Visual-olfactory Contact with a Receptive Female Reduces Anxiety in Reward Downshift and Open Field Tests in Male Rats

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ABSTRACT

Ejaculation has been shown to have anxiolytic-like effects in a consummatory Successive Negative Contrast situation. Present research was conducted with the main goal of replicating and testing whether ejaculation is a necessary factor to obtain anti-anxiety effects after socio-sexual stimulation in both a reward downshift situation and in an open field test. In Experiment 1, male rats were tested in the second post-shift session of a 32% to 4% sucrose solution downshift after having the chance of an ejaculation, visual-olfactory contact with a receptive female, or no contact with females. Similarly to treatments with anxiolytic drugs, increments in consumption after the initial consummatory reduction were equivalent for ejaculatory and visual-olfactory conditions relative to controls not exposed to females. In Experiment 2, the same treatments were applied before males were placed in an open field. Ejaculators and visual-olfactory males did not significantly differ from controls in terms of general activity, though both groups had significantly more average time in central areas of the open field than the control group. Altogether, present experiments provided evidence that socio-sexual stimulation in male rats is a sufficient factor in reducing anxiety responses in a reward downshift situation. In addition, this anxiolytic-like effect of ejaculation and socio-sexual stimulation is extensive to novel context situations.
Key words: anxiety, frustration, open field, sexual behavior, ejaculation, male rats.

RESUMEN

En un artículo previo mostramos que, en ratas, la eyaculación produjo un efecto ansiolítico en una situación de frustración usando un procedimiento de contraste sucesivo negativo consumatorio (CSNc), que implica la reducción abrupta de un reforzador apetitivo. Los experimentos que presentamos tienen como objetivo evaluar si ese efecto se extiende a una situación que implica una estimulación socio-sexual. En el Experimento 1 se evalúa el consumo mediante el tiempo de contacto con el bebedero ante la devaluación del 32% al 4% de una solución azucarada en ratas machos con experiencia sexual previa. Antes del segundo ensayo de la devaluación, las ratas se dividieron en tres condiciones: acceso y eyaculación con una hembra receptiva, contacto visual-olfativo con una hembra receptiva y ningún contacto con hembras. Durante la segunda sesión de la fase de post-cambio, los dos primeros grupos consumieron una proporción mayor de la solución devaluada en relación

con el grupo control. En el Experimento 2 se realiza el mismo tratamiento antes de colocar a los animales ante una prueba de campo abierto. Los machos sometidos a eyaculación o contacto visual -olfativo no se diferenciaron de los controles en la ambulación; sin embargo ambos grupos permanecieron en el área central significativamente más tiempo que los animales pertenecientes al grupo control. Estos resultados extienden el efecto ansiolítico de la eyaculación a la estimulación social-sexual en una condición de disminución de reforzadores apetitivos y en una situación de temor ante lugares novedosos.
Palabras clave: ansiedad, frustración, campo abierto, comportamiento sexual, eyaculación, ratas machos.

There is abundance of evidence showing that rats encountering a surprising downshift in reinforcement conditions sharply suppress the consumption of the devalued reward and develop an anxiety-related state that has been called frustration (Amsel, 1992; see Flaherty, 1996, for an extensive review on incentive relativity; see Papini & Dudley, 1997, for a review on surprising reward omission effects). For instance, when mammals face a downshift in the expected quality or quantity of an appetitive reinforcer (e.g. 32%-to-4% sucrose solution shift), they show a significant decrease in their consummatory performance and an increase in their ambulation and rearing behavior in comparison to control subjects that are trained with the lower reinforcer (Flaherty, 1996). This phenomenon has been called "consummatory Successive Negative Contrast" (SNC) effect. Moreover, rats unexpectedly shifted from a 32% sucrose solution to a less-preferred 4% solution presented a faster recovery in consumption of the devalued reinforcer when they had been administered anxiolytic drugs, such as diazepam, before the second post-shift session of a Successive Negative Contrast (SNC) relative to control animals (Flaherty, 1996).

Freidin, Kamenetzky, and Mustaca (2005) showed that the anxiolytic effect upon cSNC could be reproduced if rats were allowed to ejaculate twice before the second post-shift sessions relative to control males not exposed to females. Moreover, Fernández Guasti, Roldán Roldán, and Saldívar (1989) found a reduction in burying behavior towards a source of noxious stimulation in males that had ejaculated before the test when compared with either controls unexposed to females or animals allowed to copulate for five intromissions only. Both groups of authors (i.e., Fernández Guasti *et al*., 1989, and Freidin *et al*., 2005) concluded that ejaculation seemed to have anxiolytic-like effects.

Though the conclusion of an anxiolytic-like effect of ejaculation had some support on the burying behavior test where males that had copulated for 5 intromissions behaved similarly to controls and differently from ejaculators (Fernández Guasti *et al*., 1989), the control group where males were socio-sexually stimulated but did not ejaculate was missing when testing ejaculation effects on reward downshift situations (Freidin *et al*., 2005).

The goal of the present study was to test whether ejaculation was a necessary factor to produce an anxiolytic-like effect as tested in a reward downshift situation

and in an open field test. In Experiment 1, male rats were tested in the second post-shift session of a sucrose solution downshift situation after having the chance of an ejaculation, visual and olfactory contact with a receptive female, or no contact with females before the test. This experiment aimed to compare only the consumption rate (goal-tracking time) after experiencing a reward downshift in animals that experienced different socio-sexual conditions and thus, did not require assessing differences between downshifted and unshifted subjects. In Experiment 2, the same treatments were applied before males were placed in an open field for 5 min. This second experiment allowed us to test anxiety responses (as evidenced by exploration of central areas of the apparatus) in a different procedure, and also let us control for general activity as evidenced by total locomotor activity.

EXPERIMENT 1

The goal of the present experiment was to explore whether socio-sexual stimulation is a sufficient factor to obtain an anxiolytic-like effect in a surprising reward reduction situation. With this goal in mind, we assigned males to three independent treatments (i.e., ejaculation, visual-olfactory contact with a receptive female, and controls not exposed to females) that were applied before the second post-shift session of a 32%-to-4% sucrose downshift procedure. According to results obtained in burying behavior tests (Fernández Guasti *et al*., 1989), we expected rats in the visual-olfactory condition to consume 4% solution similarly to controls and significantly less than ejaculators during the post-shift phase.

METHOD

Subjects

Subjects were 20 male Wistar rats that achieved ejaculation during a pre-test described in the procedure section, and were bred at the *Instituto de Investigaciones Médicas Alfredo Lanari, Universidad de Buenos Aires*. Subjects had not been exposed to sucrose solutions before the present experiment. Males were approximately 120 days old at the start of the experiment and weighed between 250 and 360 g. They were housed in individual wire cages within a room with controlled temperature (23±3° C) and light: dark cycle (lights on from 06:00 to 18:00 h). Animals had ad-lib access to water throughout the experiment but were deprived of food until they reached 90% of their free-feeding weight; they were maintained at that level during the course of the experiment by daily access to food not less than 20 min after the end of the training session.

Seven ovariectomized female Wistar rats were housed in groups of four and three animals, respectively. They were used both for males' sexual pre-test and for the experimental sexual conditions applied before the second post-shift session.

Apparatus

Males' metallic home cages were 28 cm wide, 26 cm long, and 23 cm high, with bars of 0.1 cm in diameter separated 1.5 cm apart.

Consummatory training occurred in three conditioning boxes (MED Associates), each 29.2 cm long, 24.1 cm wide and 21 cm high. The floor was made of aluminium bars measuring 0.4 cm in diameter, and spaced apart 1.1 cm (from centre to centre). On one of the lateral walls there was a cubicle measuring 5 cm in width, 5 cm in height, and 3.5 cm in depth, and located 10 cm above the floor. The sipper tube was inserted into it from outside of the box, protruding approximately 2 cm inside the cubicle. Rats had to insert the head into this cubicle to reach the sipper tube from which they could drink a 32% sucrose solution (32 g of sucrose for each 68 ml of water) during the pre-shift phase and a 4% sucrose solution (4g of sucrose for each 96 ml of water) during the post-shift phase. Goal-tracking time (in 0.01 s units) was automatically registered as the amount of time a photocell located in front of the drinking tube was activated during the session.

Each box was enclosed in a sound and light-attenuating cubicle, equipped with a source of white noise and diffuse house light. The apparatus was carefully cleaned after each session. Sexual tests occurred in similar cages as males' home cages.

Animal maintenance and handling were performed according to the NIH guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23) and the UK requirements for ethics of animal experimentation (Animal Scientific Procedures, Act 1986). All the experimental procedures were approved by the Institutional Animal Ethics Committee.

Procedure

The procedure had the following sequence:

Sexual pre-test. Males were pre-tested for masculine sexual behavior. Each male was taken from the colony room to another experimental room, where it was placed in a cage that had exactly the same dimensions as home cages. Approximately two minutes later, an ovariectomized receptive female was placed with the male and their sexual behavior was observed. Males displaying ejaculation at least once in a maximum of five 20-min sessions were selected for the experiment. All females were brought into heat by administration of estradiol benzoate (50μg EB/0.1ml olive oil, 48h before testing) and progesterone (500μg P/0.1ml olive oil, 3h before testing).

Pre-shift phase. One day before the start of the consummatory training, all subjects received access to the training solution in their home cages. A drinking bottle with 40ml of 32% sucrose solution was placed in their cages for 30 min. The pre-shift phase started the following day and lasted for 6 days, with two 5-min sessions per day (i.e., overall, the pre-shift phase consisted of 12 sessions). Within a day, the inter-session interval was about 3h; between days, the inter-session interval was 20–21h. Rats were placed in their home cages during both intervals. Groups of 3 rats were trained simultaneously and the order of the squads was rotated across sessions. Pre-shift sessions consisted of

placing the males in the conditioning boxes for 5 min, where they had access to the 32% sucrose solution by licking the sipper tube.

Post-shift phase. On day 7, after the end of the pre-shift phase, all animals only accessed 4%-sucrose solution in the conditioning boxes (i.e., a reward downshift) during both sessions (i.e., sessions 13 and 14). Before the second post-shift session (i.e., session 14), rats matched for average pre-shift goal-tracking time were randomly assigned to one of three conditions: ejaculatory condition (i.e., males allowed to reach one ejaculation just before session 14, ejaculation group, *n*= 7), visual-olfactory condition (i.e., males exposed for 10 min to a receptive female while a wire mesh avoided copulation, just allowing visual and olfactory contact between the rats, visual-olfactory group, *n*= 7), and a control group (i.e., rats not exposed to females before the session, control group, *n*= 6).

Goal-tracking time was registered as the dependent measure in all pre-shift and post-shift sessions. Previous studies have shown that goal-tracking time is positively correlated with the volume of solution consumed by animals (Mustaca, Freidin, & Papini, 2002).

For statistical analyses time data was transformed to natural logarithm and analyzed with a one-way ANOVA. Afterwards, post hoc pair-wise contrasts were performed. The alpha value was set at the .05 level.

Results and Discussion

Pre-shift phase. The average goal-tracking time increased throughout the pre-shift phase for all subjects (see Figure 1). Repeated measures analysis of the 12 pre-shift sessions showed a significant effect of session [$F(11, 165)= 5.44$, $p <0.0001$], with neither an effect of treatment nor an effect of treatment x session interaction [both Fs <1].

Post-shift phase. The 32%-to-4% shift resulted in a sharp decline in goal-tracking time for all groups. Repeated measures analysis of the last pre-shift session and the first post-shift session together showed a significant effect of session [$F(1, 17)= 33.07$, $p <0.0001$], with neither an effect of treatment nor an effect of treatment x session interaction [both Fs <1]. Repeated measures analysis of the last pre-shift session and the second post-shift session together showed neither an effect of treatment, nor an effect of session [both Fs <1]. The treatment x session interaction was not significant either [$F(2, 17)= 2.39$, $p= 0.12$].

Goal-tracking time during the second post-shift session was as follows (mean ±1 SEM): control condition, 137±6 sec; visual-olfactory condition, 173±6 sec; and ejaculatory condition, 151±6 sec. The analysis of goal-tracking time for the second post-shift session showed a significant effect of treatment [$F(2, 17)= 3.49$, $p= 0.05$]. Pair-wise contrasts of the second post-shift session goal-tracking time showed no reliable differences between ejaculators and controls and between ejaculators and animals from the visual-olfactory condition (both $p >0.05$), but a reliable discrepancy between the means of the visual-olfactory condition and the control group was found ($p <0.02$).

Treatment differences in average goal-tracking time of postshift 1 were not systematic [$F(2, 17) = 2.11$, $p= 0.15$], but means were disparate enough as to seemingly
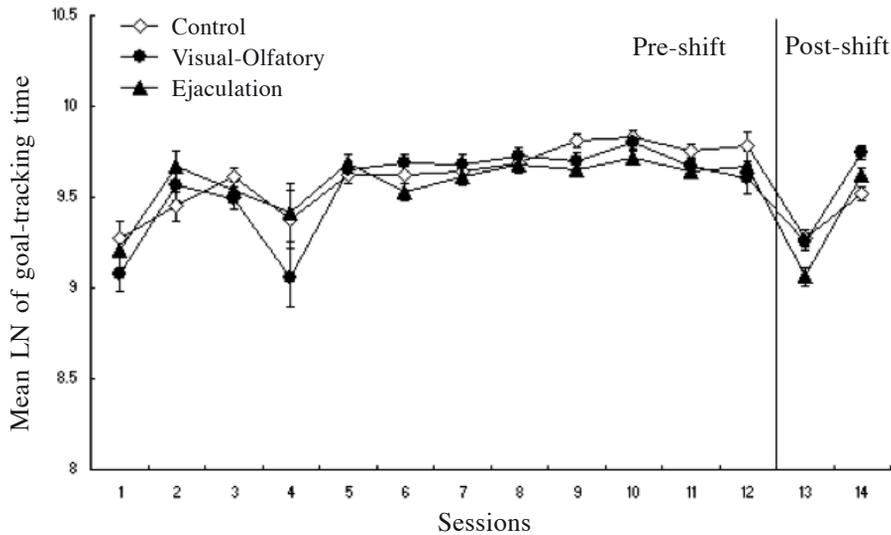
Figure 1. Experiment 1: Consummatory performance, measured in terms of natural logarithm (LN) of goal-tracking time, as a function of experimental phase and treatment. All conditions received 32%-sucrose solution during pre-shift sessions and 4% solution during post-shift sessions. Error bars indicate ± SEM.

reduce the power of finding systematic differences among treatments in post-shift 2. A way to reduce the impact of postshift 1's variability upon treatment effects was to use the proportion of change in consummatory behavior from the first to the second post-shift sessions (i.e., when the different conditions were applied) as the dependent variable. Accordingly, the one-way ANOVA of the proportion of consummatory recovery from the first to the second post-shift sessions [i.e., Post-shift 2/(Post-shift 1+ Post-shift 2)], showed a significant effect of treatment [$F_{(2, 17)}$= 4.69, p= 0.02]. The mean (±1 SEM) proportion of change from the first to the second post-shift session as a function of treatment was as follows: control condition, 0.56±0.02; visual-olfactory condition, .62±.02; and ejaculatory condition, 0.64±0.02 (see Figure 2). Pair-wise comparisons showed that ejaculation and visual-olfactory conditions presented a significantly higher proportion of change than the control group (ejaculation vs. control, p= 0.009; visual-olfactory vs. control, p <0.04), while the averages of both socio-sexual conditions did not significantly differ between themselves (p >0 .1).

Present results could not replicate exactly previous anxiolytic effects of ejaculations on goal-tracking time of the second postshift session of a surprising reward downshift procedure (Freidin et al., 2005). However, we found a similar effect as indicated by a larger increase in proportion of change from postshift 1 to postshift 2 after an ejaculation relative to controls. In addition, not only ejaculation but also visual and olfactory access to a receptive female increased the consummatory recovery relative to controls. These results suggest that the anxiolytic-like effect of ejaculation upon a surprising reward downshift as shown here and in previous reports (Freidin et al., 2005) could be in part the result of socio-sexual stimulation. Nevertheless, current findings cannot discard an
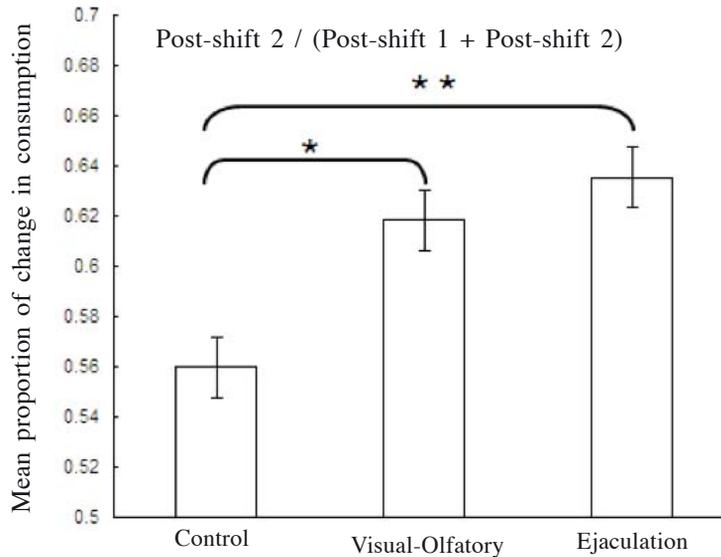
Figure 2. Experiment 1: Mean proportion of consummatory change from post-shift 1 to post-shift 2, as a function of treatment. Notice that all mean scores are above .5, which denotes an increment in consumption from post-shift 1 to post-shift 2. Error bars indicate ± SEM. * *p* <0.05; ** *p* <0 .01.

alternative interpretation, namely that those animals visually exposed to receptive females but not allowed to copulate (i.e., visual-olfactory condition) might have ended up more aroused, and hence drunk more than controls not exposed to females.

EXPERIMENT 2

The goal of this second experiment was to test socio-sexual treatments implemented in Experiment 1 (i.e., ejaculation and visual-olfactory contact with a female) in a different anxiety procedure. With this purpose in mind, we placed male rats in an open field test after allowing them to ejaculate once, to have visual-olfactory contact with a receptive female, or not exposed to females before the test. Because the open field test allows dissociating general activity (measured by the overall locomotor activity) from anxiety-related responses (measured as activity in central areas of the apparatus; e.g., Prut & Belzung, 2003), we were able to obtain further evidence relative to the potential arousing consequences of the visual-olfactory condition. Therefore, this test provided the means to make more precise conclusions about the consequences of ejaculation and visual-olfactory contact with a receptive female on frustration and anxiety-related behaviors.

<div style="text-align: center">METHOD</div>

Subjects

Subjects were 20 rats that achieved ejaculation during a similar pre-test as that described for the previous experiment. Rats were approximately 120 days old at the start of the experiment and weighed between 240 and 354g. All males were experimentally naive, and were maintained under the same environmental conditions as described for Experiment 1. We used the same female rats as before.

Apparatus

Males' home cages were the same as those in Experiment 1.

We used an open field of 97.5x97.5x25 cm (width, length, and height, respectively), made of wooden walls and a brown plastic floor marked with white lines forming 25 equal squares of 19.5cm per side. All subjects were video recorded during the open field test using a video camera (Sony digital 8 DCR-TRV310 NTSC). The open field arena was carefully cleaned after each session.

Procedure

The procedure had the following sequence:

Sexual pre-test: Males were pre-tested for sexual behavior and selected through a sexual pre-test similar to that used in the previous experiment.

Open field test: Male rats matched for weight were randomly assigned to one of three conditions: ejaculatory condition (*n*= 7), visual-olfactory condition (*n*= 7), and control condition (*n*= 6). Rats in the ejaculatory condition were allowed to reach one ejaculation before placing them in the open field; males in the visual-olfactory condition were exposed to the sight and odor of a receptive female through a wire mesh that did not allow them to copulate, during 10 min before the test; and subjects in the control group were not exposed to females. Immediately after applying the corresponding condition, each animal was taken to the experimental room where the open field was located. Each subject was placed at the central square of the open field, and left in the apparatus for 5 minutes while it was being filmed. An observer registered the rat's activity from the tape without knowing males' group assignment. Each male's test was registered twice, and reliability was above 90%.

The number of floor-lines that each animal crossed with its two fore feet (locomotor activity) and the total time that the fore feet of the animal stayed in central areas (centre time) of the open field were registered as dependent measures. We did the same statistical analyses as reported for Experiment 1. First, a one-way ANOVA was performed with locomotor activity and centre time; second, pair-wise contrasts were done. Time data were transformed to natural logarithm before statistical analyses. The alpha value was set at the .05 level.

Results

Figure 3 presents the main outcomes of the open field test. Males that ejaculated and those that had visual-olfactory access to a female did not reliable differ from controls when compared for overall locomotor activity [$F(2, 17)= 0.15$]. Nevertheless, males in the ejaculation and visual-olfactory conditions spent more time in central areas of the open field relative to controls not exposed to females before the test. This last difference was supported by a significant condition effect in the ANOVA of time spent in central areas: $F(2, 17)= 3.79$, $p= 0.04$. Pair-wise contrasts of centre time showed significant differences between ejaculators and control subjects ($p <0.02$), between the visual-olfactory condition and controls ($p <0.04$), and no significant differences between ejaculators and males from the visual-olfactory condition.

Present results showed that neither ejaculation nor visual-olfactory contact with a receptive female seemed to affect the general locomotor activity of subjects exposed to an open field test when compared with controls not exposed to females. On the other hand, the visual-olfactory group presented a similar average degree of ambulation in central areas of the open field as compared to the ejaculation group, and both groups scored significantly more than controls on this measure.

In short, taken together, the lack of effect on general activity and the increase in time spent in central areas of the apparatus after ejaculation and visual-olfactory contact with a female provided evidence of a reduction in males' anxiety relative to controls.
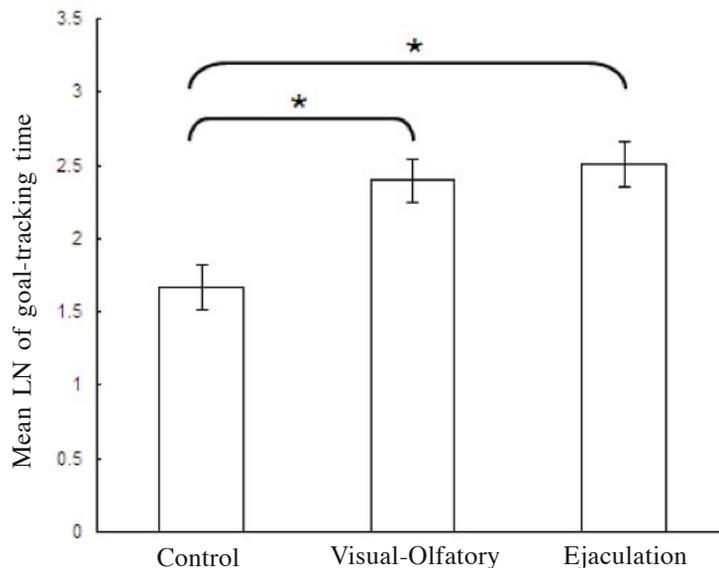

Figure 3. Experiment 2: Mean natural logarithm (LN) of time spent in central areas of the open field as a function of treatment. Error bars indicate ± SEM. * $p <0.05$.

General Discussion

Previous studies have shown that anxiety responses can be attenuated after ejaculations in male rats in a similar manner as anxiolytic drugs do. Anxiolytic drugs, such as benzodiazepines, partially reverse consummatory suppression in the second post-shift session but not in the first post-shift session of a 32%-to-4% reward downshift situation (Flaherty, 1996). Freidin *et al*. (2005) showed that previous ejaculations produced similar results in a cSNC procedure, namely no effect on the first post-shift session, and facilitation of consummatory recovery in the second post-shift session.

The goal of present experiments was to test whether social-sexual stimulation is a sufficient factor to produce an anxiolytic-like effect in a reward downshift situation and in an open field test. Similarly to Freidin *et al*.'s (2005) augmented goal-tracking time in postshift 2 after two ejaculations, results from Experiment 1 showed that males presented a larger proportion of increment in goal-tracking time from postshift 1 to postshift 2 when they were allowed to ejaculate once before postshift 2, relative to controls unexposed to females. Moreover, male rats that had only visual-olfactory contact with a receptive female before the second post-shift session recovered consumption similarly to males that had ejaculated before the test and significantly more than controls.

In Experiment 2, we applied the same conditions as in Experiment 1 but before placing the males in an open field. The open field test allowed us to dissociate between increases in general activity and anxiolytic-like effects, because it was possible to compare treatment effects upon overall ambulation vs. ambulation in central areas of the apparatus (i.e., a measure linked to anxiety and stress; see Experiment 2 for references). Results from Experiment 2 confirmed that males in the visual-olfactory condition had similar levels of activity in central areas of the open field as males that had ejaculated, and both conditions scored reliably more than controls. In addition, the three conditions did not significantly differ in terms of overall ambulation. This result extends the anxiolytic-like effect of socio-sexual stimulation to a new situation, that is, open-field tests.

In accordance with our findings, hormone studies suggest that it may not be ejaculation itself what results in anxiolytic-like consequences but the increase in testosterone as triggered by the presence of a receptive female or the start of mounting and copulation instead (e.g., Aikey *et al*., 2002; Bonilla Jaime *et al*., 2006; Kamel *et al*., 1975). For instance, Edinger and Frye (2005) and Fernandez Guasti and Martínez Mota (2005) reported anti-anxiety effects of testosterone in different animal models of stress, such as plus maze and burying behavior tests, respectively. Hence, it is possible that present reduction in anxiety-related behaviors of males in the ejaculation and visual-olfactory conditions was mediated by a rise in testosterone levels after being in contact with receptive females. On the other hand, present anti-anxiety effects may be interpretable as the outcome of social interaction, especially given that our males lived in individual cages, and hence were in partial social-deprivation. For instance, Wilson (2000) tested juvenile rats in an open field either alone or with a same-sex conspecific, and showed that social interaction with a conspecific reduced stress as suggested by a decrease in prolactin levels.

Evidence for testosterone effects on surprising reward downshift procedures is currently being investigated in our lab in order to explore potential mechanisms underlying the effects of sexual stimulation upon frustration. In addition, future experiments adding different socio-sexual conditions (e.g., contact with a non-receptive female, with another male, etc.) should be carried out to test potential anxiolytic-like effects of social interactions.

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