Hormones and Behavior 85 (2016) 30–35

Contents lists available at ScienceDirect

Hormones and Behavior

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

Social and physical environments as a source of individual variation in the rewarding effects of testosterone in male California mice (*Peromyscus californicus*)

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ARTICLE INFO

Article history:
Received 20 January 2016
Revised 21 July 2016
Accepted 27 July 2016
Available online 28 July 2016

Keywords:
Testosterone
Reinforcing effects
Territory
Conditioned place preference
Pair bonding

ABSTRACT

Despite extensive research revealing the occurrence of testosterone (T) pulses following social encounters, it is unclear how they lead to varied behavioral responses. We investigated the influence of residency (home versus unfamiliar environment) and social/sexual experience (pair-bonded, isolated or housed with siblings) on the plasticity of T's rewarding effects by measuring the development of conditioned place preferences (CPPs), a classical paradigm used to measure the rewarding properties of drugs. For pair-bonded males, T-induced CPPs were only produced in the environment wherein the social/sexual experience was accrued and residency status had been achieved. For isolated males, the T-induced CPPs only occurred when the environment was unfamiliar. For males housed with a male sibling, the T-induced CPPs were prevented in both the home and unfamiliar chambers. Our results reveal the plasticity of T's rewarding effects, and suggest that the behavioral functions of T-pulses can vary based on social/sexual experience and the environment in which residency was established. The formation of CPPs or reward-like properties of drugs and natural compounds can therefore exhibit malleability based on past experience and the current environment.

1. Introduction

The reciprocal relationship between the steroid hormone testosterone (T) and territorial aggression occurs in a wide variety of species (review see Hirschenhauser and Oliveira, 2006). Plasma T can be positively correlated with the expression of particular forms of aggression such as territorial and dominance aggression (Gesquiere et al., 2011; Wingfield and Wada, 1989) (but see Apfelbeck and Goymann, 2011), and experimental manipulations of circulating T levels can alter aggressiveness (Fuxjager et al., 2011b; Monaghan and Glickman, 1992; Trainor et al., 2004). Alternatively, T-pulses can be elicited following male-male agonistic encounters as well as male-female sexual encounters (review see Gleason et al., 2009). Post-encounter T-pulses occur in males of many vertebrate species; but the function of these T-pulses remains largely unclear. Within the context of social behavior, however, the post-encounter T-surges may reinforce learning associated with an aggressive encounter (Marler et al., 2005). The rewarding effect of T in rodents have been revealed (Packard et al., 1997; Rosellini et al., 2001; Sato et al., 2010; Zhao and Marler, 2014), but researchers have ignored the impact of the physical and social environment on the rewarding effects of T. The current study explores the plasticity of T's rewarding effects, which may provide individuals with a mechanism for altering their behavioral responses to the environment.

One important source of plasticity in T's rewarding effects may be the environmental context in which T is released, such as the location where residency has been established. Territories are critical for maintaining access to resources in many species, and the primary behavioral mechanism for retaining a territory is aggression; territorial context significantly modulates aggressive behavior and the outcome of a contest (Fuxjager et al., 2010b; Snell-Rood and Cristol, 2005). The best example of this is the phenomenon called the 'home advantage' (Schwartz and Barsky, 1977) or 'residence effect' (Kemp and Wiklund, 2004), whereby the resident has an advantage over an intruder in a territorial dispute. Manipulations of and correlations with the environmental context support this notion and in many species, males behave more aggressively toward intrusion in the home cage when residency has been established, but less aggressively in an unfamiliar environment (Fayed et al., 2008; Krebs, 1982; McGuire et al., 1992; Waage, 1988). Given the important role of T in modulating territorial aggression, if the rewarding effect of T contributes to such context-dependent behavioral responses, the rewarding effect may also rely on the environmental context.

Besides context, the social/sexual experience may also influence animals' responses to the rewarding properties of T. We previously found that pair-bonding experience dampens the rewarding effect of T in male California mice (*Peromyscus californicus*) (Zhao and Marler, 2014).

http://dx.doi.org/10.1016/j.yhbeh.2016.07.007
0018-506X/

Social interactions can also decrease the rewarding properties of amphetamine in prairie voles (*Microtus ochrogaster*) (Liu et al., 2011) and influence drug intake and susceptibility to drug abuse (review see Young et al., 2011). Moreover, the social/sexual experience accrued within the home area can shape the salience of the home environment when coupled with residency. Studies in mice (Martínez et al., 1995; Popik et al., 2003), rats (Ma et al., 2006), hamsters (Bell et al., 2010; Meisel and Joppa, 1994), European starlings (*Sturnus vulgaris*) (Kelm-Nelson et al., 2012; Riters et al., 2014), green anole lizards (*Anolis carolinensis*) (Farrell and Wilczynski, 2006) and gilthead sea bream (*Sparus aurata*) (Millot et al., 2014) demonstrate that animals can associate natural rewards such as the social/sexual experience and foods with the physical environment in which the experience was acquired, and produce conditioned place preferences (CPPs). The social/sexual experience may therefore interact with residency to modulate animals' responses to T's rewarding effect.

We investigated the plasticity of T's rewarding effects by examining the influence of residency combined with the social/sexual experience on T-induced CPPs. CPP is a classical paradigm used in examining rewarding properties of drugs (Tzschentke, 2007). Although widely used in the laboratory, exploration of its adaptive function has been neglected. Here, we hypothesized that T-induced CPPs are plastic in response to residency and social/sexual experience, thereby contributing to the behavioral flexibility in different environments; to test this, we used the California mouse because of the extensive research on interactions among residency, T and aggression in this highly territorial and monogamous species (Fuxjager et al., 2010a; Fuxjager et al., 2009; Oyegbile and Marler, 2005; Trainor et al., 2004; Trainor and Marler, 2001). According to laboratory and field studies, males appear to experience three types of home environments that differ in terms of the social/sexual experience (Ribble, 1992). First, in the natal home, the young of one litter can occupy the nest with the parents during the rearing of a second litter (Eisenberg, 1962). In this natal environment, the male response to T in the form of CPPs may be inhibited because of both the social presence of siblings/family (e.g. Bennett et al., 1999) and residency in the natal territory. Second, males then typically disperse approximately the distance of one home range (1161 m^2) (Ribble, 1992; Ribble and Salvioni, 1990) and establish their own territories. Third, a female may then disperse to the male's territory (Ribble, 1992) and a pair bond ensues. These three types of home environments may be salient to animals in different respects as the natal home could include interactions with siblings; sexually naive males need to monopolize critical resources in their own home ranges to attract females; home is imperative for pair-bonded mice to maintain bonding and breed. In the current study, we indirectly mimic aspects of the above three types of home environments and study how residency interacts with social/sexual experience to influence the T-induced CPPs.

2. Methods

2.1. Subjects

We used 120 male *P. californicus* aged 6–12 months. They were group-housed (2–3 per cage; 48 × 27 × 16 cm) under a 10L: 14D light cycle with lights off at 01:30 pm. Animals were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Males were randomly assigned to one of three groups: male-male (MM) group (n = 40), male-single (MS) (n = 40) and pair-bonded (PB) group (n = 40). For the MM group, two males were weaned, housed together (no aggression or injuries were observed) and moved to the middle chamber of the CPP apparatus three days prior to the CPP trial; one of the two males was randomly selected as the focal animal. For the MS group, a randomly selected sexually naive male was separated from its cage mate and moved to the middle chamber three days prior to the CPP trial. For the PB group, each male was paired with a female 1-week before being housed in the middle chamber for three days before the CPP trial. The paired male and female mice were huddling side-by-side after 24 h of pairing, which is a well-accepted indicator of partner preference in monogamous prairie voles (Ahern et al., 2009; Liu et al., 2001; Williams et al., 1992). We did not record the mating behavior of paired animals, but typically a majority has mated within 10 days (Gleason and Marler, 2010). Pairs were observed for compatibility and if fighting occurred then the pair was separated and excluded from the experiment (n = 3).

2.2. Testosterone dose

We used 36 μg/kg T-injections (T-cyclodextrin inclusion complex) because in a previous study this dose produced an increase in T-levels approximately 3–5 times higher than the baseline, reaching a maximum of 4–5 ng/ml and lasting for approximately 10 min (Trainor et al., 2004). Moreover this dose produces CPPs in male California mice (Zhao and Marler, 2014). While the dose in the current study is lower than those used to identify CPPs in rats and mice, it mimics natural changes in male T-levels found in intact California mice after winning an aggressive encounter (Oyegbile and Marler, 2005) and male-female encounters (Zhao and Marler, 2014); in keeping with this, the same dosage enhances aggression and future winning ability (Fuxjager et al., 2011a; Fuxjager et al., 2011b; Gleason et al., 2009; Trainor et al., 2004). In the current study, half of the males were randomly selected to receive T-injections during the conditioning phase (T-group). As T-cyclodextrin was dissolved in saline, the other half of the males constituted the controls and received injections of saline (saline group).

2.3. CPP apparatus and procedure

Conditioning took place in large polycarbonate testing cages (91 cm long × 46 cm wide × 43 cm high) divided into three equal chambers. The two side chambers were connected to the middle chamber by manually controlled, sliding guillotine doors. To elicit the residence effect, we housed animals in the middle chamber for three days. We have used three days and fewer (24–48 h) to establish residency status (Fuxjager et al., 2010a; Oyegbile and Marler, 2005). For the unfamiliar environment, the middle chamber was again used but without prior residency, and therefore no odor cues. The focal males in each of the three groups were randomly assigned to be conditioned to either the home environment (home group) or the unfamiliar environment (unfamiliar group) (Fig. 1).

The CPP procedure was conducted over eight days (see Zhao and Marler, 2014). On day 1, a male was allowed to explore all three chambers for 30-min and was excluded if all three were not investigated. During this 30-min period, the female partner or the male cage mate was removed from the apparatus. The training phase occurred from days 2–7. On days 2, 4 and 6, each male received a T-injection and was placed into the home or unfamiliar middle chamber for 45-min. On days 3, 5 and 7, the male explored all three chambers for 45-min. The order of injections (T or saline) and exploration were also randomly assigned to the focal males but counterbalanced to reduce the chances of the order of treatment or other factors adversely influencing the results. On day 8, we tested the male's preference by recording time spent in each chamber as males explored all three chambers for 30-min. The female partner or the male cage mate was temporarily removed from the apparatus during the 30-min adaptation, the 30-min test and each 45-min conditioning session (Fig. 2).

2.4. Data analysis

Time spent in the middle chamber was used for analysis. The normality of the data was determined by the Shapiro-Wilk test. Three