Running title: Estratetraenol and sexual reward

Estratetraenol increases preference for large sexual reward but not impulsivity among heterosexual males

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Abstract:

There is increasing evidence suggesting that estratetraenol, a human chemosignal deemed a putative sex pheromone, affects social cognition and sexual behavior. The present study investigates the effects of estratetraenol on preference for sexual rewards in heterosexual males. Seventy-six male participants received either estratetraenol or a control carrier in a double-blind, placebo-controlled, within-participant design. Participants underwent a sexual delay discounting task, in which they were asked to make a choice between a variable larger-later option (i.e., waiting longer to view a sexual picture for a longer duration) and a smaller-sooner option (i.e., waiting for a fixed shorter period of time to view the same picture for a shorter duration). Results revealed that, compared to the control solution, estratetraenol selectively increases preference for larger-later sexual rewards. Computational modelling showed that estratetraenol has no observable influence on impulsivity, as indexed by the discounting rate. These findings suggest that estratetraenol could increase men's sexual motivation, possibly facilitating behavioral processes associated with the pursuit of a sexual partner.

Keywords: chemosignaling; estratetraenol; sexual reward; impulsivity

1. Introduction

A ubiquitous feature of animal communication within species is chemosensory communication. Animals can draw upon chemosignals given off by conspecifics to prime the reproductive system (Whitten, 1956), inducing preference and reproductive behavior toward potential mates (Dorries et al., 1997). Human beings communicate through olfactory signals as well. Social chemosignaling is linked to human reproduction such that women with unexplained repeated pregnancy loss have altered olfactory responses to men's body odor (Rozenkrantz et al., 2020), and men's brains tune differently to the body odor of women in different reproductive states (Habel et al., 2021). Women living together tend to have synchronized menstrual cycles, and this effect is further mediated by odorless compounds from the armpits of women (Stern and McClintock, 1998). Human brain has shown differentiated activations for sexual sweat and non-social odors (Zhou and Chen, 2008). Moreover, tears from women, which convey a chemosignal, modulate sexual arousal, physiological response, testosterone levels, and brain activity in men (Gelstein et al., 2011).

Among the many components of human secretions, one endogenous steroid estra-1,3,5(10),16-tetraen-3-ol (estratetraenol or EST), has been found to exert effects on male recipients. First identified in female late-pregnancy urine, estratetraenol is related to the estrogen sex hormones but with no known estrogenic effects (Thysen et al., 1968). Previous evidence has suggested a link between estratetraenol and human reproductive function. In particular, exposure to estratetraenol affects self-reported sexual arousal and cognition among males (Oren et al., 2019). Furthermore, estratetraenol communicates gender information such that exposure to estratetraenol biases heterosexual males towards perceiving individuals as

more feminine (Zhou et al., 2014) and primes the identification of emotionally receptive states for potential mates (Ye et al., 2019). Moreover, neuroimaging studies have shown that exposure to estratetraenol in heterosexual males and homosexual females activates the anterior hypothalamus, a key brain region associated with sexual behavior (Savic et al., 2001). To elucidate the relationship between estratetraenol and human reproduction, it's critical to investigate its effect on human sexual behavior and motivation. Nevertheless, relevant studies have been scarce, partly due to the difficulty of measuring sexual behavior and motivation directly and accurately in the laboratory setting.

To fill this gap, the present study investigates the effects of estratetraenol on preference for sexual rewards in heterosexual men, which was assessed by a sexual delay discounting task (SDDT). In a typical delay discounting task, the participants are asked to choose between two monetary options, a smaller reward (SS reward) available sooner and a larger reward (LL reward) available later (Bickel and Marsch, 2001). As delay increases, the value of the larger reward decreases. This decrease corresponds to discounting and is typically well captured by hyperbolic functions (Kable and Glimcher, 2007). In our SDDT, participants were asked to choose between two options: (a) briefly viewing a sexual picture right now; or (b) waiting longer to view the sexual picture for a longer duration (Prevost et al., 2010).

Previous research has employed SDDTs to investigate the psychological and neural mechanisms underlying processing sexual rewards. For instance, Parkinson's patients with hypersexuality were more likely to wait longer to see the erotic image for a longer period of time in the SDDT, due to the enhanced incentive salience of erotic rewards lasting longer (Girard et al., 2019). This effect was paralleled by a negative correlation between subjective value of the delayed reward and brain activity in the medial prefrontal cortex and ventral

striatum. We hypothesized that if estratetraenol is associated with mating motivation and conveys information regarding a female's sexuality, then estratetraenol administration among heterosexual males would increase their preference for LL sexual rewards. The preference for LL sexual rewards may be confounded by the impulsivity of getting the sexual rewards. Here we adopted a classical hyperbolic discounting model to derive the discounting rate, which was an index representing an individual's impulsivity level.

2. Materials and methods

2.1. Participants

Sample size was determined by G*Power 3.1 to detect a moderate effect (d = 0.4) with the power of 0.95 ($\alpha = 0.05$), resulting in a sample size of 68. Nevertheless, we recruited 95 healthy male non-smokers (mean age = 21.57 years, *SD* = 2.32, range = 18-33), to allow for possible non-compliance or impossibility of model fit. All the participants were undergraduate students and none of them was married (64 single and 31 in a relationship). Nineteen participants were excluded from data analyses: Three participants did not follow the instructions, seven participants chose the same option across the task (two participants chose the LL option exclusively and five chose the SS option exclusively), seven participants did not complete the task due to program failure, and two participants reported current neurological illness. There were 76 participants for the final analysis (mean age = 20.46 years, *SD* = 2.09, range = 18-33; 54 single and 22 in a relationship). All participants reported to be Han Chinese, heterosexual (Kinsey score = 0), to have normal or corrected-to-normal vision, a normal sense of smell and no respiratory allergy or upper respiratory infection. Each participant was exposed to either estratetraenol or a control in a crossover, double-blind and

within-participant design. Two experimental sessions were separated by a 1-week interval (range = 6-9 days). The experimenter for all the experimental sessions was a female research assistant, and she was not present in the room when the participants were completing the experimental task. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local research ethics committee. All participants provided written informed consent and were paid a RMB 140 (~$20) participant fee.

2.2. Olfactory stimuli

We followed the standard practice in this field (Hare et al., 2017; Ye et al., 2019; Zhou et al., 2014). The olfactory stimuli consisted of estratetraenol (500μM in 1% v/v clove oil propylene glycol solution; Steraloids Inc.) and the carrier solution alone (control, 1% v/v clove oil in propylene glycol). Previous research has shown the estratetraenol solution with such a formulation is indiscriminable and without discernable difference in perceived intensity, pleasantness or familiarity (Ye et al., 2019; Zhou et al., 2014). They were presented in identical 40ml polypropylene jars, each containing 5ml of clear liquid and connected with two Teflon nosepieces via a Y-structure. The olfactory stimuli were prepared before the experiment as mother solutions to ensure the accuracy of the concentrations. The prepared mother solutions were then split (one jar per participant per olfactory condition) and stored in the 40ml sterilized polypropylene jars at 4℃ until use.

To assess the distinguishability of the olfactory stimuli in our sample, besides rating the perceived intensity and pleasantness of the olfactory stimuli, participants were asked to complete one trial of forced choice task. At the beginning of this trial, they were presented with one smell (estratetraenol or control, counterbalanced between participants). Then two

olfactory stimuli were presented sequentially (order counterbalanced between participants). Participants were asked to report which one smelled more like the first odor. Thus, the probability of arriving at a correct response by chance was 0.5.

2.3. Sexual stimuli

The pictures were selected from various websites. An independent group of heterosexual male participants (n = 32, mean age ± SD = 20.38 ± 1.07) rated the clear form of the pictures in attractiveness (mean ± SD, 6.28 ± 1.91), arousal (5.70 ± 2.17), and familiarity (4.33 ± 2.47), using a 9-point Likert scale (1 = least attractive/aroused/familiar, 7 = most attractive/aroused/familiar).

2.4. General procedure

We employed a within-participant design, in which participants returned on separate days, at the same time of day, to be tested once with estratetraenol and once with the control solution. Upon arrival, the participants first completed the questionnaires (basic demographic information and Kinsey scale) and signed the informed consent. Then they got used to the odor presentation device (see Figure S1 in the Supplementary Material) and performed the forced choice task (to determine the distinguishability of the olfactory stimuli at the group level). After that, the experimental instruction was given, and each participant had to perform a six-trial practice session to get familiar with the experimental procedure. It took about 15 minutes from the arrival to the start of the formal Sexual Delay Discounting Task (SDDT). The SDDT took 15-20 minutes for each participant. While performing the SDDT, the participants were continuously exposed to estratetraenol or the carrier solution alone, one on each day in a counterbalanced manner. Participants were asked to hold the jar with their non-

dominant hand, position the nosepieces inside their nostrils and continuously inhale through the nose and exhale through their mouth while performing the task. To ensure the participants followed the experimental instructions, their activities were continuously monitored from the adjacent room via a video monitor.

2.5. Sexual delay discounting task (SDDT)

Participants completed 60 trials of the SDDT adapted from Prevost (2010) and Girard et al. (2019). Each trial started with the presentation of a fuzzy sexual picture for 0.5s (see Figure 1). Next, participants were presented with an option of larger-later reward (*LL*) and an option of smaller-sooner reward (*SS*). A thermometer representing one of six possible delay levels was presented in each option (i.e., 1s, 3s, 6s, 10s, 15s, 21s for the *LL* option; 0s for the *SS* option). Once the *LL* option was selected, the *LL* option was framed by a red rectangle, and participants had to wait for the duration indicated on the thermometer before seeing the clear version of the sexual picture for 3s. If participants chose the *SS* option, they saw the clear version of the picture for 0.5s immediately after the *SS* option was framed by a red rectangle. Participants had to make a choice within 3.5s. If they failed to respond, the trial was aborted and then a warning sign "Pay attention" was presented for 2s before the task proceeded to the next trial (number of missing trials for each participant at each session, estratetraenol: *mean* = 0.63, *SD* = 1.55, range = 0-10; control: *mean* = 0.79, *SD* = 2.09, range = 0-15). To prevent the strategy of choosing the *SS* option more often so as to see more pictures, the reward delivery stage and the intertrial interval were presented for a fixed duration (*mean* = 4.5 s, *SD* = 0.82; range: 3.5-5.5 s). For the formal experiment, there were 10 trials at each delay level, and all the trials were presented in a random order.

----------insert Figure 1 about here----------

2.6. Statistical analysis

We used both model-free and model-based analyses to investigate choice behavior. All the statistical analyses were performed using R 4.1.1 and SPSS 22.0. The area under the curve (AUC) of *LL* choices was calculated as the model-free measure of the willingness to obtain the *LL* reward (Myerson et al., 2001; Ou et al., 2021; Vekaria et al., 2017). For each participant and odorant condition, we calculated AUC by using proportions of *LL* choices (*p*), normalizing delay time (D) as a percentage of maximum D, connecting the *p* points by straight lines, and then summing the trapezoids formed (see Figure S2 in the Supplementary Material). Following standardization, AUC can vary from 1 (no discounting) to 0 (maximal discounting). A larger AUC value indicates a stronger preference of waiting for *LL*. We used t-test to assess the effect of estratetraenol on AUC. In addition, we looked at the proportion of LL choices as a function of olfactory stimuli and delay level using repeated measures ANOVA.

Model-based analysis allow us to characterize psychological processes with distinct parameters in a detailed way, which helps to decompose the psychological processes that underpin complex social and moral behavior (Crockett, 2016; Konovalov et al., 2018). In the model-based analyses, participants' impulsivity level was depicted by the canonical hyperbolic discounting rate *k,* which indicates the trend of participants' choice behavior as a function of delays (Peters and Büchel, 2011). Reward amount was defined as the duration of seeing the clear pictures. Specifically, the decision utility (*DU*) was assumed in a form of hyperbolic discounting, with $f(t)$ capturing the relative difference in utility between *SS* and *LL*

(Equation (1)). The binary choice was linked to *DU* using logistic regression, with the inverse temperature parameter β capturing the decision accuracy (Equation (2)).

$$DU = \frac{reward\ amount}{1 + k \cdot delay} \qquad \text{Equation (1)}$$

$$logit\ p(LL) = \beta(DU_{LL} - DU_{SS}) \qquad \text{Equation (2)}$$

2.7. Open practice statement

All the erotic stimuli, data and analysis scripts are available on the Open Science Framework (OSF) page: https://osf.io/z5d47/.

3. Results

Olfactory awareness check. We found that participants were at chance level in discriminating the olfactory stimuli [*mean* accuracy = 0.46, *SD* = 0.50; chance = 0.50; $t(75) = -0.69$, $p = 0.495$] and there was no reliable difference among the stimuli in perceived intensity [estratetraenol: $M = 4.74$, $SD = 1.92$; Control: $M = 4.81$, $SD = 2.02$; $t(75) = 0.25$, $p = 0.807$], pleasantness [estratetraenol: $M = 4.20$, $SD = 2.05$; control: $M = 4.36$, $SD = 1.99$; $t(75) = 0.65$, $p = 0.517$].

Model-free analyses. We calculated the area under the curve (AUC) of the proportion of *LL* choices over *SS*. We found that individuals with estratetraenol administration exhibited significantly larger AUC than those administered the control solution [estratetraenol: $M = 0.44$, $SD = 0.26$; control: $M = 0.38$, $SD = 0.21$; Paired *t*-test: $t(75) = 2.11$, $p = 0.039$, Cohen's $d = 0.242$], indicating that estratetraenol enhanced the preference for *LL* rewards (see Figure 2A).

----------insert Figure 2 about here----------

Next, we looked at whether the proportion of choosing LL reward differed across conditions (see Figure 2B). We performed a repeated measures ANOVA with olfactory condition and delay (6 levels) as within-subject factors, and the results revealed a significant main effect of olfactory condition ($F(1,75) = 4.32$, $p = 0.041$), with estratetraenol ($M = 0.47$, $SD = 0.25$) inducing more LL choices compared with the control solution ($M = 0.42$, $SD = 0.20$). The main effect of delay level was significant ($F(5,375) = 87.64$, $p < 0.001$), with large delay inducing fewer LL choices. The interaction between olfactory condition and delay level was not significant ($F(5, 375) = 0.80$, $p = 0.55$), suggesting that the estratetraenol had a comparable effect across each delay level.

We further tested if the estratetraenol effect depended on the sexual nature of the rewards. In a repeated measures ANOVA with picture groups (4 levels, by their ratings order), olfactory stimuli and delay level as three independent variables, we only observed significant main effects of rating order [$F(3,225) = 52.17$, $p < 0.001$] and EST [$F(1,75) = 4.37$, $p = 0.040$], suggesting that more attractive pictures and EST elevated the proportion of LL choices. Importantly, no interaction effect between was picture groups and olfactory stimuli was found, [$F(3,225) = 0.34$, $p = 0.799$], suggesting that EST really affects choice behavior irrespective of the attractiveness of pictures.

Model-based analyses. To further understand whether the preferences for *LL* rewards was caused by impulsivity, we conducted the model-based analyses. We first used the Shapiro-Wilk test of normality on the parameter distributions. Results showed that both parameter

distributions deviated from normality (k: W = 0.915, p = 0.001; b: W = 0.888, p < 0.001). Neither the discounting rate (k, capturing impulsivity) [estratetraenol: M = 0.68, SD = 0.39; control: M = 0.75, SD = 0.35; Wilcoxon signed rank test: W = 1770, Z = 1.59, p = 0.113] nor the inverse temperature (β, capturing decision accuracy) [estratetraenol: M = 2.02, SD = 2.53; Control: M = 2.17, SD = 2.83; Wilcoxon signed rank test: b: W = 1596, Z = 0.69, p = 0.493] showed significant differences (see Figure 2C), indicating that estratetraenol did not change participants' impulsivity or decision accuracy.

4. Discussion

Accumulating evidence from animals and humans has linked olfaction to reproduction. In rodents, female body odors stimulate the male reproductive system and induce increased sexual arousal (Richardson et al., 2004). The present finding that estratetraenol induces preference for large sexual rewards is consistent with the growing literature that estratetraenol, a putative human sex pheromone, increases sexual motivation among heterosexual males (Oren et al., 2019). In particular, when viewing photos with two humans either romantically touching or not, estratetraenol increased individuals' perceived emotionality of romantically touching, suggesting that estratetraenol conveys mating-related information such as the presence of women and their reproductive readiness. These effects held despite that the olfactory stimuli were not explicitly discriminable, which is consistent with previous evidence that the social odor cues take effect without the involvement of consciousness (Zhou et al., 2014).

These findings offer insight into the evolutionary significance of estratetraenol in human social behavior. Previous research has shown that exposure to estratetraenol activates the

anterior hypothalamus of heterosexual males, a key brain region associated with sexual behavior (Savic et al., 2001). From the present study, estratetraenol may communicate information regarding women's readiness for reproduction, and the preference for larger-later sexual rewards as depicted in the erotic picture would give men more time to evaluate the potential mates so as to maximize their reproductive fitness.

Some issues warrant further discussion. First, previous research has shown that in humans, the scent cues of women near peak levels of fertility increase salivary testosterone levels as well as accessibility to sexual concepts and perceptions of women's sexual arousal in men (Cerda-Molina et al., 2013; Miller and Maner, 2010). We encourage future estratetraenol research to measure testosterone levels before and after the estratetraenol exposure and test whether the estratetraenol-induced changes are mediated by testosterone fluctuation. Second, although there is growing recognition of chemosensory communication in humans, this effect has been challenged by statistical methods (Strassmann, 1999). Future studies could fruitfully address this issue by employing a more refined experimental design. Third, the effects of estratetraenol have been reported to be sexually dimorphic. Hence, this effect on homosexual men and heterosexual women awaits further investigation.

Declaration of competing interest

None of the authors have conflicts of interests to declare.

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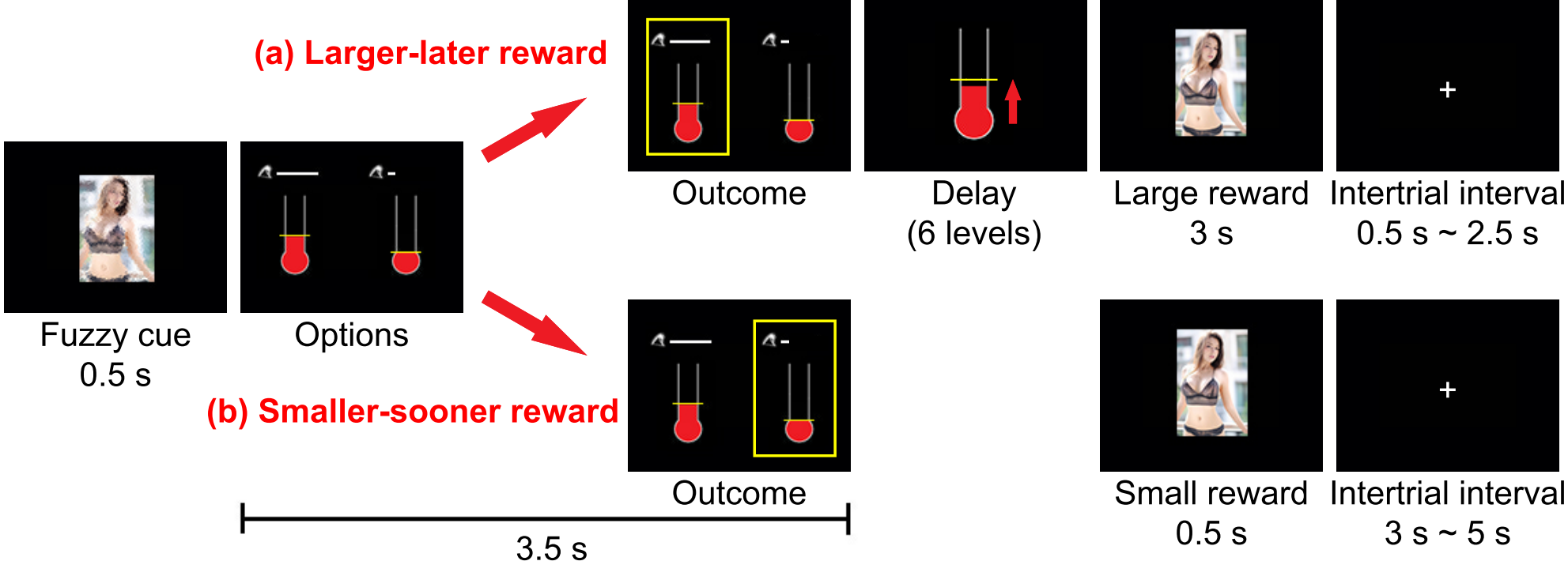
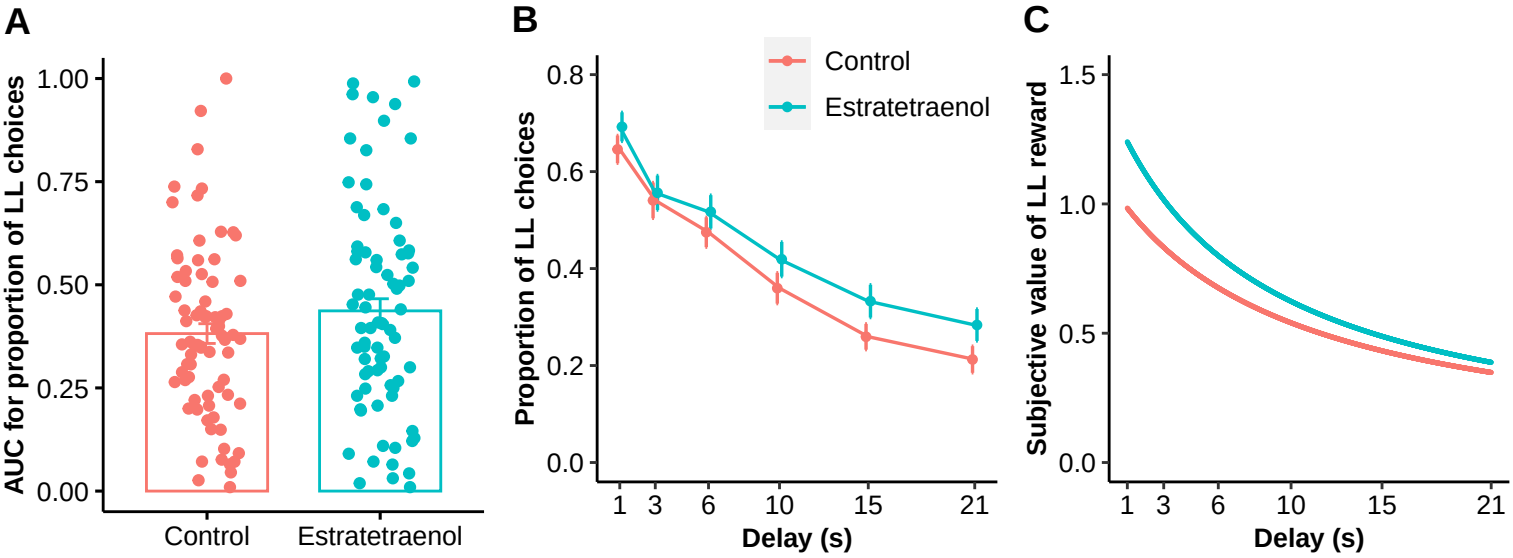
Figure captions

Figure 1. Sexual delay discounting task. In each trial, a fuzzy erotic picture briefly appeared on the screen and was followed by the instruction 'Wait?', along with a thermometer indicating one of six possible levels of the proposed delay period to wait. Participants had to decide between a variable larger-later option (i.e. waiting longer to view a sexual picture for a longer duration) and a smaller-sooner option (i.e. waiting for a fixed shorter period of time to view the same picture for a shorter duration), depending on the incentive cue and the level of waiting proposed. If they accepted to wait the cost proposed, they had to wait passively during the proposed delay period before seeing the erotic picture for a longer time (large reward). Otherwise, if they refused, they saw the erotic picture clearly for a short time period (small reward) immediately.

Figure 2. (A) AUC for proportion of LL choices between conditions, the error bars represent SE. **(B)** Proportion of LL choices at different delay levels in SDDT. **(C)** Subjective values of the LL reward as a function of delay intervals in SDDT. Error bars represent standard errors of means.


(a) Larger-later reward
Fuzzy cue
0.5 s
Options
Outcome
Delay
(6 levels)
Large reward
3 s
Intertrial interval
0.5 s ~ 2.5 s
+
(b) Smaller-sooner reward
Outcome
Small reward
0.5 s
Intertrial interval
3 s ~ 5 s
+
3.5 s


A
AUC for proportion of LL choices
1.00
0.75
0.50
0.25
0.00
Control
Estratetraenol
B
Proportion of LL choices
0.8
0.6
0.4
0.2
0.0
Control
Estratetraenol
1
3
6
10
15
21
Delay (s)
C
Subjective value of LL reward
1.5
1.0
0.5
0.0
1
3
6
10
15
21
Delay (s)

Ratings of the fuzzy cues

In order to reveal the variability of participants' motivation to see each picture, participants were asked to rate the 60 fuzzy cue pictures after completing the sexual delay discounting task. They were asked to rate "To what extent would you like to see this picture in its clear form" from 1 to 9 with an increment of 0.1, with 1 indicating not at all, 5 indicating neutral and 9 indicating very much.

The ratings of the 60 fuzzy cue pictures did not differ between the two conditions [estratetraenol: $M = 5.36$, $SD = 0.99$; control: $M = 5.50$, $SD = 1.21$, $t(75) = 1.26$, $p = 0.211$, Cohen's $d = 0.145$].

Order effect between session days

In a mixed ANOVA with olfactory condition (EST, CON) and delay (6 levels) as within-subject factors and order (estratetraenol or control first) as a between-subject factor, the main effect of order was not significant ($F(1,74) = 0.049$, $p = 0.83$), suggesting that there was no order effect between session days. Importantly, the main effect of olfactory condition remained significant, ($F(1,74) = 4.28$, $p = 0.042$, partial $\eta^2 = 0.055$).

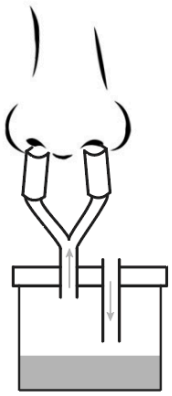


Figure S1. Illustration of the device for odor presentation

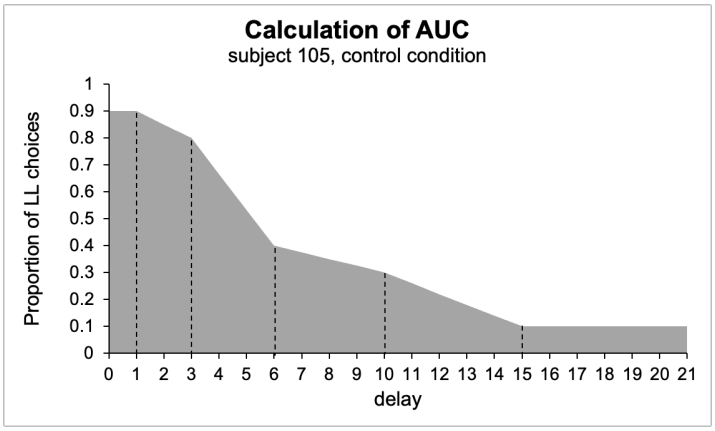


Figure S2. Example of AUC calculation. The AUC was calculated by using proportions of *LL* choices (*p*), normalizing delay time (D) as a percentage of maximum D, connecting the *p* points by straight lines, and then summing the trapezoids formed.

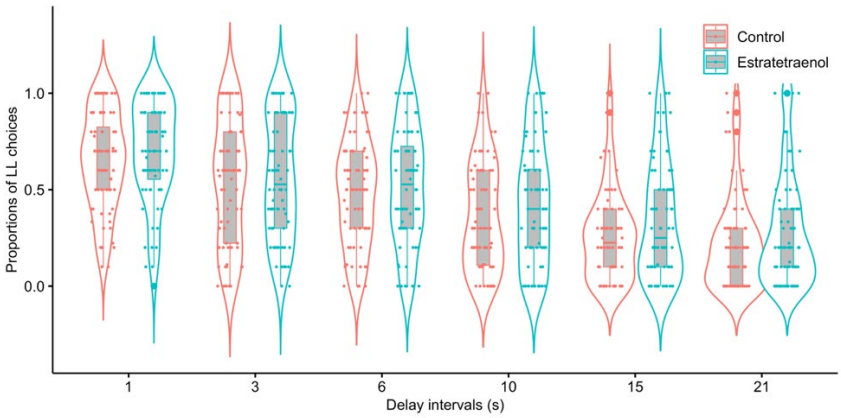


Figure S3. Proportions of larger-later choices of each individual and the distributions in each treatment condition at each delay level.

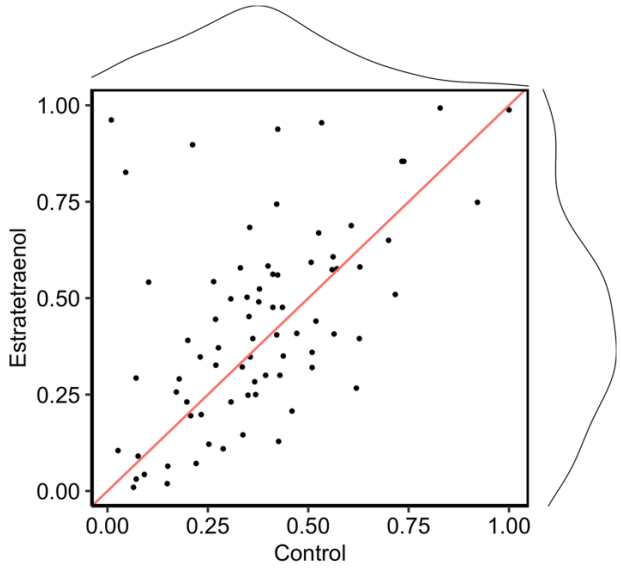


Figure S4. Scatter plot of the AUCs distribution with control condition as X-axis and EST condition as Y-Axis.

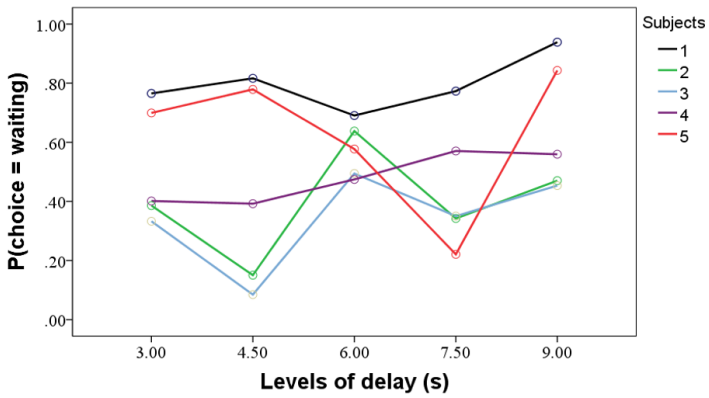


Figure S5. Pilot study data. We tested 5 participants using delay levels of 3-9 s with an increment of 1.5 s, there was no general trend of discounting (i.e., participants made choices independent of delay).