, $\eta_p^2 = 0.34$). Exposure to chocolate ($p < 0.05$, d = 1.04) and white noise ($p < 0.05$, d = 1.13) increased ambulatory activity in the burrow compared to baseline (Fig. 4 C).

There was an effect of experimental condition on the time spent in the open area ($F_{5,170}$ = 7.73, $p < 0.001$, $\eta_p^2 = 0.19$). Music ($p < 0.05$, d = 0.65) and white noise ($p < 0.05$, d = 0.62; Fig. 4 D) reduced the time spent in the open area compared to baseline, whereas the other conditions had no effect. Logically, the time spent in the burrow was also affected by experimental condition ($F_{5,170} = 7.73$, $p < 0.001$, $\eta_p^2 = 0.19$) in a way opposite to the open area. Music ($p > 0.05$, d = 0.65) and white noise (p <0.05, d = 0.62) enhanced the time spent in the burrow (Fig. 4 E).

The frequency of rearing was also modified by the experimental condition ($F_{5,170}$ = 5.81, $p < 0.001$, $\eta_p^2 = 0.15$). More rearing episodes were observed during exposure to white noise than at baseline ($p < 0.05$, d = 0.70; Fig. 4 F). The experimental conditions also altered the frequency of sniffing the floor ($F_{5,170} = 26.57$, $p < 0.001$, $\eta_p^2 = 0.44$). Exposure to chocolate ($p < 0.05$, d = 1.00) and white noise ($p < 0.05$, d = 1.42) enhanced the frequency relative to baseline (Fig. 4 G).

3.1.4 Female prosocial behaviors.

These behaviors could, obviously, be directed towards the other females in the group or to the males. We found it useful to analyze female-female and female-male interactions separately. The frequency of resting with another female did not change between experimental conditions ($F_{(5,170)} = 2.19$, $p = 0.058$, $\eta_p^2 = 0.06$). To the contrary, the time resting with males differed between experimental conditions ($F_{(5,170)} = 23.88$, $p < 0.001$, $\eta_p^2 = 0.41$) The females rested more with males during exposure to chocolate than at baseline ($p < 0.05$, d = 0.70). These data are illustrated in Fig. 5 A. We also found main effects of experimental condition both on the frequency of female sniffing another female ($F_{5,170} = 12.66$, $p < 0.001$, $\eta_p^2 = 0.27$) and a male ($F_{5,170} = 10.37$, $p < 0.001$, $\eta_p^2 = 0.23$). The frequency was always higher

during exposure to chocolate than at baseline (female-female, $p < 0.05$, d = 0.56; female-male, $p < 0.05$, d = 0.53). Data are shown in Fig. 5 B.

3.1.5 Female antisocial behavior towards males and females

Only the female nose-off behavior satisfied the criteria for parametric analysis. All the other antisocial behaviors were analyzed using non-parametric statistics.

The nose-off frequency varied between the experimental conditions regardless of the sex of the other party (males, $F_{5,170} = 3.95$, $p = 0.002$, $\eta_p^2 = 0.10$; females, $F_{5,170} = 7.32$, $p < 0.001$, $\eta_p^2 = 0.18$). More nose-off episodes occurred during exposure to chocolate than at baseline in both cases (males, $p < 0.05$, d = 0.35; females, $p < 0.05$, d = 0.61). White noise increased nose-off of other females ($p < 0.05$, d = 0.97) but not of males ($p > 0.05$, d = 0.24). Data are found in Fig. 6 A. As can be seen in Fig. 6 B, the frequency of fleeing from the males as well as from other females differed between conditions (males, $\chi^2_{DF=5} = 29.89$, $p < 0.001$, W = 0.16; females, $\chi^2_{DF=5} = 48.90$, $p < 0.001$, W = 0.26). The fleeing frequency was higher during exposure to chocolate and white noise than at baseline (males, chocolate-baseline: $p = 0.001$, $\delta = 0.48$; white noise-baseline: $p < 0.001$, $\delta = 0.41$; females, chocolate-baseline: $p = 0.001$, $\delta = 0.52$; white noise-baseline: $p < 0.001$, $\delta = 0.58$).

3.1.6 Non-social behaviors. There was no systematic effect of experimental condition on drinking, self-grooming or resting alone (data not shown).

3.1.7 Summary of the effects of experimental condition

The only experimental condition with an effect on the display of female sexual behaviors was white noise. The stimulus reduced these behaviors. The other conditions were ineffective with regard to sex behaviors, and none of the conditions modified female

attractivity. The availability of chocolate pellets stimulated ambulatory activity throughout the seminatural environment and enhanced both prosocial and antisocial interactions with both sexes. White noise was associated with avoidance of the open area and increased ambulatory activity in the burrow only. Antisocial behaviors were also enhanced in this condition. The other purportedly fear-inducing stimulus, fox odor, had no consistent effect. This was also the case for lavender odor and music.

3.2 Effects of hormone treatment

3.2.1 Female sexual behaviors

When the treatment effect was collapsed over all experimental conditions, it was found that the frequency of lordosis differed between treatments ($H_{3,\ N=38}$ =22.71, $p < 0.001$, $\eta^2_H = 0.60$). The Oil group displayed less lordoses than the EB group ($p < 0.001$, $\delta = 0.90$) and the PPT group ($p = 0.001$, $\delta = 0.60$). There was no difference between the Oil group and the DPN group ($p = 1$, $\delta = 0.00$). When evaluating treatment effects within each of the experimental conditions it was found that the number of lordoses differed between treatments at baseline ($H_{3,\ N=38} = 10.34$, $p = 0.016$, $\eta^2_H = 0.27$), during exposure to lavender odor ($H_{3,\ N=38} = 10.42$, $p = 0.015$, $\eta^2_H = 0.27$), music ($H_{3,\ N=38} = 12.14$, $p = 0.007$, $\eta^2_H = 0.32$) and chocolate ($H_{3,\ N=38} = 19.25$, $p < 0.001$, $\eta^2_H = 0.51$). No difference between the treatments was observed during exposure to the negative conditions, white noise ($H_{3,\ N=38} = 5.76$, $p = 0.124$, $\eta^2_H = 0.15$) and TMT odor ($H_{3,\ N=38} = 6.30$, $p = 0.098$, $\eta^2_H = 0.17$). The EB group showed more lordoses than the Oil group at baseline ($p = 0.006$, $\delta = 0.50$), during exposure to lavender odor ($p = 0.038$, $\delta = 0.40$), music ($p = 0.003$, $\delta = 0.40$) and chocolate ($p < 0.001$, $\delta = 0.60$). White noise and TMT odor suppressed responding, since the EB and Oil groups did no longer differ. The PPT group displayed more lordoses than the Oil group only during exposure to lavender

odor ($p = 0.009$, $\delta = 0.500$). DPN failed to stimulate lordosis in all conditions. Results are illustrated in Fig. 7 A.

There was a treatment effect on the LQ ($H_{3,\ N=38} = 13.50$, $p = 0.004$, $\eta^2_H = 0.36$) when all experimental conditions were collapsed. Only the EB group differed significantly from Oil ($p = 0.001$, $\delta = 0.60$). Treatment with PPT failed to significantly enhance the LQ ($p = 0.053$, $\delta = 0.40$), whereas DPN was completely inactive ($p = 1$, $\delta = 0$). The treatment effect was absent during exposure to white noise ($H_{3,\ N=38} = 2.80$, $p = 0.424$, $\eta^2_H = 0.07$) or fox odor ($H_{3,\ N=38} = 6.30$, $p = 0.098$, $\eta^2_H = 0.17$), but present in all other conditions (baseline, $H_{3,\ N=38} = 10.36$, $p = 0.016$, $\eta^2_H = 0.27$; lavender odor, $H_{3,\ N=38} = 10.81$, $p = 0.013$, $\eta^2_H = 0.29$; music, $H_{3,\ N=38} = 12.14$, $p = 0.007$, $\eta^2_H = 0.32$; chocolate, $H_{3,\ N=38} = 19.43$, $p < 0.001$, $\eta^2_H = 0.51$). During these conditions, EB-treated females systematically had a higher LQ than oil-treated females (baseline: $p = 0.006$, $\delta = 0.50$; lavender: $p = 0.006$, $\delta = 0.50$; music: $p = 0.003$, $\delta = 0.40$; chocolate: $p < 0.001$, $\delta = 0.60$). Exclusively during exposure to lavender, the PPT group displayed a higher LQ than the Oil group ($p = 0.050$, $\delta = 0.40$). Data are illustrated in Fig. 7 B.

There was also an effect of treatment on the frequency of paracopulatory behaviors when all experimental conditions were included in the analysis ($H_{3,\ N=38} = 21.06$, $p < 0.001$, $\eta^2_H = 0.55$). The Oil group displayed less paracopulatory behaviors than the EB group ($p < 0.001$, $\delta = 0.90$) and the PPT group ($p < 0.001$, $\delta = 0.80$). There was no difference between the Oil group and the DPN group ($p = 0.273$, $\delta = 0.30$).

We then proceeded to evaluate treatment effects on paracopulatory behavior under each of the experimental conditions. There was a difference between treatments at baseline ($H_{3,\ N=38} = 10.34$, $p = 0.016$, $\eta^2_H = 0.27$), during exposure to lavender odor ($H_{3,\ N=38} = 12.68$, $p = 0.005$, $\eta^2_H = 0.33$) and chocolate ($H_{3,\ N=38} = 11.84$, $p = 0.021$, $\eta^2_H = 0.26$. No difference

was found between the treatments in the frequency of paracopulatory behaviors during the exposure to music ($H_{3,\ N=38} = 6.95$, $p = 0.074$, $\eta^2_H = 0.18$), white noise ($H_{3,\ N=38} = 5.75$, $p = 0.124$, $\eta^2_H = 0.15$) and fox odor ($H_{3,\ N=38} = 7.46$, $p = 0.059$, $\eta^2_H = 0.20$). The EB group showed more paracopulatory behaviors than the Oil group at baseline ($p = 0.003$, δ=0.60), during exposure to lavender odor ($p = 0.002$, $\delta = 0.60$) and chocolate ($p = 0.003$, $\delta = 0.60$). The PPT group displayed more paracopulatory behaviors than the Oil group during exposure to lavender odor ($p = 0.038$, $\delta = 0.40$) but not during the other conditions. The DPN group did not differ from the Oil group in any condition. These data are found in Fig. 7 C. The frequency of rejections was not modified by the treatments ($H_{3,\ N=38} = 3.30$, $p = 0.347$, $\eta^2_H = 0.09$; data not shown).

3.2.2 Female attractivity to males

All experimental conditions collapsed, there was a main effect of treatment on the number of mounts received $H_{3,\ N=38} = 20.03$, $p < 0.001$, $\eta^2 = 0.53$). The females in the EB group were mounted more than the females in the Oil group ($p < 0.001$, $\delta = 0.90$) and so were females treated with PPT ($p = 0.024$, $\delta = 0.40$). The DPN group ($p = 0.678$, $\delta = 0.10$) did not differ from the Oil group.

The number of mounts received differed between treatments during exposure to lavender odor ($H_{3,\ N=38} = 10.25$, $p = 0.017$, $\eta^2_H = 0.27$), music ($H_{3,\ N=38} = 8.86$, $p = 0.031$, $\eta^2_H = 0.23$) and chocolate ($H_{3,\ N=38} = 15.60$, $p = 0.001$, $\eta^2_H = 0.41$). No difference was found at baseline, during exposure to white noise or fox odor (all ps > 0.103). The EB group received more mounts than the Oil group during exposure to lavender odor ($p = 0.012$; $\delta = 0.50$), music ($p = 0.016$; $\delta = 0.30$) and chocolate ($p < 0.001$, $\delta = 0.50$). Only during exposure to lavender odor, females treated with PPT were more mounted than those treated with Oil ($p = 0.031$;

δ=0.50). The DPN group was never different from the Oil group (all *p*s = 1). Data are summarized in Fig. 8 A.

All experimental conditions collapsed, there was an effect of the treatment on the frequency of male pursuit of the females ($H_{3,\ N=38} = 13.73$, $p = 0.003$, $\eta^2_H = 0.36$). The males pursued females given EB more than those given oil ($p = 0.002$, $\delta = 0.78$). Neither PPT- nor DPN-treated females differed from Oil (ps > 0.120).

The number of pursuit episodes differed between the treatments during exposure to lavender odor ($H_{3,\ N=38} = 8.24$, $p = 0.041$, $\eta^2_H = 0.22$). There was no difference between the treatments in the frequency of male pursuit at baseline, during exposure to music, chocolate, white noise and fox odor (all p's > 0.053). During exposure to lavender odor the EB group was more pursued by the males than the Oil group ($p = 0.017$, $\delta = 0.55$). The other treatment groups were not different from the Oil group (all ps > 0.129; Fig. 8 B).

The frequency of male anogenital sniffing of the females was unaffected by the treatments ($H_{3,\ N=38} = 4.67$, $p = 0.198$, $\eta^2_H = 0.12$) when all experimental conditions were considered. We then proceeded with an analysis of the effects of EB in each of the experimental conditions. It turned out that the frequency of male anogenital sniffing did not differ between treatments at any of the experimental conditions (ps > 0.058). Neither the frequency of male resting with females nor the frequency of sniffing the females differed between treatments at any of the experimental conditions (all ps > 0.100; data not shown).

3.2.3 Exploratory behaviors

There was no main effect of treatment for any of the exploratory behaviors (all ps > 0.071), and no interaction treatment * condition (ps > 0.286).

3.2.4 Female prosocial behavior

There was no main effect of treatment on the frequency of resting with another female ($F_{3,34} = 1.33$, $p = 0.281$, $\eta^2 = 0.11$) nor with a male ($F_{3,34} = 1.60$, $p = 0.207$, $\eta^2 = 0.12$). The interaction treatment * experimental condition was, however, significant with regard to resting with a male ($F_{15,170} = 2.99$, $p < 0.001$, $\eta_p^2 = 0.21$). This prompted tests for simple main effects of treatment within each of the experimental conditions. It turned out that there was a treatment effect during exposure to chocolate. The Tukey HSD test revealed that the females treated with PPT rested less with males than the females treated with oil ($p = 0.006$, d = 2.13). There was no effect at any other experimental condition. Data are shown in Fig. 9.

None of the treatments affected the frequency of sniffing another female or of the males. Likewise, the interactions between treatment and experimental condition was nonsignificant (all *p*s > 0.605; data not shown).

3.2.5 Female antisocial behavior towards males and females

The frequency of nose-off or of fleeing involving other females or males was not affected by treatment and there was no interaction between treatment and experimental condition (*p*s > 0.075; data not shown).

3.2.6 Female non-social behavior

There was no main effect of treatment on drinking, resting alone and self-grooming and there was no interaction between treatment and experimental condition (all *p*s > 0.230; data not shown).

3.2.7 Treatment effects on white-noise specific behaviors

Data for all the condition specific behaviors, except “hide alone” and “hide with another rat”, greatly deviated from the normal distribution and were analyzed with non-

parametric statistics. There was no treatment effect on the frequency of hiding alone, hiding with another rat, freezing, or startle (all *p*s > 0.662). The proportion of females fleeing from the noise differed between the treatments ($\chi^2_3 = 8.43$, $p = 0.038$; V = 0.47) and so did the latency to flee ($H_{3, N=38} = 8.23$, $p = 0.041$, $\eta^2_H = 0.22$). More females in the PPT group fled from the noise (Fisher exact test, $p = 0.038$, d = 0.66; Fig. 10 A) and they had a shorter latency to flee than the Oil group ($p = 0.008$, $\delta = 0.50$; Fig. 10 B). None of the other treatments differed from oil (*p*s > 0.512).

3.2.8 Treatment effects on chocolate-specific behaviors

The treatments did not influence the frequency of grabbing a chocolate pellet, of eating or sniffing the chocolate or the latency to approach the chocolate (all ps > 0.210; data not shown).

3.2.9 Weight gain

We calculated the weight difference between the moment the females were introduced into the seminatural environment and the moment they were removed. The weight change was expressed as a proportion of initial weight. After the 8 days spent in the environment, the weight gain was not evenly distributed among the females ($F_{3,34} = 9.62$, $p < 0.001$ $\eta^2_p = 0.46$). The EB group gained less weight than the Oil group ($p < 0.001$, $d = 2.36$). The PPT and the DPN groups did not differ from the Oil group (PPT-Oil: p = 0.068, $d = 1.22$; DPN-Oil: p = 0.852, $d = 0.36$). Data are shown in Fig. 11.

3.2.10 Summary of the treatment effects

EB, and to some extent PPT, stimulated the sexual behaviors. These treatments also enhanced some aspects of females' attractivity. DPN had no effect. Pro- or antisocial

behaviors were not modified by any of the treatments. This was also the case for exploratory behaviors.

EB stimulated sexual behaviors at baseline, during exposure to lavender odor and music, and when chocolate was available. During white noise or fox odor, EB-treated females did not differ from those treated with oil. PPT enhanced these behaviors only during exposure to lavender odor. During exposure to lavender odor and music, as well as when chocolate was available, EB enhanced female attractivity. PPT did so only during exposure to lavender odor. When chocolate was available, PPT reduced the time spent resting with males, and during white noise this compound facilitated the flight reaction. DPN did not affect any behavior in any condition.

4. Analysis of co-occurrences

Co-occurrence analysis identified the behavioral associations typical of the experimental condition without considering treatment. The baseline condition showed modest associations between sniffing another rat, nose off and fleeing. In a common cluster, we find white noise and chocolate availability. Associated to the white noise we find rearing. The main behavior in this cluster is sniffing the floor, probably a result of the enhanced exploratory behavior observed during these two conditions. Lavender odor is associated with the sexual behaviors as well as with male pursuit of the female, anogenital sniffing, and rejection. Finally, there is a cluster containing the conditions of fox odor and music, and the behaviors of resting alone and resting with another rat. Perhaps this illustrates that these conditions somewhat reduced social behaviors, making the non-social activity of resting more preeminent.

Analysis of treatment effects, ignoring experimental conditions, revealed that EB and PPT each belonged to a different cluster whereas Oil and DPN were found in the same

cluster. EB is associated with the sexual behaviors and drinking, whereas PPT is associated with exploratory behaviors, grooming, chocolate related behaviors and behavior patterns indicative of fear like freezing, hiding and startle. Oil and DPN are mainly related to sniffing other rats and nose off.

We then evaluated the treatments in each experimental condition. During baseline, Oil and DPN were found in the same cluster, whereas EB and PPT were in separate clusters (Fig. 12 A). During exposure to lavender odor, Oil and DPN were found in the same cluster, while EB was clearly associated with sexual behaviors, and PPT was related to exploratory behaviors and grooming. Data are found in Fig. 12 B. During music, a separate cluster containing EB and sexual behavior was found. PPT was found in another cluster, together with nose-off and fleeing. DPN formed a cluster together with rejection and drinking, whereas Oil was associated with exploratory and social behaviors (Fig. 12 C). Chocolate exposure again made Oil and DPN appear in the same cluster, with EB and PPT clearly separated. EB was associated with sexual behaviors, whereas PPT had a strong association with chocolate-related and exploratory behaviors (Fig. 12 D). White noise seemed to have altered behavior. All treatments now belonged to different clusters, and some behavior patterns formed clusters unrelated to the treatments. However, behaviors associated with white noise avoidance were found in the same cluster as DPN (Fig. 12 E). Fox odor also caused each of the treatments to belong to different clusters, with EB having a minor association with lordosis, and PPT with paracopulatory behavior (Fig. 12 F). DPN appeared in a separate cluster during exposure to TMT.

4. Discussion

4.1 General considerations

The data obtained in this study are meaningful only if two requirements are met: That the emotion-inducing procedures actually induced the intended emotion and that the experimental treatments were active with about the same intensity during the 5.83 h between the beginning of the baseline observation and the end of the observation during fox odor exposure. If one or both of the requirements fail, then we cannot determine the effects of negative and positive emotions on female sexual behavior. Likewise, it would be impossible to determine the role of the estrogen receptors in the responses to the experimental conditions.

Concerning the effectiveness of the experimental conditions, it is evident that some of them indeed altered behavior in the expected way. This is particularly the case for chocolate availability and white noise. The data show that the availability of chocolate had clear behavioral effects. Both prosocial, antisocial, and exploratory behaviors were stimulated. All these responses may be interpreted as manifestations of increased arousal. It is unlikely that the enhanced arousal was caused by a fear reaction, since the females made more visits to the open area than at baseline. It is known that food reward causes increased arousal (Killeen et al., 1978), often called food arousal (Tuersley & McCrohan, 1987), in addition to positive affect. The response to chocolate observed here is, then, what would be expected if this stimulus indeed induced positive affect. White noise clearly caused a fear reaction, manifested as avoidance of the open area and enhanced antisocial behavior. The effect of lavender odor on non-sexual behaviors was limited to enhanced activity in the open area. Whether this is a manifestation of positive affect, as we expected during lavender exposure, is an open question. However, the fact that lavender odor altered sexual behavior reinforces the notion that this stimulus might have had the desired effect. To the contrary, music did not produce any behavioral manifestation of positive affect. It rather appeared to cause a fear reaction, since the time in the open area was reduced. Finally, fox odor had very slight behavioral effects, and it cannot be concluded that the females responded with fear to this odor. It is

worth noting, though, that the co-occurrence analysis localized music and fox odor to the same cluster. Perhaps recent data showing that TMT is inferior to fox feces for producing fear responses in rats (Rampin et al., 2018) could explain the modest effect of this compound. This proposal is, of course, at variance to a substantial number of reports on the effectiveness of TMT (reviewed in Rosen et al., 2015).

In sum, the data allow us to suggest that chocolate availability, white noise, and perhaps lavender odor, had the intended effects, whereas music rather seemed to have an effect opposite to what we expected. Fox odor had slight effects, especially when compared to the other aversive stimulus, white noise. Nevertheless, we conclude that the first requirement mentioned above is, at least partially, satisfied.

The second requirement, the constant effect of the treatments during the entire observation period, is only possible to answer for oil and EB. The no-effect of oil is obviously constant, and there are good reasons to believe that the effects of EB outlast the observation period. We have earlier reported that female sociosexual behaviors are remarkably stable during the entire period of behavioral estrus in intact, cycling females in a seminatural environment. There is no significant change in lordosis frequency or LQ from the moment the first lordosis of the estrus period is displayed until the last lordosis (Chu & Ågmo, 2014). The change from non-receptivity to full receptivity and vice versa is almost instantaneous (Chu & Ågmo, 2015a). Similar data have been obtained in ovariectomized females, given the same doses of EB and P as used here. The duration of behavioral estrus in those females was 6.35 ± 0.42 h (mean ± SEM) (Le Moëne et al., 2015). This is longer than the duration of the present observation. Thus, the intrinsic effects of EB should have remained constant under all experimental conditions. The duration of the effects of PPT and DPN is unknown, but there is no compelling reason to assume that it is much different from that of EB. The same molecular events underlying sexual behavior are probably activated both by EB and PPT, and once

activated these events will have a similar time course (see Pfaff, 2017 for an extensive review of the molecular events underlying estrogen-induction of lordosis). Other actions, as well as those of DPN, can have a different time course, so present data need to be interpreted with some caution. Nevertheless, we propose that the effects of the treatments remained reasonably stable throughout the observation period.

In addition to the two requirements discussed above, there are to caveats to the meaningfulness of the data obtained in this experiment. The first is the possibility that one or several of the experimental conditions influenced the subsequent condition or conditions. We have no data to refute this possibility. However, it can also be argued that a sequence of events probably is part of rats' nightly experience in their natural habitat. Consequently, our design would contribute to enhance external validity compared to an experiment consisting of a single event. Nevertheless, it cannot be excluded that the specific sequence used here somewhat affected the results.

A second caveat concerns the confounding effects of potential circadian rhythms causing variations in behavior during the rather long observation. This, however, is highly unlikely. There is no change in receptivity from the beginning to the end of estrus (Chu and Ågmo, 2014). Locomotor activity shows peaks at both ends of the dark period, but it remains at a stable, high level during the middle part (Spiteri et al., 2012). Also food intake remains stable during that period (e.g. Kersten et al., 1980). Thus, circadian variations cannot explain the differences between the experimental conditions. Finally we would like to point out that the males' sexual activity was as high at the end of the observation period as at baseline (mean ± SEM number of mounts was 0.38 ± 0.18 at baseline vs. 0.31 ± 0.22 during exposure to TMT, $V = 36$, $p = 0.412$).

4.2 Negative and positive emotions and sexual behavior

One aim of the present study was to determine the effects of fear on female sexual behavior. White noise strongly inhibited sexual behaviors. In fact, females treated with EB did not show more of these behaviors in the presence of the noise than females treated with oil. It is interesting to note that fox odor also eliminated the difference between oil- and EB-treated females, even though this odor had no observable effects on behaviors indicating fear or anxiety. Perhaps female sexual behaviors are more sensitive to potential threats than other behaviors, at least in the seminatural environment. Little is known about the differential effect of stress on the entire behavioral repertoire, and the relative sensitivity of each behavior to the environmental context. It could even be proposed that observations of sex behavior in the seminatural environment are a potential, externally valid procedure for evaluating anxiolytic and anxiogenic drugs. This possibility should be further explored. We also want to point out that this is the first study in which the effects of aversive or fearful stimulation present in the test situation on sexual behavior have been evaluated.

While fear-inducing situations inhibited female sexual behavior, it appears that situations putatively leading to positive affect enhance these behaviors. This is evident for lavender odor, in which both EB- and PPT-treated females showed a non-significant tendency to display more sexual behaviors than in at baseline. These treatments also enhanced female attractivity to the males during the presence of lavender odor. Considering that lavender odor might induce a state of positive affect, it could be suggested that such affect facilitates female sexual behaviors, and makes the female more attractive to males. Whether the enhanced female attractiveness was due to factors intrinsic to the female or to lavender-induced, enhanced male responsivity to the females is not known. However, since female receptivity also was increased during exposure to lavender odor, it is likely that lavender-induced changes in the females was the main factor behind the observed behavioral changes. Chocolate availability did not have any particular effect on the sexual behaviors, despite the

fact that its consumption should have caused positive affect, just as lavender odor. The fact that our observations were limited to the moment when chocolate was available may, however, obscure any possible effect. The behavioral consequences of the positive affect caused by chocolate availability might have been counteracted by the urge to collect and consume the pellets.

Too little is known about the actions of lavender odor and chocolate to make any informed speculation about the causes of differences in effects on female behavior. Furthermore, as was the case with aversive stimuli, the influence of positive affect on sexual behavior has not been studied before, rendering any effort to propose explanations for these discrepancies still more difficult.

The many studies of the effects on female sexual behavior of drugs producing positive or negative affect (e.g. Guarraci & Bolton, 2014; Ågmo, 2014) are not directly relevant for the issue of how emotional state might alter sexual behaviors. The drugs have many effects in addition to altering the emotional state (e.g. Paredes & Ågmo, 2004; López, 2010), making such studies difficult to interpret. In fact, drug effects are usually explained in terms of altered neurotransmission rather than in terms of altered emotional states.

4.3 Anxiogenic and anxiolytic effects of estrogen receptor activation

We did not obtain much evidence for estrogen effects on fear behavior. EB and DPN had no effect whatsoever on the frequency of individual behavioral items, whereas PPT showed two signs of having produced or enhanced fear reactions. It reduced female resting with males in the presence of chocolate, and it enhanced the flight reaction in the presence of white noise. Both these effects can be interpreted as manifestations of fear or anxiety. PPT would then be anxiogenic in the chocolate and noise conditions. These conditions were associated with heightened arousal, and it has been shown that PPT indeed is anxiogenic in

such situations (Lund et al., 2005; Morgan et al., 2004; Spiteri et al., 2010a; Spiteri et al, 2010b; see also Borrow & Handa, 2017, for a review). It is also noteworthy that PPT belonged to a cluster separate from the other treatment clusters at all experimental conditions. This shows that the females treated with this compound had a behavioral structure different from all other treatments. This fact can probably be attributed to the fact that PPT stimulated sexual behaviors under some conditions and enhanced anxiety-like behaviors under others.

The complete lack of effect of DPN on the frequency and duration of the behaviors recorded here would indicate that the ERβ receptor is of little or no importance for sexual activity as well as for fear and anxiety in test procedures with external validity. However, the co-occurrence analysis showed that DPN belonged to a separate cluster in the situations that might be considered aversive, i.e. during exposure to music, white noise and fox odor. In the neutral or positive conditions, i.e. at baseline, during exposure to lavender odor and chocolate, DPN and oil belonged to the same cluster. This important observation suggests that actions at the ERβ only becomes apparent in contexts being aversive or even inducing fear. It appears, then, that present data confirm the lack of a role for the ERβ in sexual behavior as well as its importance for anxiety-related behaviors.

The potential role of the membrane receptor GPER1 has not been mentioned. This receptor is obviously activated in the EB-treated females, and perhaps also in the females treated with PPT. High concentrations of this ERα agonist bind to the GPER1, and DPN might be still less active (Petrie et al., 2013). Since the GPER1 has been implicated in fear responses as well as in female sexual behavior (reviewed in Hadjimarkou and Vasudevan, 2018), and since there is evidence for crosstalk between GPER1 and the ERα, it is possible that the GPER1 may have contributed to the effects observed in the present study. This issue is, however, too complex for being analyzed here.

Another issue not addressed here is the possible contribution of local synthesis of estrogens. Although such synthesis has been suggested to affect some of the behaviors studied here (reviewed in Cornil, 2018), present data have no relevance for this question.

4.4 On the utility of a seminatural environment and the problem of opposing effects of ERα and ERβ

When the experimental subjects are given the opportunity to express a substantial proportion of their natural behavioral repertoire, the multitude of data generated needs to be made comprehensible in some way or another. Moreover, the frequency or duration of behavioral items give only a rudimentary description of behavior. Behavior patterns are displayed in a continuous flow, and the sequence of behavior is completely ignored in frequency and duration analysis. The co-occurrence analysis and the clustering and visualization techniques employed here makes the patterning of behavior intelligible, and subtle modifications can be discovered. This, for example, made it possible to see that DPN affected behavior in aversive situations, even though the frequency and duration of none of the behaviors was altered.

At the time of the experimental manipulations, the rats had lived in the seminatural environment for 7 days. Consequently, they have had plenty of time to familiarize themselves to the environment and to the other rats. One manifestation of this is the almost complete absence of aggressive interactions. It is reasonable to assume that the subjects considered the environment as a safe place. We introduced the experimental conditions upon this baseline. A similar approach was employed in the studies of fear and aggression in the visual burrow system (Blanchard et al., 1995; Blanchard et al., 2001; Blanchard & Blanchard, 1989), a procedure not entirely different from the one used here. The present results may be most

illustrative with regard to the behavioral consequences of the activation of the estrogen receptors.

In nature, rats live and copulate in groups, and most of their activities are localized within the well-known home range. These characteristics are preserved in the seminatural environment but entirely absent in most other tests. It can be maintained that our procedure satisfies the requirements for a representative design in brunswikian terms (see Brunswik, 1955; Petrinovich, 1980, also Chu & Ågmo, 2016). Data from such designs have external validity in the sense that they might be applicable to situations other than the same in which the data were obtained. Therefore, we propose that activation of the ERα in female rats leads to the display of sexual behaviors and enhanced fear in unsafe or novel situations, even outside the laboratory setting. The ERβ does not modify the sexual behaviors, but it may be important for reducing fear in fear-inducing contexts, even outside of the laboratory setting.

Unfortunately, opposing actions of the ERα and ERβ would complicate the understanding of the behavioral actions of estrogens. In the intact animal, both receptors would be stimulated simultaneously, and opposing actions would then be nulled out. It is difficult to find a situation in which circulating estradiol would stimulate one receptors and not the other, meaning that opposing actions would not be physiologically relevant. The solution to this conundrum is not immediately apparent.

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References

Ågmo, A., 1997. Male rat sexual behavior. Brain Res. Prot. 1**,** 203-209.

Ågmo, A. (2014). Animal models of female sexual dysfunction: Basic considerations on drugs, arousal, motivation and behavior. *Pharmacology Biochemistry and Behavior, 121,* 3-15.

Antal, M. C., Petit-Demoulière, B., Meziane, H., Chambon, P., & Krust, A. (2012). Estrogen dependent activation function of ERβ is essential for the sexual behavior of mouse females. *Proceedings of the National Academy of Sciences of the United States of America, 109,* 19822-19827.

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., Mcewen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. *Psychoneuroendocrinology, 20,* 117-134.

Blanchard, R. J. & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. *Journal of Comparative Psychology, 103,* 70-82.

Blanchard, R. J., Dullogg, L., Markham, C., Nishimura, O., Compton, J. N., Jun, A. & Han, C., Blanchard, D. C. (2001). Sexual and aggressive interactions in a visible burrow system with provisioned burrows. *Physiology and Behavior, 72,* 245-254.

Borrow, A. P. & Handa, R. J. (2017). Estrogen receptors modulation of anxiety-like behavior. *Vitamins and Hormones, 103,* 27-52.

Boswell, K. J., Reid, L. D., Caffalette, C. A., Stitt, K. T., Klein, L. A., Lacroix, A. M. & Reid, M. L. (2006). Estradiol increases consumption of a chocolate cake mix in female rats. *Pharmacology Biochemistry and Behavior, 84,* 84-93.

Brotto, L. A., Hanson, L. A., & Gorzalka, B. B. (1999). Nefazodone attenuates the stress-induced facilitation of wet dog shaking behaviour but not the facilitation of sexual behaviour in female rats. European Journal of Pharmacology, 381(2–3), 101–104.

Brunswik, E. (1955). Representative design and probabilistic theory in a functional psychology. *Psychological Review, 62,* 193-217.

Butera, P. C. (2010). Estradiol and the control of food intake. *Physiology and Behavior, 99,* 175-180.

Chikahisa, S., Sano, A., Kitaoka, K., Miyamoto, K. I., & Sei, H. (2007). Anxiolytic effect of music depends on ovarian steroid in female mice. *Behavioural Brain Research, 179,* 50-59.

Chu, X. & Ågmo, A. (2014). Sociosexual behaviours in cycling, intact female rats (*Rattus norvegicus*) housed in a seminatural environment . *Behaviour, 151,* 1143-1184.

Chu, X. & Ågmo, A. (2015a). Sociosexual behaviors during the transition from non-receptivity to receptivity in rats housed in a seminatural environment. *Behavioural Processes, 113,* 24-34.

Chu, X. & Ågmo, A. (2015b). Sociosexual behaviors of male rats (*Rattus norvegicus*) in a seminatural environment. *Journal of Comparative Psychology, 129,* 132-144.

Chu, X. & Ågmo, A. (2016). Sociosexual interactions in rats: Are they relevant for understanding human sexual behavior? *International Journal of Psychological Research, 9,* 76-95.

Chu, X., Snoeren, E., & Ågmo, A. (2017). Functions of vocalization in sociosexual behaviors in rats (*Rattus norvegicus*) in a seminatural environment. *Journal of Comparative Psychology, 131,* 10-18.

Clarke, S. N. D. A. & Ossenkopp, K. P. (1998). Taste reactivity responses in rats: influence of sex and the estrous cycle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 274,* R718-R724.

Cliff, N. (1996). *Ordinal methods for behavioral data analysis* . Mahwah, NJ: Lawrence Erlbaum.

Conover, W. J. (1999). *Practical nonparametric statistics, 3rd ed.* New York, NY: Wiley.

Cornil, C.A., 2018. On the role of brain aromatase in females: why are estrogens produced locally when they are available systemically? Journal of Comparative Physiology A - Neuroethology, Sensory, Neural, and Behavioral Physiology 204**,** 31-49.

Cruz, J. N., Lima, D. D., Dal Magro, D. D., & Cruz, J. G. P. (2015). Anxiolytic effect of Mozart music over short and long photoperiods as part of environmental enrichment in captive *Rattus norvegicus* (Rodentia: Muridae). *Scandinavian Journal of Laboratory Animal Science, 41,* Article number 7.

de Oliveira Andrade Jr., E. & de Oliveira Andrade, E. (2016). Lexical analysis of the Code of Medical Ethics of the Federal Council of Medicine. *Revista da Associação Médica Brasileira, 62,* 123-130.

Díaz-Véliz, G., Alarcón, T., Espinoza, C., Dussaubat, N., & Mora, S. (1997). Ketanserin and anxiety levels: Influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. *Pharmacology Biochemistry and Behavior, 58,* 637-642.

Endres, T., Apfelbach, R., & Fendt, M. (2005). Behavioral changes induced in rats by exposure to trimethylthiazoline, a component of fox odor. *Behavioral Neuroscience, 119,* 1004-1010.

Escribano, B., Quero, I., Feijoo, M., Tasset, I., Montilla, P., & Tunez, I. (2014). Role of noise and music as anxiety modulators: Relationship with ovarian hormones in the rat. *Applied Animal Behaviour Science, 152,* 73-82.

Fendt, M., Endres, T., Lowry, C. A., Apfelbach, R., & McGregor, I. S. (2005). TMT-induced autonomic and behavioral changes and the neural basis of its processing. *Neuroscience and Biobehavioral Reviews, 29,* 1145-1156.

Flanagan-Cato, L. M., Grigson, P. S., & King, J. L. (2001). Estrogen-induced suppression of intake is not mediated by taste aversion in female rats. *Physiology & Behavior, 72,* 549-558.

Frasnelli, J., Hummel, C., Bojanowski, V., Warr, J., Gerber, J., & Hummel, T. (2015). Food-related odors and the reward circuit: Functional MRI. *Chemosensory Perception, 8,* 192-200.

Frye, C. A., Rhodes, M. E., Petralia, S. M., Walf, A. A., Sumida, K., & Edinger, K. L. (2006). 3α-hydroxy-5α-pregnan-20-one in the midbrain ventral tegmental area mediates social, sexual, and affective behaviors. *Neuroscience, 138,* 1007-1014.

Geary, N., Asarian, L., Korach, K. S., Pfaff, D. W., & Ogawa, S. (2001). Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-alpha null mice. *Endocrinology, 142,* 4751-4757.

Guarraci, F. A. & Bolton, J. L. (2014). Sexy stimulants: The interaction between psychomotor stimulants and sexual behavior in the female brain. *Pharmacology Biochemistry and Behavior, 121,* 53-61.

Hadjimarkou, M.M., Vasudevan, N., 2018. GPER1/GPR30 in the brain: Crosstalk with classical estrogen receptors and implications for behavior. The Journal of Steroid Biochemistry and Molecular Biology 176**,** 57-64.

Homiack, D., O'Cinneide, E., Hajmurad, S., Barrileaux, B., Stanley, M., Kreutz, M. R. & Schrader, L. A. (2017). Predator odor evokes sex-independent stress responses in male and female Wistar rats and reduces phosphorylation of cyclic-adenosine monophosphate response element binding protein in the male, but not the female hippocampus. *Hippocampus, 27,* 1016-1029.

Hrupka, B. J., Smith, G. P., & Geary, N. (1997). Ovariectomy and Estradiol Affect Postingestive Controls of Sucrose Licking. *Physiology & Behavior, 61,* 243-247.

Kersten, A., Strubbe, J. H., & Spiteri, N. J. (1980). Meal patterning of rats with changes in day length and food availability. *Physiology and Behavior*, *25*(6), 953–958. https://doi.org/10.1016/0031-9384(80)90316-9

Killeen, P. R., Hanson, S. J., & Osborne, S. R. (1978). Arousal: Its genesis and manifestation as response rate. *Psychological Review, 85,* 571-581.

Krezel, W., Dupont, S., Krust, A., Chambon, P., & Chapman, P. F. (2001). Increased anxiety and synaptic plasticity in estrogen receptor beta-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 12278-12282.

Kudwa, A. E., McGivern, R. F., & Handa, R. J. (2014). Estrogen receptor β and oxytocin interact to modulate anxiety-like behavior and neuroendocrine stress reactivity in adult male and female rats. *Physiology & Behavior, 129,* 287-296.

La Mela, I., Latagliata, E. C., Patrono, E., Puglisi-Allegra, S., & Ventura, R. (2010). Olfactory priming reinstates extinguished chocolate-induced conditioned place preference. *Appetite, 54,* 237-240.

Lampert, C., Arcego, D. M., Laureano, D. P., Diehl, L. s. A., da Costa Lima, I. F., Krolow, R., Pettenuzzo, L. F., Dalmaz, C. & Vendite, D. (2013). Effect of chronic

administration of tamoxifen and/or estradiol on feeding behavior, palatable food and metabolic parameters in ovariectomized rats. *Physiology & Behavior, 119,* 17-24.

Le Moëne, O. & Ågmo, A. (2017). The neuroendocrinology of sexual attraction. *Frontiers in Neuroendocrinology, in press*.

Le Moëne, O., Snoeren, E., Chu, X., & Ågmo, A. (2015). Changes in socio-sexual interactions during transition from non-estrus to estrus in devocalized as well as vocalizing hormone-treated, ovariectomized rats housed in a semi-natural environment . *Society for Neuroscience Abstracts, 715.08*.

LePape, G., Reinert, M., Blois-Heulin, C., & Belzung, C. (1997). Dissection of free exploratory activity into sub-units of behavior in mice. *Sciences et Techniques de l'Animal de Laboratoire, 22,* 131-139.

Li, W. J., Yu, H., Yang, J. M., Gao, J., Jiang, H., Feng, M., Zhao, X. Y. & Chen, Z. Y. (2010). Anxiolytic effect of music exposure on BDNFMet/Met transgenic mice. *Brain Research, 1347,* 71-79.

Linck, V. M., da Silva, A. L., Figueiro, M., Caramao, E. B., Moreno, P. R. H., & Elisabetsky, E. (2010). Effects of inhaled linalool in anxiety, social interaction and aggressive behavior in mice. *Phytomedicine, 17,* 679-683.

López, H. H. (2010). Cannabinoid-hormone interactions in the regulation of motivational processes. *Hormones and Behavior, 58,* 100-110.

Lund, T. D., Rovis, T., Chung, W. C. J., & Handa, R. J. (2005). Novel actions of estrogen receptor-beta on anxiety-related behaviors. *Endocrinology, 146,* 797-807.

Marchand, P. & Ratinaud, P. (2012). L'analyse de similitude appliquée aux corpus textuels?: les primaires socialistes pour l'élection présidentielle française (septembre-octobre 2011). In A. Dister, D. Longrée, & G. Pumelle (Eds.), (pp. 687-698). Liège, Belgium: LASLA-SESLA.

Martínez-Mota, L., Estrada-Camarena, E., López-Rubalcava, C., Contreras, C. M., & Fernández-Guasti, A. (2000). Interaction of desipramine with steroid hormones on experimental anxiety. *Psychoneuroendocrinology, 25,* 109-120.

Mazzucco, C. A., Walker, H. A., Pawluski, J. L., Lieblich, S. E., & Galea, L. A. M. (2008). ERα, but not ERβ, mediates the expression of sexual behavior in the female rat. *Behavioural Brain Research, 191,* 111-117.

Meyers, M. J., Sun, J., Carlson, K. E., Marriner, G. A., Katzenellenbogen, B. S., & Katzenellenbogen, J. A. (2001). Estrogen receptor-β potency-selective ligands: Structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *Journal of Medicinal Chemistry*, *44*, 4230–4251.

Miller, N. R., Jover, T., Cohen, H. W., Zukin, R. S., & Etgen, A. M. (2005). Estrogen can act via estrogen receptor α and β to protect hippocampal neurons against global ischemia-induced cell death. *Endocrinology, 146,* 3070-3079.

Mora, S., Dussaubat, N., & Díaz-Véliz, G. (1996). Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. *Psychoneuroendocrinology, 21,* 609-620.

Morgan, M. A. & Pfaff, D. W. (2001). Effects of estrogen on activity and fear-related behaviors in mice. *Hormones and Behavior, 40,* 472-482.

Morgan, M. A., Schulkin, J., & Pfaff, D. W. (2004). Estrogens and non-reproductive behaviors related to activity and fear. *Neuroscience and Biobehavioral Reviews, 28,* 55-63.

Morrow, B. A., Elsworth, J. D., & Roth, R. H. (2002). Fear-like biochemical and behavioral responses in rats to the predator odor, TMT, are dependent on the exposure environment. *Synapse, 46,* 11-18.

Nomikos, G. G. & Spyraki, C. (1988). Influence of estrogen on spontaneous and diazepam-induced exploration of rats in an elevated plus maze. *Neuropharmacology, 27,* 691-696.

Ogawa, S., Chan, J., Gustafsson, J. Å., Korach, K. S., & Pfaff, D. W. (1999). Survival of reproductive behaviors in estrogen receptor β gene-deficient (βERKO) male and female mice. *Proceedings of the National Academy of Sciences of the United States of America, 96,* 12887-12892.

Ogawa, S., Eng, V., Taylor, J., Lubahn, D. B., Korach, K. S., & Pfaff, D. W. (1998). Roles of estrogen receptor-α gene expression in reproduction-related behaviors in female mice. *Endocrinology, 139,* 5070-5081.

Oyola, M. G., Portillo, W., Reyna, A., Foradori, C. D., Kudwa, A., Hinds, L., Hand, R. J. & Mani, S. K. (2012). Anxiolytic effects and neuroanatomical targets of estrogen receptor-β (ERβ) activation by a selective ER β agonist in female mice. *Endocrinology, 153,* 837-846.

Paredes, R. G. & Ågmo, A. (2004). Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. *Progress in Neurobiology, 73,* 179-226.

Petrie, W.K., Dennis, M.K., Hu, C., Dai, D., Arterburn, J.B., Smith, H.O., Hathaway, H.J., Prossnitz, E.R., 2013. G protein-coupled estrogen receptor-selective ligands modulate endometrial tumor growth. Obstet Gynecol Int 2013**,** Article number 472720.

Petrinovich, L. (1980). Brunswikian behavioral biology. In K. R. Hammond & N. E. Wascoe (Eds.), *Realizations of Brunswik's representative design* (pp. 85-93). San Francisco, CA: Jossey-Bass.

Pfaff, D. (2017). *How the vertebrate brain regulates behavior. Direct from the lab.* Cambridge, MA: Harvard University Press.

Pisani, S. L., Neese, S. L., Katzenellenbogen, J. A., Schantz, S. L., & Korol, D. L. (2016). Estrogen receptor-selective agonists modulate learning in female rats in a dose- and task-specific manner. *Endocrinology, 157,* 292-303.

Rampin, O., Jerôme, N., Saint-Albin, A., Ouali, C., Boué, F., Meunier, N. & Nielsen, B. L. (2018). Where is the TMT? GC-MS analyses of fox feces and behavioral responses of rats to fear-inducing odors. *Chemical Senses, 43,* 105-115.

Reinert, A. (1983). Une méthode de classification descendante hiérarchique: Application à l'analyse lexicale par contexte. *Les Cahiers de l'analyse des Données, 8,* 187-198.

Reinert, M. (1990). Alceste. Une méthodologie d'analyse des données textuelles et une application: Aurélia de Gérard de Nerval. *Bulletin of Sociological Methodology/Bulletin de Méthodologie Sociologique, 26,* 24-54.

Reynaert, M. L., Marrocco, J., Mairesse, J., Lionetto, L., Simmaco, M., Deruyter, L., Allorge, D., Moles, A., Pittaluga, A., Maccari, S., Morley-Fletcher, S., Van Camp, G. & Nicoletti, F. (2016). Hedonic sensitivity to natural rewards is affected by prenatal stress in a sex-dependent manner. *Addiction Biology, 21,* 1072-1085.

Rissman, E. F., Wersinger, S. R., Taylor, J. A., & Lubahn, D. B. (1997). Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Hormones and Behavior, 31,* 232-243.

Rosen, J. B., Asok, A., & Chakraborty, T. (2015). The smell of fear: innate threat of 2,5-dihydro-2,4,5-trimethylthiazoline, a single molecule component of a predator odor. *Frontiers in Neuroscience, 9,* Article number 292.

Sepehr, E., Lebl-Rinnova, M., Mann, M. K., Pisani, S. L., Churchwell, M. I., Korol, D. L., Katzenellenbogen, J. A. & Doerge, D. R. (2012). Pharmacokinetics of the estrogen receptor subtype-selective ligands, PPT and DPN: Quantification using UPLC-ES/MS/MS. *Journal of Pharmaceutical and Biomedical Analysis, 71,* 119-126.

Shaw, D., Annett, J. M., Doherty, B., & Leslle, J. C. (2007). Anxiolytic effects of lavender oil inhalation on open-field behaviour in rats. *Phytomedicine, 14,* 613-620.

Shen, L., Wang, D. Q. H., Xu, M. F., Woods, S. C., & Liu, M. (2017). BDNF/TrkB signaling mediates the anorectic action of estradiol in the nucleus tractus solitarius. *Oncotarget, 8,* 84028-84038.

Snoeren, E. M. S., Antonio-Cabrera, E., Spiteri, T., Musatov, S., Ogawa, S., Pfaff, D. W. & Ågmo, A. (2015). Role of oestrogen receptors in sociosexual behaviour in female rats housed in a seminatural environment. *Journal of Neuroendocrinology, 27,* 803-818.

Södersten, P. & Eneroth, P. (1982). Estradiol and progesterone in the control of sexual receptivity in female rats. *Scandinavian Journal of Psychology, 23, Suppl. 1,* 127-132.

Spiteri, T. & Ågmo, A. (2006). Modèles precliniques du désir sexuel. *Sexologies, 15,* 241-249.

Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D. W., & Ågmo, A. (2010b). The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behavioural Brain Research, 210,* 211-220.

Spiteri, T., Musatov, S., Pfaff, D. W., Ogawa, S., & Ågmo, A. (2010a). Preoptic estrogen receptor alpha and anxiety-like behavior in female rats. *Society for Neuroscience Abstracts*.

Spiteri, T., Ogawa, S., Musatov, S., Pfaff, D. W., & Ågmo, A. (2012). The role of the estrogen receptor α in the medial preoptic area in sexual incentive motivation, proceptivity and receptivity, anxiety, and wheel running in female rats. *Behavioural Brain Research, 230,* 11-20.

Stauffer, S. R., Coletta, C. J., Tedesco, R., Nishiguchi, G., Carlson, K., Sun, J., Katzenellenbogen, B. S., Katzenellenbogen, J. A. (2000). Pyrazole ligands: Structure-

affinity/activity relationships and estrogen receptor-α-selective agonists. *Journal of Medicinal Chemistry*, *43*, 4934–4947.

Tomczak, M. & Tomczak, E. (2014). The need to report effect size estimates revisited. An ovreview of some recommended measures of effect size. *Trends in Sport Sciences, 1,* 19-25.

Truitt, W., Harrison, L., Guptarak, J., White, S., Hiegel, C., & Uphouse, L. (2003). Progesterone attenuates the effect of the 5-HT1A receptor agonist, 8-OH-DPAT, and of mild restraint on lordosis behavior. *Brain Research, 974,* 202-211.

Tsang, H. W. H. & Ho, T. Y. C. (2010). A systematic review on the anxiolytic effects of aromatherapy on rodents under experimentally induced anxiety models. *Reviews in the Neurosciences, 21,* 141-152.

Tsang, H. W. H., Lo, S. C. L., Chan, C. C. H., Ho, T. Y. C., Fung, K. M. T., Chan, A. H. L. , Ho, T. Y. C., Fung, K. M. T., Chan, A. H. L. & Au, D. W. H. (2013). Neurophysiological and behavioural effects of lavender oil in rats with experimentally induced anxiety. *Flavour and Fragrance Journal, 28,* 168-173.

Tuersley, M. D. & McCrohan, C. R. (1987). Food arousal in the pond snail, *Lymnaea stagnalis*. *Behavioral and Neural Biology, 48,* 222-236.

Umezu, T., Nagano, K., Ito, H., Kosakai, K., Sakaniwa, M., & Morita, M. (2006). Anticonflict effects of lavender oil and identification of its active constituents. *Pharmacology Biochemistry and Behavior, 85,* 713-721.

Uphouse, L., Hiegel, C., Sarkar, J., Hurlburt, J., Templeton, C., Guptarak, J. & Maswood, N. (2008). Female gonadal hormones, mild restraint, and male preference. *Pharmacology Biochemistry and Behavior, 90,* 758-762.

Uphouse, L., Selvamani, A., Lincoln, C., Morales, L., & Comeaux, D. (2005). Mild restraint reduces the time hormonally primed rats spend with sexually active males. *Behavioural Brain Research, 157,* 343-350.

Valax, M. F., Marine, C., & Reinert, M. (1990). Traitement de données structurées par un ordre temporel ou hiérarchique : Utilisation dans l'analyse de l'activité . *Le Travail Humain, 53,* 79-89.

Walf, A. A., Ciriza, I., Garcia-Segura, L. M., & Frye, C. A. (2008a). Antisense oligodeoxynucleotides for estrogen receptor-β and α attenuate estradiol's modulation of affective and sexual behavior, respectively. *Neuropsychopharmacology, 33,* 431-440.

Walf, A. A. & Frye, C. A. (2005). Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. *Neuropsychopharmacology, 30,* 1288-1301.

Walf, A. A. & Frye, C. A. (2007). Estradiol decreases anxiety behavior and enhances inhibitory avoidance and gestational stress produces opposite effects. *Stress-the International Journal on the Biology of Stress, 10,* 251-260.

Walf, A. A. & Frye, C. A. (2008). Parity and estrogen-administration alter affective behavior of ovariectomized rats. *Physiology and Behavior, 93,* 351-356.

Walf, A. A., Koonce, C. J., & Frye, C. A. (2008b). Estradiol or diarylpropionitrile administration to wild type, but not estrogen receptor beta knockout, mice enhances performance in the object recognition and object placement tasks. *Neurobiology of Learning and Memory, 89,* 513-521.

Weyers, P., Janke, W., Macht, M., & Weijers, H. G. (1994). Social and nonsocial open-field behavior of rats under light and noise stimulation. *Behavioural Processes, 31,* 257-267.

Williams, G. W., McGinnis, M. Y., & Lumia, A. R. (1992). The effects of olfactory bulbectomy and chronic psychosocial stress on serum glucocorticoids and sexual behavior in female rats. *Physiology and Behavior, 52,* 755-760.

Table 1. Summary of the experimental procedure on the test day.

	Experimental condition					
	Baseline	Lavender odor	Music	Chocolate	White noise	Fox odor
Time of the day	12:30-13:00	13:00-13:30	14:20-14:44	15:35-16:05	16:55-17:10	18:00-18:30
Observation time	12:45-13:00	13:15-13:30	14:29-14:44	15:35-15:50	16:54-17:09	18:15-18:30
Duration	30 min	30 min	24 min 18 s	30 min	15 min	30 min

Table 2. Definition of recorded behaviors.

f = frequency; d = duration; l = latency; o = occurrence.

Category	Behavior pattern	Definition
Female sexual behaviors	*Lordosis; f*	Posture of the female arching her back, exposing her vagina.
	Paracopulatory behaviors; f,d	Approach to a male followed by runaway, often associated with hops, darts, and ear wiggling.
Female attractivity	*Rejection; f*	Female kicks, boxes or assumes a belly up posture.
	Mounts received; f	Male catches the female by her waist and puts his belly over her back, with pelvic thrusting.
Prosocial behaviors	*Male pursuit; f,d*	Male runs after a female with his snout in close to the anogenital zone of the female.
	Male sniffing female; f,d	Snout close to a female, sniffing the fur
	Male sniffing anogenital area, f,d	Male sniffs the anogenital zone of a female by putting his snout under her tail.
	Resting with other females; f,d	Rests immobilized in relaxed position at a distance shorter than one rat to one or several females.
	Resting with males,f,d	Rests immobilized in relaxed position at a distance shorter than one rat to one or several males.

		Snout close to a female, sniffing the fur
	Sniffing other females,f,d	Snout close to a male, sniffing the fur
	Sniffing males; f,d	
Antisocial behaviors	*Hiding with another rat[b]; f,d*	Immobilized in a corner or in a nest box within one body length of the other rat.
	Nose-off male; f,d	The female faces a male, nose to nose, heads up, with or without boxing.
	Nose-off female; f,d	The female faces another female, nose to nose, heads up, with or without boxing.
	Flee from male; f	Escapes from agonistic interaction by running away or simply turning head away from a male.
Solitary behaviors	*Flee from another female; f*	Escapes from agonistic interaction by running away or simply turning head away from a female.
	Resting alone; f,d	Rests immobilized in relaxed position at a distance longer than one rat to a conspecific.
	Drinking; f,d	Self explanatory.
	Selfgrooming and scratching; f,d	Self explanatory.

	Hide alone[b]; f,d	Immobilized in a corner or nest box at a distance longer than one body length to another rat.
	Approach to chocolate[a]; f,l	Coming close enough for making snout or paw contact with the chocolate pellets. The latency is the time between putting the petri dish on the floor of the open area and the first approach.
	Grabbing[a]; f	Grabbing chocolate with paws or mouth.
	Eating[a]; f,d	Chew on chocolate.
	Freezing[b]; f,d	Immobilized in rigid position without any movement including those of vibrissa.
	Startle[b]; o	Sudden reflex contractions of the major muscles of the body, leading to a little jump on the spot. Only observed in response to onset of the white noise.
Exploratory behaviors and ambulatory activity	*Flee from noise[b]; o,l*	Rush into the burrows at the onset of the white noise. The latency is the time from onset of the noise until the rat escapes from the open field into the burrow.
	Sniffing the floor, f,d	Sniffs the floor material with all four paws on the floor
	Rearing; f,d	Sniffs the air while standing on the hind legs.
	Transitions; f	Displays a behavior in a zone different from the one in which the previous behavior was displayed.

[a], behavior observed only in the presence of chocolate. [b], behavior only observed during exposure to white noise.

Figure legends:

Figure 1. A. Picture of the seminatural environment. B. The division in zones.

Figure 2. Effect of the experimental conditions on female sexual behavior, for EB- and PPT-treated groups only, both treatments collapsed. A. Lordosis frequency. B. Lordosis quotient. C. Frequency of paracopulatory behaviors. D. Rejection frequency. Data are mean ± SEM. Friedman test, post hoc: Conover-Iman test. *, different from baseline. N=20.

Figure 3. Effect of the experimental conditions on female attractivity to males, all treatments collapsed. A. Pursuit frequency. B. Frequency of male sniffing of a female. Data are mean ± SEM. Friedman test, post hoc: Conover-Iman test. *, different from baseline. N=38.

Figure 4. Effect of the experimental conditions on female exploratory behavior, all treatments collapsed. A. Frequency of transition between the zones of the seminatural environment. B. Frequency of transition between the zones of the open area. C. Frequency of transition between the zones of the burrow system. D. Time spent in the open area. E. Time spent in the burrow system. F. Rearing frequency. G. Frequency of sniffing the floor. Data are mean ± SEM. Repeated measures two-way ANOVA, post hoc: Tukey HSD. *, different from baseline. N=38.

Figure 5. Effect of the experimental conditions on female social behavior directed to males (black) and to other females (white), all treatments collapsed. **A.** Frequency of resting with another rat. B. Frequency of sniffing another rat. Data are mean ± SEM. Repeated measures two-way ANOVA, post hoc: Tukey HSD. *, different from baseline. N=38.

Figure 6. Effect of the experimental conditions on female anti-social behavior directed to males (black) and to other females (white), all treatments collapsed. A. Nose-off frequency; repeated measures two-way ANOVA, post hoc: Tukey HSD. B. Frequency of fleeing from

another rat; Friedman test, post hoc: Conover-Iman test. Data are mean ± SEM. *, different from baseline; N=38.

Figure 7. Effect of the treatment on female sexual behavior. A. Lordosis frequency. B. Lordosis quotient. C. Frequency of paracopulatory behaviors. Kruskal-Wallis test, post hoc: Conover test. Data are mean ± SEM. *, different from the Oil group. Oil: n=8, EB: n=10, PPT: n=10, DPN: n=10.

Figure 8. Effect of the treatment on female attractivity to males. A. Mount frequency. B. Pursuit frequency. Kruskal-Wallis test, post hoc: Conover test. Data are mean ± SEM. *, different from the Oil group. Oil: n=8, EB: n=10, PPT: n=10, DPN: n=10.

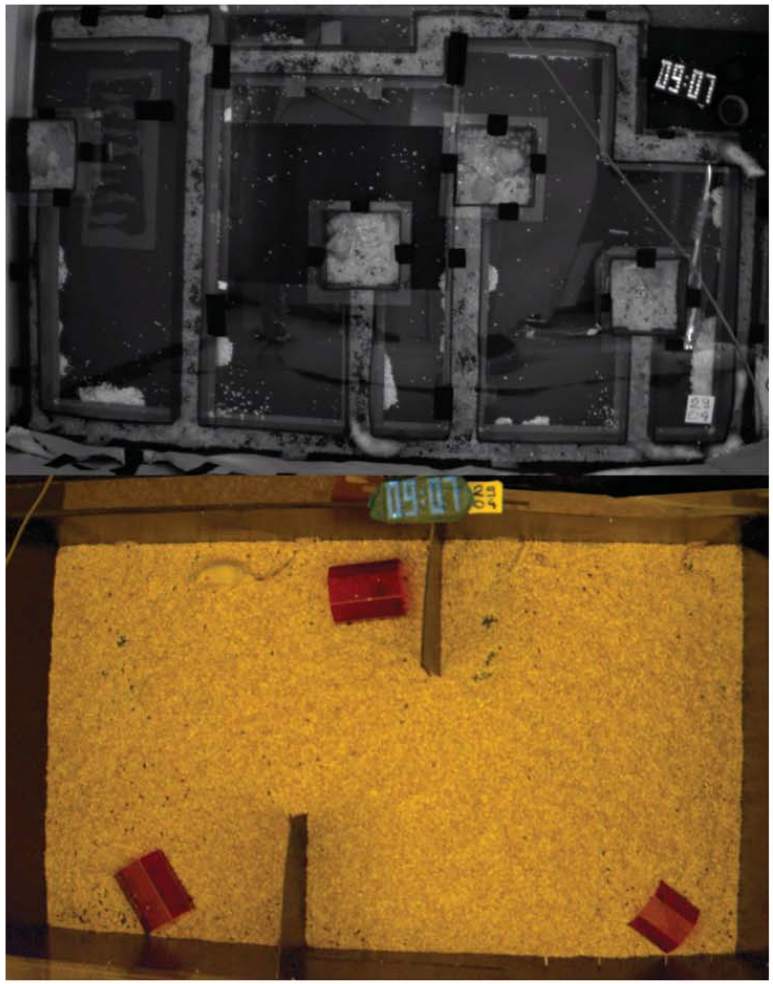
Figure 9. Effect of the interaction between the treatment and the experimental condition on the frequency of female resting with a male. Repeated measures two-way ANOVA, post hoc: Tukey HSD. *, different from the Oil group. Oil: n=8, EB: n=10, PPT: n=10, DPN: n=10.

Figure 10. Effect of the treatment on the response to the onset of white noise. A. Probability for an individual to flee from the white noise; Fisher exact test; statistical significance: *, different from the Oil group. Latency to flee the noise at its onset (B); Kruskal-Wallis test, post hoc: Conover test; statistical significance: * different from the Oil group. Oil: n=8, EB: n=10, PPT: n=10, DPN: n=10.

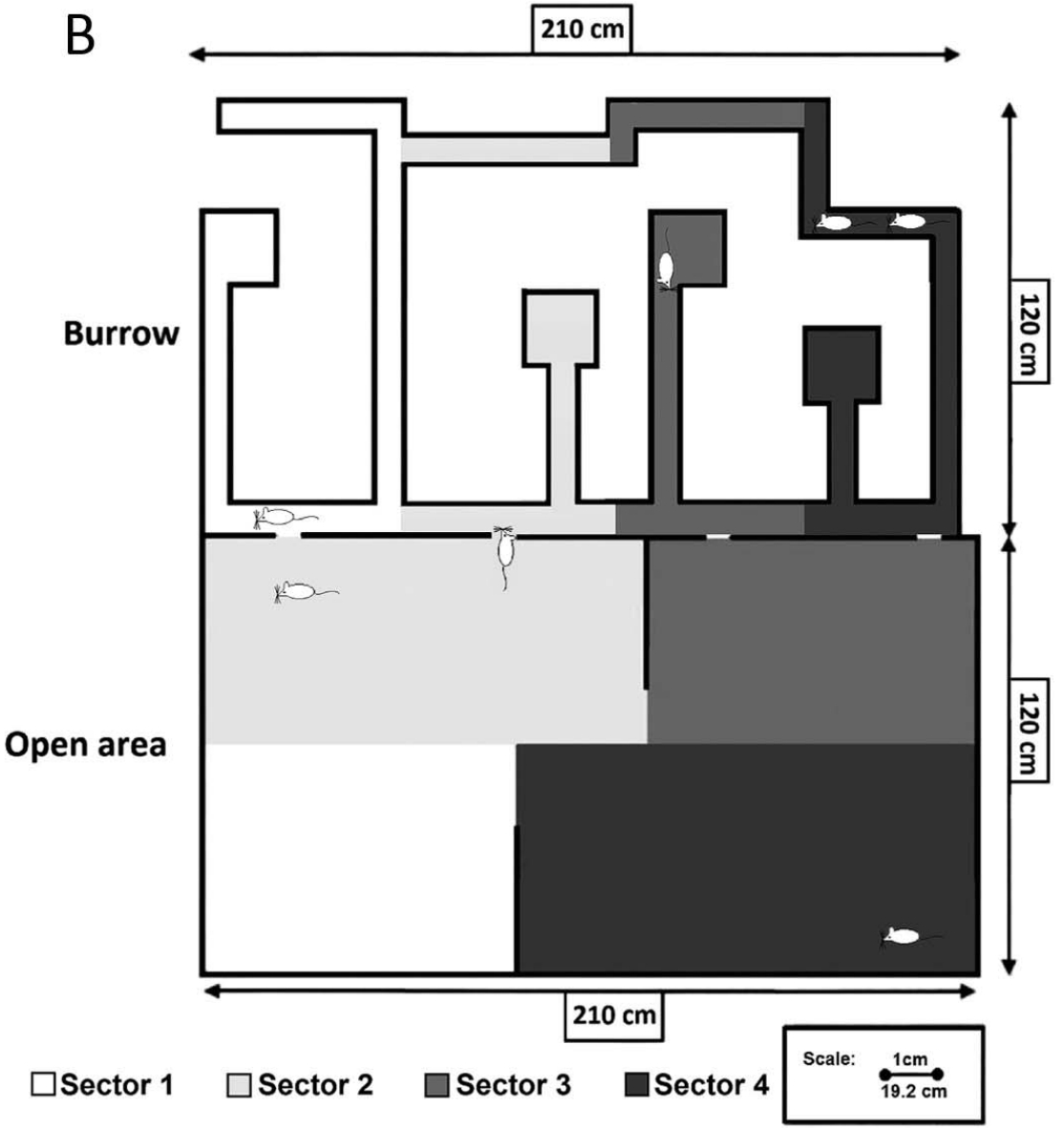
Figure 11. Effect of the treatment on weight gain. Repeated measures two-way ANOVA, post hoc: Tukey HSD. Data are mean ± SEM. *, different from the Oil group. Oil: n=8, EB: n=10, PPT: n=10, DPN: n=10.

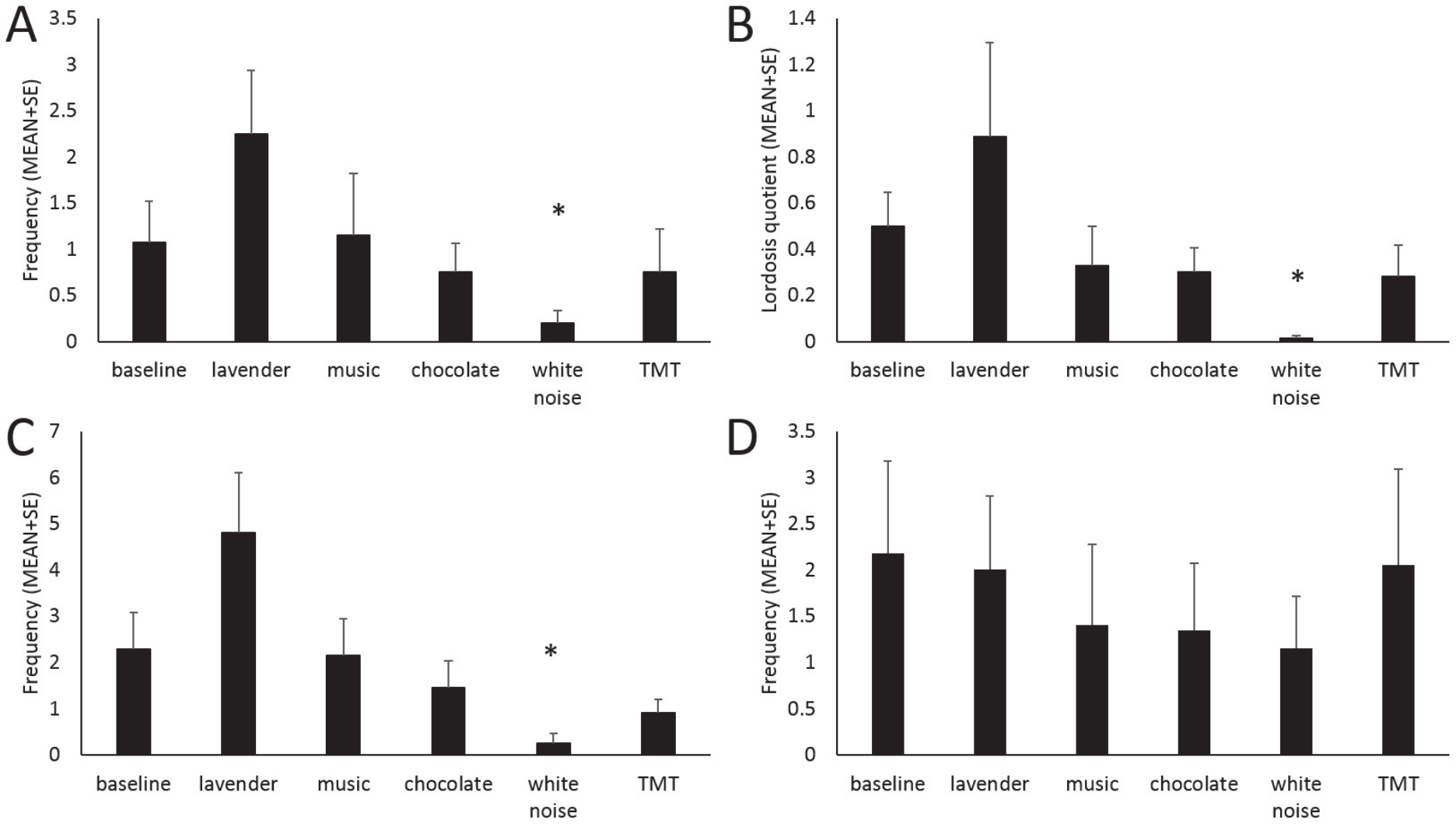
Figure 12. Co-occurrence analysis showing main behavioral associations typical of each of the treatments. A. Baseline. B. Exposure to lavender odor. C. Music. D. Chocolate. E. White noise. F. Fox (TMT) odor. Clusters of behavioral association are represented in halos of different colors.

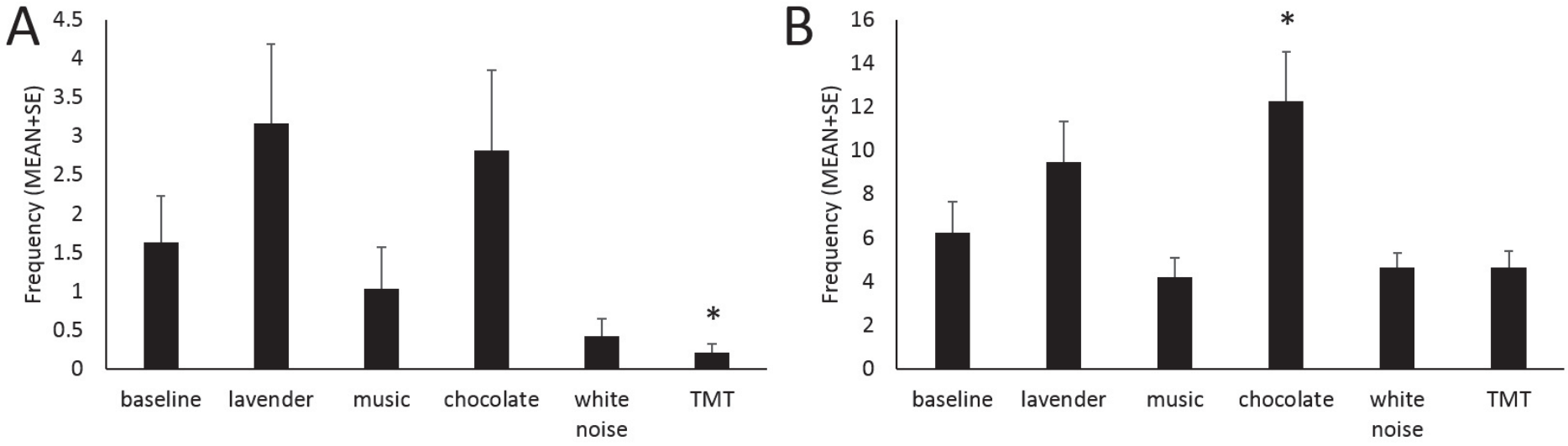
A

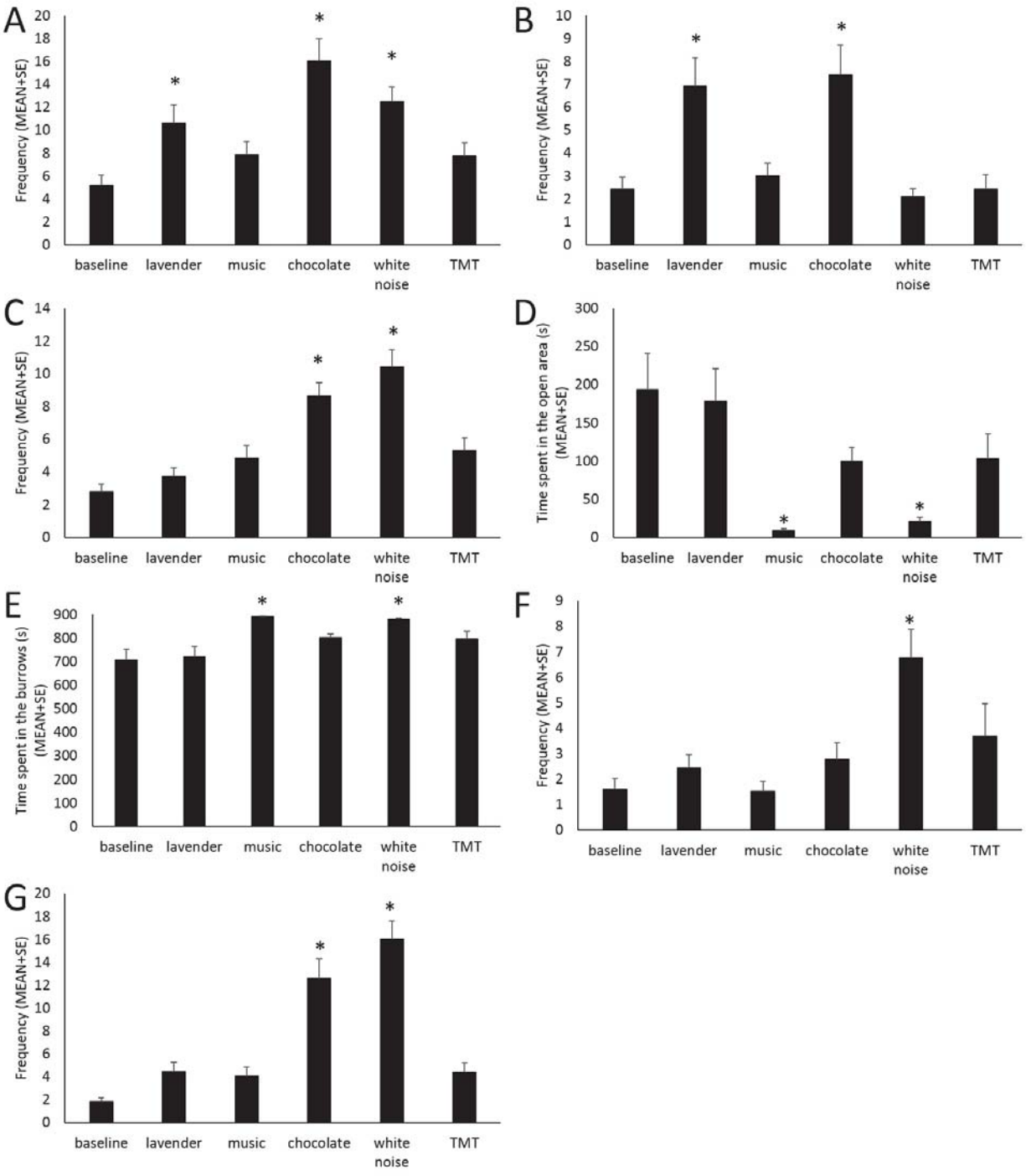

09:07
09:07

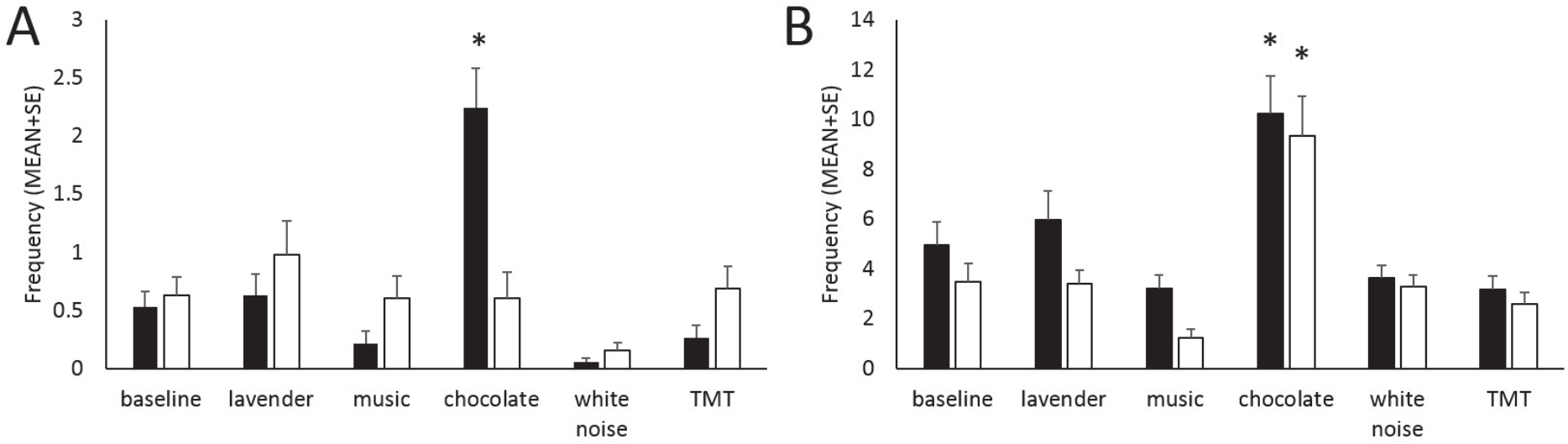
B

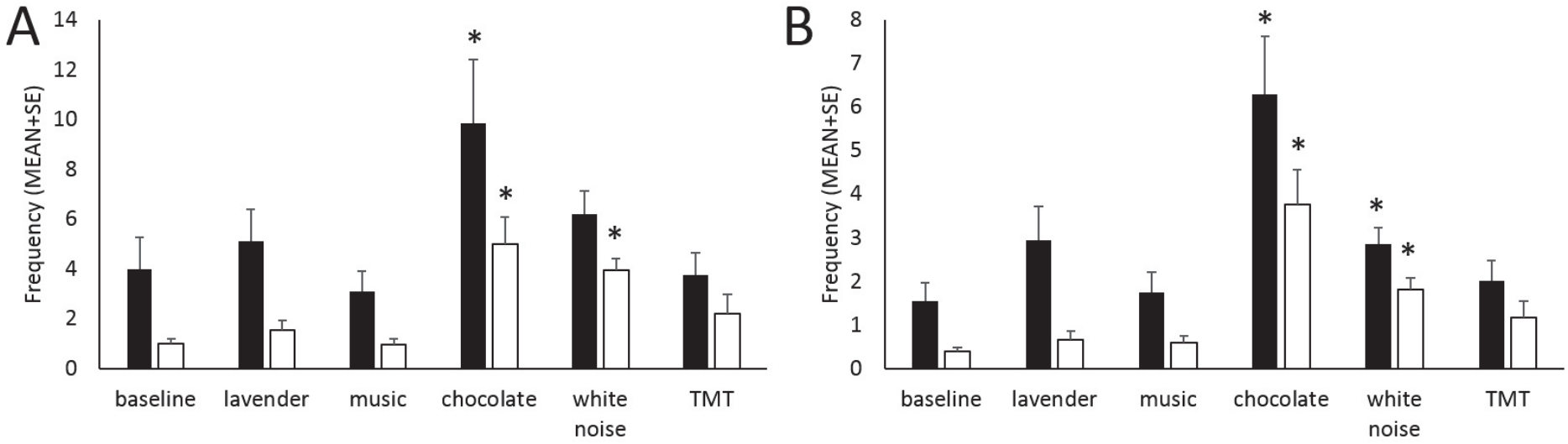

210 cm
Burrow
120 cm
Open area
120 cm
210 cm
Sector 1
Sector 2
Sector 3
Sector 4
Scale:
1cm
19.2 cm

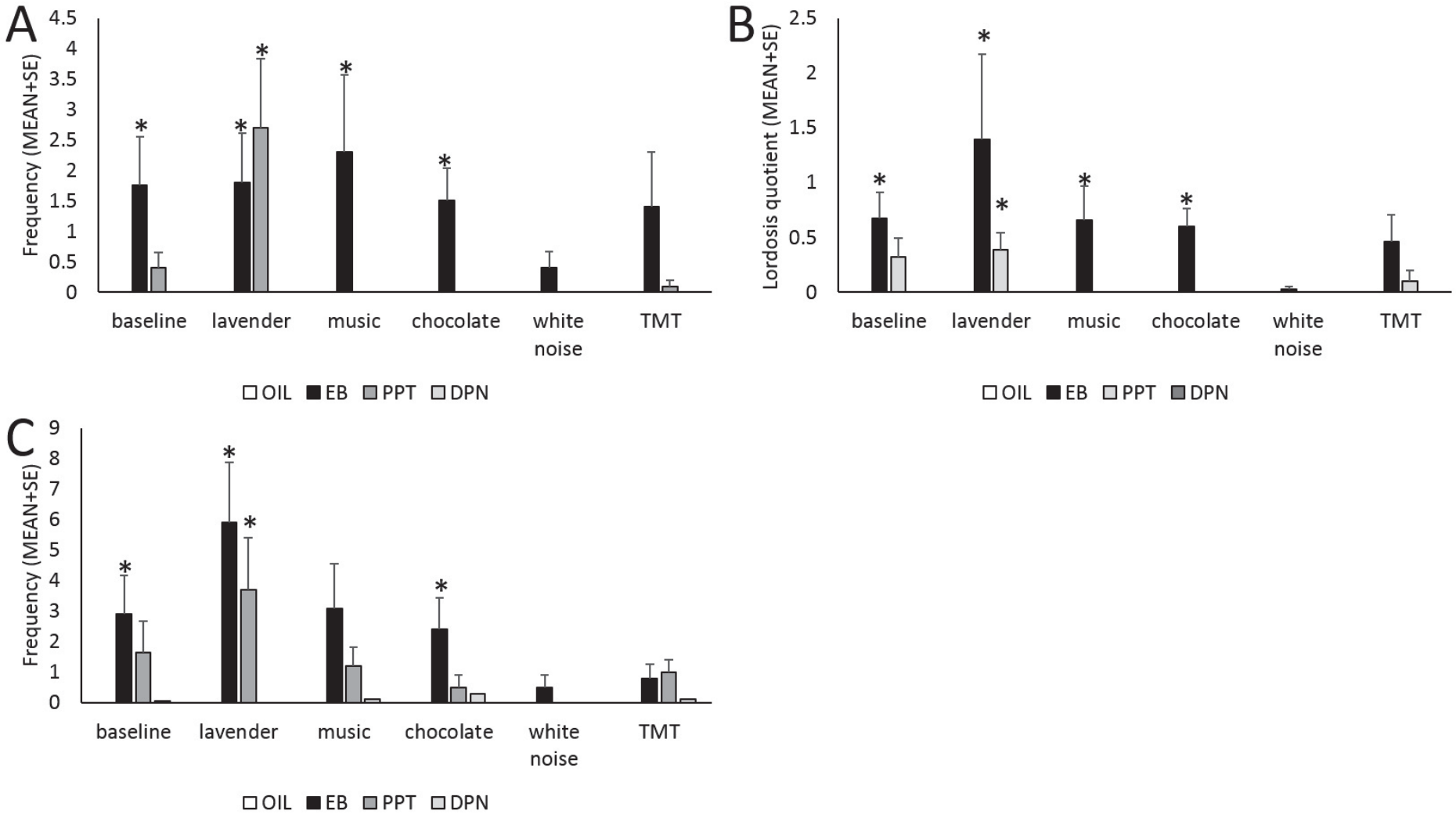

A
Frequency (MEAN+SE)
3.5
3
2.5
2
1.5
1
0.5
0
*
baseline
lavender
music
chocolate
white noise
TMT
B
Lordosis quotient (MEAN+SE)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
baseline
lavender
music
chocolate
white noise
TMT
C
Frequency (MEAN+SE)
7
6
5
4
3
2
1
0
*
baseline
lavender
music
chocolate
white noise
TMT
D
Frequency (MEAN+SE)
3.5
3
2.5
2
1.5
1
0.5
0
baseline
lavender
music
chocolate
white noise
TMT

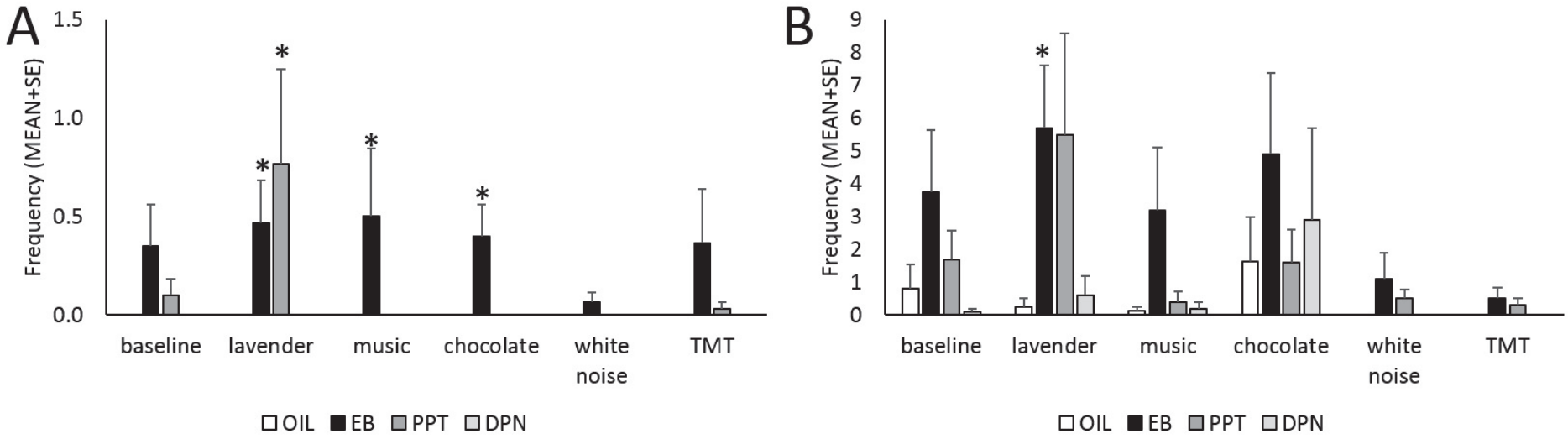

A
Frequency (MEAN+SE)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
baseline
lavender
music
chocolate
white noise
TMT
B
Frequency (MEAN+SE)
16
14
12
10
8
6
4
2
0
*
baseline
lavender
music
chocolate
white noise
TMT

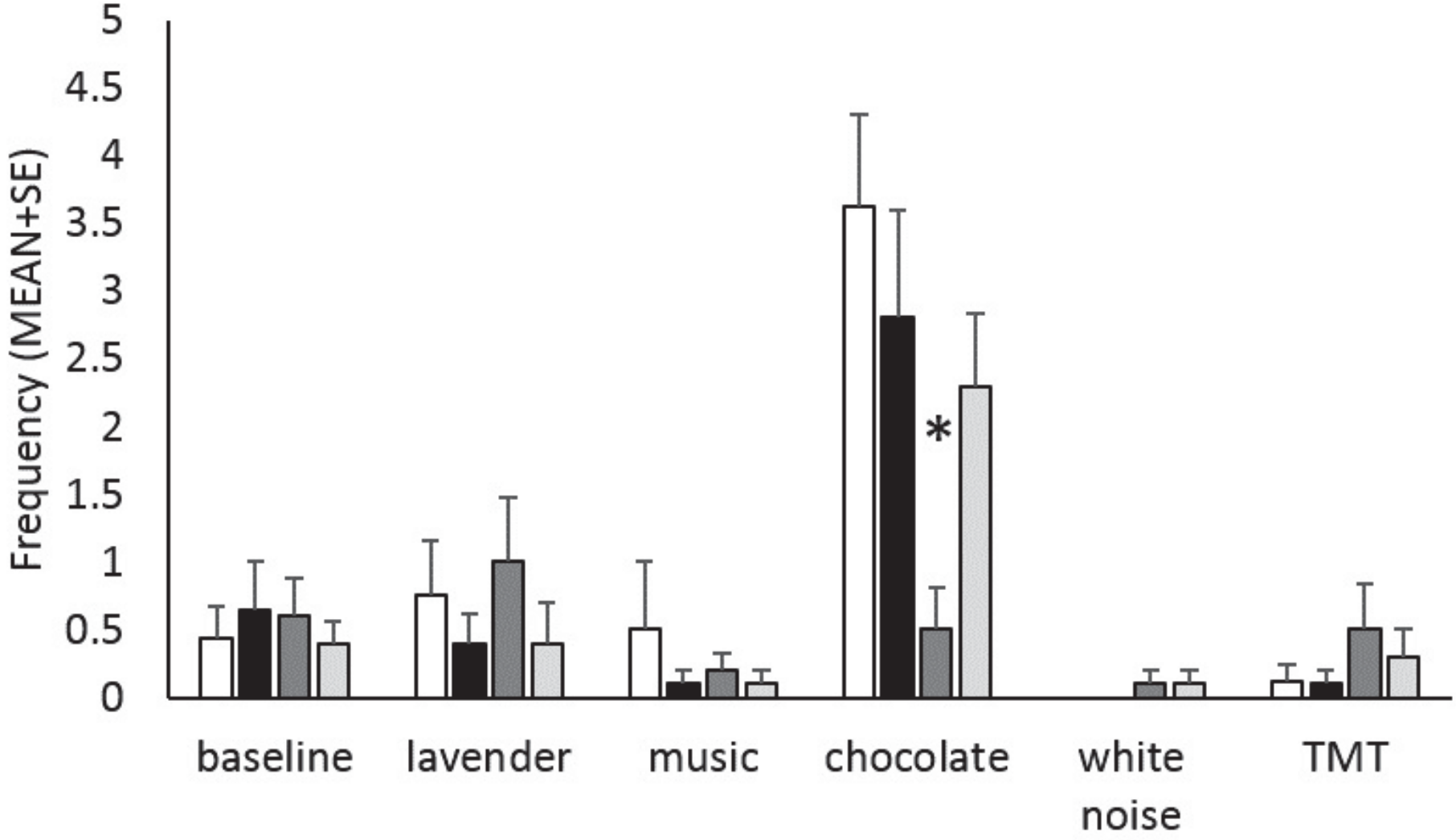

A
Frequency (MEAN+SE)
baseline
lavender
music
chocolate
white noise
TMT
B
Frequency (MEAN+SE)
C
Frequency (MEAN+SE)
D
Time spent in the open area (s) (MEAN+SE)
E
Time spent in the burrows (s) (MEAN+SE)
F
Frequency (MEAN+SE)
G
Frequency (MEAN+SE)

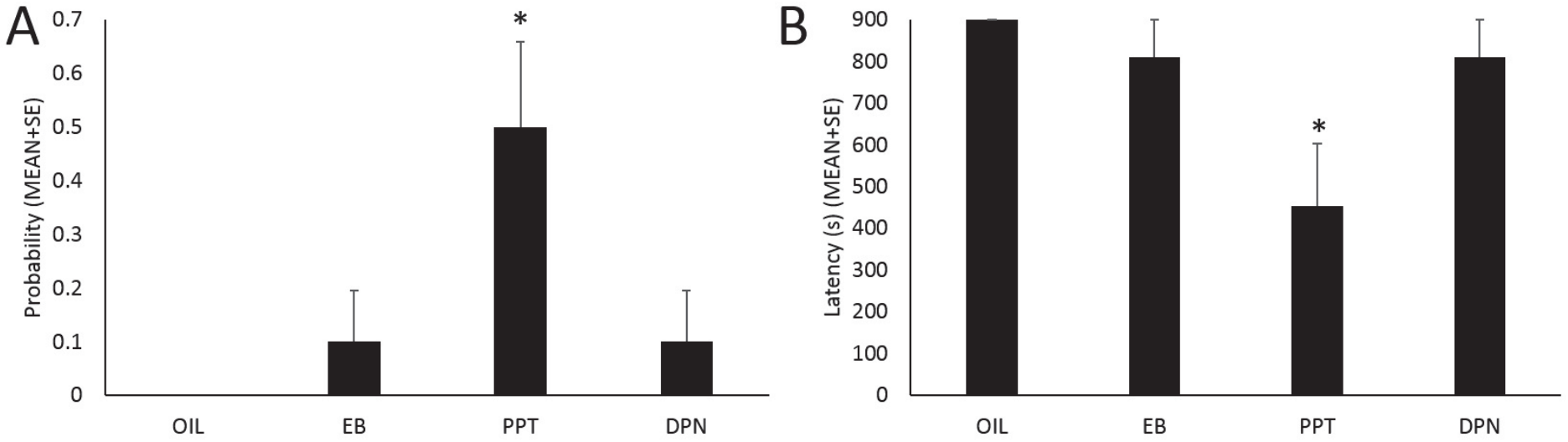

A
Frequency (MEAN+SE)
3
2.5
2
1.5
1
0.5
0
*
baseline
lavender
music
chocolate
white noise
TMT
B
Frequency (MEAN+SE)
14
12
10
8
6
4
2
0
*
*
baseline
lavender
music
chocolate
white noise
TMT

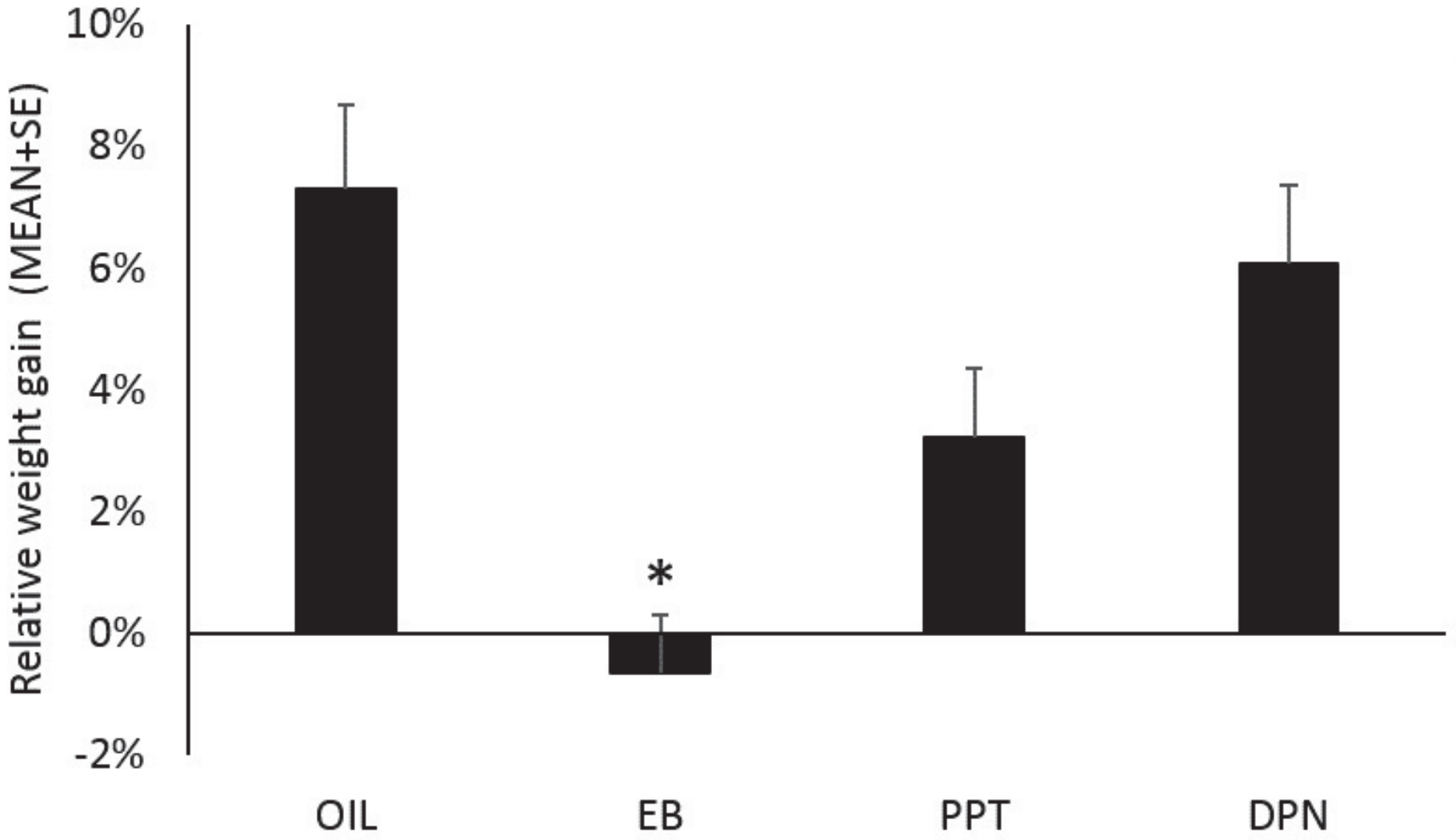

A
Frequency (MEAN+SE)
14
12
10
8
6
4
2
0
*
*
*
baseline
lavender
music
chocolate
white noise
TMT
B
Frequency (MEAN+SE)
8
7
6
5
4
3
2
1
0
*
*
*
*
baseline
lavender
music
chocolate
white noise
TMT

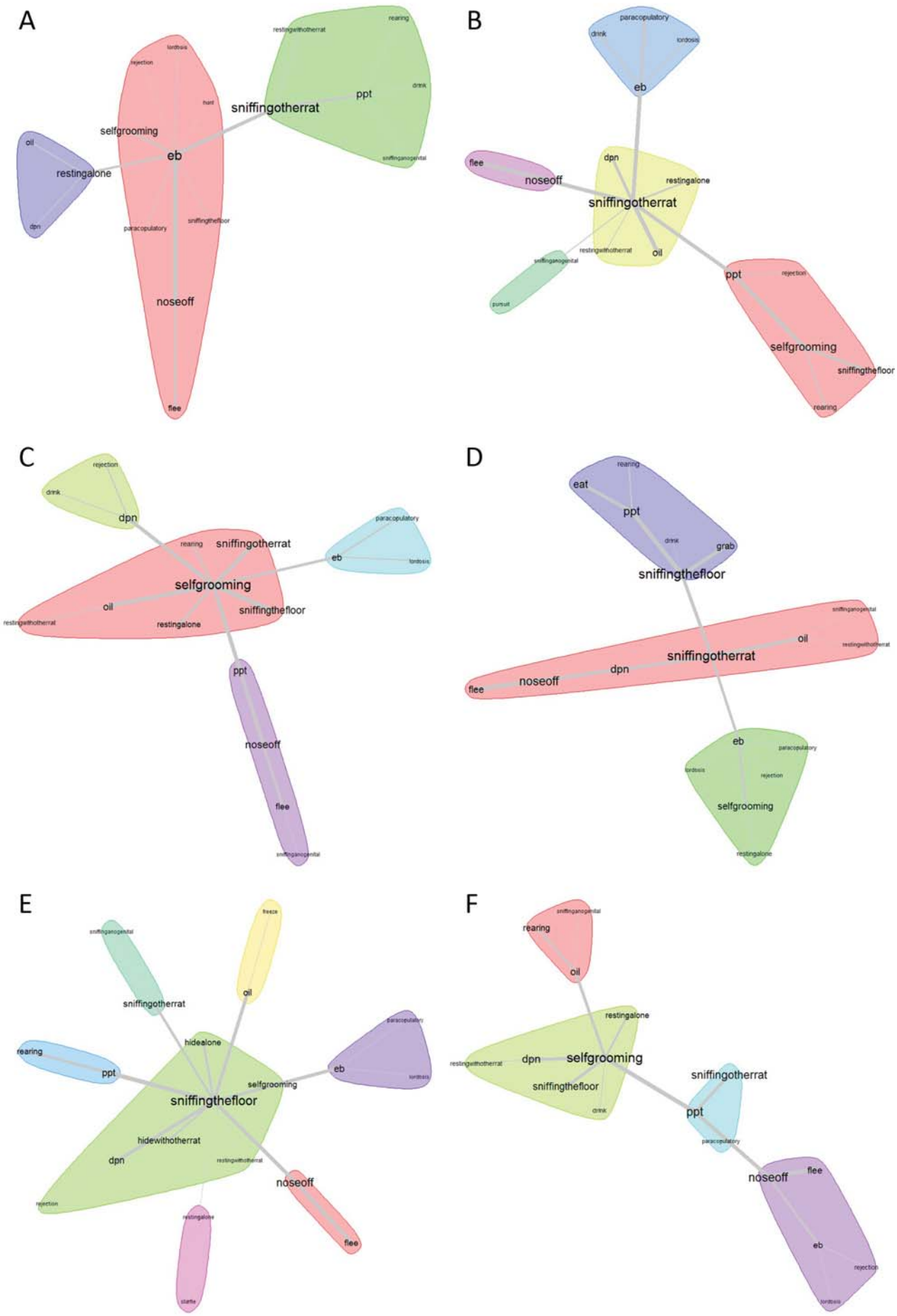

A
Frequency (MEAN+SE)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
baseline
lavender
music
chocolate
white noise
TMT
OIL
EB
PPT
DPN
B
Lordosis quotient (MEAN+SE)
2.5
2
1.5
1
0.5
0
baseline
lavender
music
chocolate
white noise
TMT
OIL
EB
PPT
DPN
C
Frequency (MEAN+SE)
9
8
7
6
5
4
3
2
1
0
baseline
lavender
music
chocolate
white noise
TMT
OIL
EB
PPT
DPN


A
Frequency (MEAN+SE)
1.5
1.0
0.5
0.0
*
*
*
*
baseline
lavender
music
chocolate
white noise
TMT
OIL
EB
PPT
DPN
B
Frequency (MEAN+SE)
9
8
7
6
5
4
3
2
1
0
*
baseline
lavender
music
chocolate
white noise
TMT
OIL
EB
PPT
DPN


Frequency (MEAN+SE)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
baseline
lavender
music
chocolate
white noise
TMT


A
Probability (MEAN+SE)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
OIL
EB
PPT
DPN
B
Latency (s) (MEAN+SE)
900
800
700
600
500
400
300
200
100
0
*
OIL
EB
PPT
DPN


10%
8%
6%
4%
2%
0%
-2%
Relative weight gain (MEAN+SE)
*
OIL
EB
PPT
DPN


A
rearing
restingwithotherrat
lordosis
rejection
drink
ppt
hunt
sniffingotherrat
selfgrooming
oil
eb
restingalone
sniffinganogenital
dpn
paracopulatory
sniffingthefloor
noseoff
flee
B
paracopulatory
drink
lordosis
eb
flee
dpn
noseoff
restingalone
sniffingotherrat
restingwithotherrat
oil
sniffinganogenital
ppt
rejection
pursuit
selfgrooming
sniffingthefloor
rearing
C
rejection
drink
dpn
paracopulatory
rearing
sniffingotherrat
eb
lordosis
selfgrooming
oil
sniffingthefloor
restingwithotherrat
restingalone
ppt
noseoff
flee
sniffinganogenital
D
rearing
eat
ppt
drink
grab
sniffingthefloor
sniffinganogenital
oil
restingwithotherrat
sniffingotherrat
dpn
noseoff
flee
eb
paracopulatory
lordosis
rejection
selfgrooming
restingalone
E
sniffinganogenital
freeze
oil
sniffingotherrat
paracopulatory
hidealone
rearing
ppt
eb
selfgrooming
lordosis
sniffingthefloor
hidewithotherrat
dpn
restingwithotherrat
noseoff
rejection
flee
restingalone
startle
F
sniffinganogenital
rearing
oil
restingalone
dpn
selfgrooming
restingwithotherrat
sniffingotherrat
sniffingthefloor
drink
ppt
paracopulatory
noseoff
flee
eb
rejection
lordosis