Animal Behaviour 100 (2015) 8–14

Contents lists available at ScienceDirect

Animal Behaviour

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

A single testosterone pulse rapidly reduces urinary marking behaviour in subordinate, but not dominant, white-footed mice

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ARTICLE INFO

Article history:
Received 5 August 2014
Initial acceptance 29 August 2014
Final acceptance 31 October 2014
Published online
MS. number: A14-00636R2

Keywords:
androgen
communication
dominant/subordinate
mating strategy
Peromyscus
territoriality
urinary scent marking

Androgenic hormones can rapidly influence how animals behave, although the dynamics of these effects remain elusive. In particular, we know little about whether androgens rapidly impact behavioural strategizing or whether such effects vary among individuals of the same species. Here, we examine these issues by testing how transient testosterone (T) surges, which normally occur in response to social stimuli, rapidly alter the urinary marking behaviour of socially subordinate and dominant male white-footed mice, *Peromyscus leucopus*. Marking behaviour in mice is important in reproductive competition and advertisement; thus, how an individual marks its environment with urine likely reflects the strategy it uses to find a mate. Moreover, urine-marking patterns are indicative of social status and territorial propensity. Our results show that a single T pulse, within minutes, suppresses all measures of urinary marking behaviour in subordinate mice, including amounts of perimeter (territorial) marking and total (status-related) marking. These effects are not observed in dominant mice. The data collectively suggest that androgens rapidly change behaviour tightly associated with reproductive strategy and decision making. Moreover, these effects depend on an individual's social status, which suggests that there is intraspecific variation in the way that androgens affect marking behaviour on short timescales. This study fills a gap in our understanding about the plasticity of rapid androgenic effects on behaviour, and how these responses can influence adaptive reproductive tactics.

Androgens regulate adaptive behaviour by integrating endogenous physiological processes with the external environment (Adkins-Regan, 2005). Under this paradigm, it is often assumed that (1) the effects of androgens on behavioural output occur over extended timescales and (2) the adaptive benefits of such hormone action are similar for all individuals of a given species. These assumptions, however, many not apply to all species. For instance, in animals like bluehead wrasse, *Thalassoma bifasciatum*, and African cichlids (*Haplochromis burtoni*), individuals constantly fine-tune their behavioural tactics in response to fluid social and/or environmental conditions (Godwin, Crews, & Warner, 1996; Hofmann, Benson, & Fernald, 1999). In other animals, such as prairie voles, *Microtus ochrogaster*, alpine marmots, *Marmot marmota*, and spear-nosed bats, *Phyllostomus hastatus*, conspecifics within a local population differ dramatically in terms of the behavioural strategy used to acquire mates (Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993; Goossens et al., 1998; McCracken & Bradbury, 1981). In cases like these, the effects of androgens on behavioural output may need to be rapid and/or different among individuals. Little research has explored the nature of such 'nontraditional' androgen–behaviour interactions, and this has contributed to a substantial gap in our understanding of the way in which androgenic hormones mediate suites of dynamic reproductive behaviour.

Growing research suggests that androgens can affect behaviour within minutes of cellular activation (Cross & Roselli, 1999; Mangiamele & Thompson, 2012; Remage-Healey & Bass, 2006a, 2006b). These so-called rapid effects of androgens are thought to support behavioural flexibility by acting as the mechanism through which sudden changes in the environment can quickly modify individual behaviour (Mangiamele & Thompson, 2012; Remage-Healey & Bass, 2006a, 2006b). Support for this idea comes primarily from work illustrating that specific social or environmental cues can trigger spikes in plasma testosterone (T) levels (Gleason, Fuxjager, Oyegbile, & Marler, 2009). For example, in rodents like laboratory mice, *Mus musculus*, California mice, *Peromyscus*

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http://dx.doi.org/10.1016/j.anbehav.2014.11.006
0003-3472/

californicus, and Norwegian rats, *Rattus norvegicus*, males that either detect a receptive female (Amstislavskaya & Popova, 2004; Gleason & Marler, 2010; Nyby, 2008) or win a competitive bout (Marler, Oyegbile, Plavicki, & Trainor, 2005; Oyegbile & Marler, 2005) experience a transient surge of T. This suggests that a single T pulse generated in response to a salient cue has the capacity to change subsequent behaviour quickly, particularly behaviour related to reproduction and competition. However, few studies have experimentally manipulated the occurrence of these singular T pulses to measure their direct and immediate effect on an individual's sociosexual repertoire.

One factor that may determine how androgens rapidly alter the way an individual behaves is perceived social status. Animals of differing ranks or statuses often use distinct behavioural tactics that maximize their own chances for survival and reproduction (Oliveira, 2009), and past work shows that accruing such social experiences that help determine position within a hierarchy may modify androgenic signalling systems in the brain (Fuxjager, Forbes-Lorman, et al., 2010; Holmes, Goldman, & Forger, 2008). Behavioural acquisition of one's status may therefore affect how androgens act centrally to differentially influence behaviour across separate individuals. From a functional standpoint, this may allow individuals to respond to the bioactivity of androgenic hormones in a manner that is most suitable to their own condition or state. Whether such socially selective effects of androgens occur is poorly understood.

Building on the information described above, we hypothesize that certain rapid effects of androgens on reproductive behaviour depend on social status. We test this hypothesis by studying the rapid effects of a single T pulse on urinary scent-marking behaviour in white-footed mice, *Peromyscus leucopus*. In murid rodents, urinary marking behaviour is androgen dependent, as T acts on discrete brain nuclei that control sexual motivation and arousal to impact how and when males deposit urine throughout their environment (Matochik, Sipos, Nyby, & Barfield, 1994; Sipos & Nyby, 1996, 1998). This provides a clear pathway through which androgens can modulate reproductive decision making and the urinary marking behaviour that corresponds to such processes. In addition, murid rodents differentially mark substrates according to their own perceived social status, with dominant males marking novel arenas more abundantly and subordinate males marking the same type of arena more sparsely (Desjardins, Maruniak, & Bronson, 1973; Drickamer, 2001). To this end, recent work in California mice attributes this variation in marking behaviour to differences in territoriality. Males that are pair-bonded, and thus maintain a territory, scent-mark like dominant males; exposing these males to a novel female consequently does little to change this behaviour. By contrast, males that are unpaired dramatically increase their overall marking behaviour when they come into contact with a novel female, suggesting that the males begin acting in a territorial manner through urinary marking to attract the 'available' female (Becker, Petruno, & Marler, 2012).

In white-footed mice, evidence suggests that urinary marking similarly helps mediate male–female attraction (Drickamer, 1984) and male–male competition (Fuxjager, Montgomery, et al., 2010). Adult males mate promiscuously (Xia & Millar, 1991) but rely on different tactics to acquire mates; that is, some males guard territories to attract mates, whereas other males adopt wandering search strategies (Wolff, 1986; Wolff & Cicirello, 1990). Scent marking may therefore not only reflect these tactical differences, but also help mediate them (Becker et al., 2012; Fuxjager, Montgomery, et al., 2010). Interestingly, past work suggests that adult males do not overtly change their marking behaviour in response to either sexual or competitive contexts (Becker, Moore, Auger, & Marler, 2010). This past study, however, examined adult males as a whole and did not account for interspecific differences in personality or social rank, which are known to influence behavioural decision making in this species (Fuxjager, Montgomery, et al., 2010). Furthermore, research in this species suggests that T plays an important role in regulating male reproductive behaviour, as circulating T increases during the periods of sexual activity and breeding (Pyter, Neigh, & Nelson, 2004). This opens the door to the possibility that pulsatile release of T may affect behaviour associated with reproduction, such as urinary marking.

Thus, in our current study, we test two specific hypotheses: (1) a single pulse of T, similar to that produced by a male after he detects a receptive female in the vicinity, has the ability to modulate urinary marking behaviour rapidly in adult male mice; and (2) these effects can vary based on an individual's social status. We predicted that one injection of T would change marking behaviour within minutes of its administration, but that the way in which T influences this behaviour would differ between dominant and subordinate males either in the degree or the presence/absence of urinary marks.

METHODS

Animals

We used sexually naïve male white-footed mice (age 6–12 months) reared in a laboratory colony at the University of Wisconsin, Madison. Mice were provided Purina 5050 mouse chow and water ad libitum, and individuals were kept under a 14:10 h light:dark cycle (lights on: 0300 hours Central Standard Time, CST). After weaning, mice were housed in same-sex groups of three to four individuals per cage (30 × 19 × 13 cm), typically mixing young males from different litters. Rodents housed in such conditions often form clear dominance hierarchies (Davis, 1958; Haemisch, Voss, & Gartner, 1994; Poole & Morgan, 1973); thus, measurement of an individual's social status when housed in these conditions is directly related to the experiences it accrues over time (Drickamer, 2001). Only one randomly selected animal per cage was used in the study.

Ethical Note

Mice were maintained according to the recommendations of the National Research Council (2010). All appropriate institutional authorities approved of the experimental protocol described herein, and the mice used in this study suffered no negative effects from either the hormone treatment or the behavioural assay.

Baseline Marking Patterns

We first measured the social status of each male used in our study ($N = 29$) by collecting a baseline urinary marking pattern. Prior work has shown that socially dominant mice deposit numerous, widely scattered urine marks within a marking arena, whereas socially subordinate males deposit only a few pools of urine in the same space (Desjardins et al., 1973; Drickamer, 2001). All marking behaviour was collected according to methods described in detail elsewhere (Becker et al., 2012; Fuxjager, Montgomery, et al., 2010). Briefly, we placed each male by itself in a large glass aquarium (i.e. marking arena; 60 × 30 × 30 cm) that contained a single sheet of unsoiled filter paper on the bottom surface (Fisher Brand; Qualitative P8; flow rate: fast). A screen top was placed over the marking arena to prevent the mouse from escaping. Each male was given 20 min to explore and mark the arena in darkness under dim red light. Once this time expired, the male was removed from the arena and put back in its home cage