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# Testosterone and nucleus accumbens dopamine in the male Syrian hamster

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## Summary

Most drugs of abuse increase dopamine (DA) in nucleus accumbens (Acb). However, the effects of anabolic androgenic steroids (AAS) on Acb DA have not been examined. We determined the effects of subcutaneous (sc) testosterone (T) on Acb DA in male hamsters. The effects of sc amphetamine were also examined for comparison. In addition, Acb DA was evaluated during intracerebroventricular (ICV) T infusion, designed to mimic T intake during ICV T self-administration in drug-naïve and drug-preexposed animals. Acb DA was measured using *in vivo* microdialysis and HPLC-EC. T (7.5 or 37.5 mg/kg), amphetamine (1 or 5 mg/kg), or vehicle was injected sc and Acb DA monitored for 4 hrs. In the ICV experiment, T (1 or 2 μg/infusion) or vehicle was infused ICV every 6 min for 4 hrs and Acb DA monitored. ICV T preexposure was accomplished by repeating the same ICV T infusion (1 μg/infusion) daily for 14 days, and T infusion was accompanied by microdialysis on 15$^{th}$ day. Neither sc nor ICV T administration increased Acb DA. At high dose (2 μg/infusion), ICV T decreased Acb DA. Likewise, daily ICV infusion of T for 15 days did not alter Acb DA. In contrast, sc amphetamine significantly increased Acb DA at both doses. Therefore, unlike many drugs of abuse, AAS does not increase Acb DA levels. The reduction in DA at high T doses is likely due to autonomic depressant effects of AAS. We suggest that AAS act via mechanism distinct from those of stimulants, but may share neural substrates with other drugs of abuse.

### Keywords

anabolic androgenic steroid; testosterone; amphetamine; hamster; nucleus accumbens; dopamine; microdialysis; HPLC-EC; intracerebroventricular; subcutaneous

Androgenic anabolic steroid (AAS) use is widespread among athletes and non-athletes (Yesalis et al., 1993). Physical (Leshner, 2000) and psychological (Brower, 2002; Pope and Katz, 1994) effects of AAS use are of significant concern from a public health perspective. Brower (2002) has recently suggested that most AAS users initiate use for the anabolic properties, but many subsequently develop physical and psychological dependence. However, the addictive potential of AAS has received little attention so far. The results of studies using animal models of drug abuse indicate that AAS are reinforcing. For example, AAS induces conditioned place preference (CPP) in rats (Packard et al., 1997) and mice (Arnedo et al., 2000). In addition, AAS

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are self-administered through various routes in rats (Sato et al., 2006; Wood et al., 2004) and hamsters (Ballard and Wood, 2005; DiMeo and Wood, 2006b; Frye et al., 2007; Johnson and Wood, 2001; Peters and Wood, 2005; Wood, 2002). However, it is not known how AAS affect neural circuitry underlying the reinforcing effects of other drugs of abuse.

The mesolimbic dopamine (DA) system, the DAergic projection from the ventral tegmental area (VTA) to the nucleus accumbens (Acb), is a major substrate for drugs of abuse (Berridge and Robinson, 1998; Koob and Nestler, 1997). Selective lesion of DAergic fibers disrupts self-administration of stimulants (Roberts et al., 1977). DA antagonists also have been shown to attenuate the reinforcing effects of cocaine during self-administration (Caine & Koob, 1994). In addition, most commonly-abused drugs are known to increase DA levels in Acb, including stimulants (Di Chiara and Imperato, 1988), opiates (Di Chiara and Imperato, 1988), ethanol (EtOH, Di Chiara and Imperato, 1985), and nicotine (Imperato et al., 1986).

There is some evidence implicating a role for the mesolimbic DA system in AAS abuse. For example, AAS induces CPP when injected into Acb (Packard et al., 1997), an effect blocked by the DA antagonist α-flupenthixol (Packard et al., 1998). Furthermore, acute intracerebroventricular (ICV) administration of AAS induces c-Fos expression in the VTA (Dimeo and Wood, 2006a). Based on these data, we have hypothesized that the reinforcing effects of AAS are mediated by the mesolimbic DA system. If AAS utilize the same neural substrates as other drugs of abuse, then AAS should also increase DA in the Acb. The current study was designed to examine Acb DA release in response to AAS administration. Acb DA release was measured in hamsters, using *in vivo* microdialysis with high performance liquid chromatography with electrochemical detection (HPLC-EC). We examined the effects of acute systemic administration of testosterone (T) on Acb DA release. As a control, we also examined the effects of acute amphetamine administration on Acb DA. Furthermore, we examined the effects of ICV T infusions designed to mimic drug intake during self-administration. Finally, we tested the effects of ICV T following repeated (15 day) ICV T administration, in order to control for possible interference from the autonomic depressant effects of T.

## Materials and Methods

### Animals

Adult male Syrian hamsters (120–160g BW) were obtained from Charles River Laboratories (Wilmington, MA). Hamsters were housed individually under a reversed long-day photoperiod (14L: 10D) with lights off at 9 AM. Food and water were available *ad libitum*. All tests were conducted during the dark phase of their light cycle.

### Experimental Design

In the first experiment, we examined the effects of an acute injection of systemic T on Acb DA release. Following baseline sample collection, hamsters received a subcutaneous injection of 7.5 mg/kg T (n = 5), 37.5 mg/kg T (n = 5), or vehicle (n = 5). Acb DA levels were monitored for 4 hrs following the injection. The lower dose (7.5 mg/kg) has been used previously in rats and mice to test the effects of androgens on seizure activity (Frye and Reed, 1998), anxiety (Rojas-Ortiz et al., 2006), aggression (Martinez-Sanchis et al., 1998), and social behavior (Barreto-Estrada et al., 2004). Furthermore, 7.5 mg/kg testosterone is comparable to doses tested in human volunteers. According to the National Center for Health Statistics (NHANES, 2007), the average American man weighs 86 kg. Thus, a typical human dose of 600 mg testosterone (Bhasin et al., 1996; Kouri et al., 1995; Tricker et al., 1996) is equivalent to 7 mg/kg.

The Acb DA responses to drugs of abuse have not been previously examined in Syrian hamsters. Therefore, we examined Acb DA response to amphetamine in hamsters as a control. Hamsters were subcutaneously injected with either 1 or 5 mg/kg amphetamine, and Acb DA was monitored for 4 hrs. These doses of amphetamine are known to induce robust increases in Acb DA in rats (Birgner et al., 2007; Di Chiara and Imperato, 1988).

In the second set of experiments, Acb DA levels were examined using a drug infusion paradigm similar to ICV self-administration. Following baseline sample collection, T was infused through a modified microdialysis probe inserted into the lateral ventricle. Drug-naïve animals received vehicle (n = 5), 1 μg/infusion T (40 μg total; n = 6), or 2 μg/infusion T (80 μg total; n = 4) every 6 min over 4 hrs. Solutions were delivered as 1 μl infusions every 6 min (40 μl/4hrs), using a programmable syringe pump (BS-8000, Braintree Scientific, Braintree, MA). Acb DA was monitored throughout the 4 hr infusion. Forty μg (1 μg/infusion) T was designed to approximate a heavy dose of T during ICV T self-administration, while 80 μg (2 μg/infusion) T is a maximal dose. We have previously used this method to examine ICV T-induced c-Fos expression (Dimeo and Wood, 2006a) and physiologic effects of T (Peters and Wood, 2005).

As we have previously demonstrated, T exerts autonomic depressant effects when administered ICV (Peters and Wood, 2005). It is possible that any influence of T on Acb DA may be masked by its autonomic depressant effects. In order to control for this possibility, we infused 1 μg/infusion T (n = 5) ICV daily for 14 days as described above. This duration is sufficient for hamsters to develop tolerance to autonomic depressant effects of T (Peters and Wood, 2005). On 15$^{th}$ day, T infusion was accompanied by microdialysis sampling.

## Surgery

Surgical procedures were carried out under aseptic conditions according to “Principles of laboratory animal care” (NIH publication NO. 86-23, revised 1985). Hamsters were anesthetized with sodium pentobarbital (80 mg/kg) and secured in a Kopf stereotaxic apparatus with lambda and bregma in the same horizontal plane. A microdialysis guide cannula (CMA/12, CMA, N. Chelmsford, MA) was lowered to 1 mm above Acb. Stereotaxic coordinates were: AP: +3.3 mm, ML: +1.1 mm, DV: − 6.0 mm from bregma, (Fig. 1a, Morin and Wood, 2001). For ICV infusion, another guide cannula was implanted into the lateral ventricle (AP: +0.8 mm, ML, −1.2 mm, DV: −5.0 mm from bregma). Dummy cannulae were inserted to prevent the entry of foreign material. The cannula assemblies were secured to the skull with stainless steel screws and dental acrylic. After surgery, males were allowed to recover for at least 5 days before microdialysis sampling.

## Drugs

Testosterone (Steraloids, Newport, RI) was dissolved in an aqueous vehicle of 13% 2-hydroxyorpyl-β-cyclodextrin (βCD, RBI, Natick, MA). Glass was used for preparation and delivery of testosterone since steroids readily adsorb to plastic (Bruning et al., 1981). D-amphetamine (Sigma-Aldrich, St. Louis, MO) was dissolved in the same vehicle.

## Microdialysis and DA analysis

Testing was performed in a 30 cm$^2$ glass aquarium. To obtain a stable baseline of DA release, the microdialysis probe (CMA/12, 1 mm dialysis membrane) was inserted at least 12 hrs before testing, and perfused with artificial cerebrospinal fluid (aCSF, pH 7.4, Harvard Apparatus, Holliston, MA). The flow of aCSF (1.5 μl/min) began immediately after probe insertion, and was controlled by a Harvard Model 11 syringe infusion pump. A 15–22.5 μl dialysate was collected every 10–15 min. Baseline samples were collected for at least 50 min before drug infusion or injection, and sampling continued for the next 4 hrs. Dialysates were collected into a centrifuge tube and frozen until analysis.

Dialysates were loaded via a Rheodyne injector valve (Model #9125, Rheodyne Inc., Cotati, CA), isolated using a reverse-phase column (ESA MD-150, Chelmsford, MA) and quantified using an ESA Coulochem II detector (ESA model 5200) comprising a guard cell (+350 mV, ESA model 5020) and an analytical cell (ESA model 5014B) with two electrodes in series. The potentials of the electrodes were set at −125 mV and +125 mV to detect DA at the second electrode. Flow rate of the mobile phase (ESA MD-TM) was 0.6 ml/min using an ESA pump (Model 582). Data were collected using PowerChrom software (AD instruments, Mountain View, CA) linked to a Macintosh computer. The detection threshold for DA was ≥ 100 fg at 3:1 SNR. Due to the low levels of DA observed in some groups, the system was optimized for detection of DA. As a result, DA metabolites were often out of detection range, thus not analyzed in the current study.

### Statistical Analysis

Basal DA release was determined from the average of the last 3 baseline samples. Subsequent samples were expressed as a percentage of the mean baseline value. The percent changes from the baseline were calculated for each sample, and consecutive samples were pooled to produce percent baseline values for 30 min samples. Each treatment condition was analyzed with 1-way repeated measures ANOVA, followed by Tukey's multiple-comparions test when appropriate. In all analyses, $p < 0.05$ was considered significant.

### Histology

At the end of the experiment, each male was deeply anesthetized with sodium pentobarbital and perfused through the aorta with 150 ml of 0.1 M phosphate-buffered saline containing 0.1 % sodium nitrite for vasodilation, followed by 250 ml of 0.1 M sodium phosphate buffer containing 4 % paraformaldehyde. Brains were removed and post-fixed in the same fixative for 1 h at room temperature and then cryoprotected overnight in buffer with 20 % sucrose at 4°C. Probe placement was verified histologically in 60 μm coronal brain sections stained with cresyl violet. The placement of a representative microdialysis probe is shown in Fig. 1b.

## Results

### A bolus subcutaneous testosterone injection

The effects of an acute sc injection of T (7.5 and 37.5 mg/kg) or βCD vehicle on Acb DA levels are shown in Fig. 2. At a dose (7.5 mg/kg) known to induce CPP in rats, the sc injection of T did not significantly change DA levels ($F_{8, 32} = 0.67$, ns). Even at a much higher dose (37.5 mg/kg), sc T did not significantly alter DA levels ($F_{8, 32} = 1.32$, ns). Likewise, animals injected with βCD did not show any changes in Acb DA levels ($F_{8, 32} = 0.80$, ns). No apparent behavioral effect was observed in any group.

### A bolus subcutaneous amphetamine injection

The effects of an acute sc injection of amphetamine are shown in Fig. 3. Amphetamine dose-dependently increased Acb DA levels. High dose amphetamine (5 mg) induced a significant DA increase up to 500 % of baseline levels, peaking at 1h after injection. At this dose, all animals exhibited increased locomotion and stereotypy. The DA increase followed a similar pattern at 1 mg/kg, but the peak level was lower: 250 % of baseline. At this dose, most animals showed little behavioral effects of amphetamine.

### ICV testosterone infusion in drug-naïve animals

Fig. 4 shows the effects of ICV infusions of the βCD vehicle, low dose (1 μg/infusion) T, and high dose (2 μg/infusion) T on Acb DA in drug-naïve animals. In the animals receiving vehicle, the extracellular DA levels declined slightly, but non-significantly during the 4 hr infusion

($F_{8, 32} = 0.77$, ns). Similarly, with the low dose T (1 μg/infusion), the extracellular DA levels showed a slight decline in the first 30 min, but did not decline further. Instead, DA levels remained at 70 to 80 % of baseline ($F_{8, 40} = 1.39$, ns). In the animals receiving 2 μg/infusion T, Acb DA levels significantly decreased from baseline ($F_{8, 24} = 15.80$, $p < 0.0001$). The DA level decreased gradually during the first 2 hrs of infusion, reaching significance by 1hr. For the last 2 hrs of infusion, DA remained at approximately 30 % of baseline.

### ICV testosterone infusion following 15-day daily ICV T infusion

Fig. 5 shows the effect of ICV T infusion on Acb DA levels following 14 days of daily 4 hr T-infusion. Similar to acute T infusion of the same dose (1 μg/infusion), ICV T infusion failed to alter Acb DA levels ($F_{8, 32} = 0.83$, ns), even after 14 days of daily ICV T infusions. The DA levels were unchanged from the baseline level for the duration of the test.

## Discussion

### Exogenous testosterone has little influence on Acb DA levels

The results of the current study suggest that testosterone does not stimulate Acb DA release at physiologically- and behaviorally-relevant doses. A bolus injection of systemic 7.5 or 37.5 mg/kg T failed to induce Acb DA increase. No sign of stereotypy or increased locomotion was apparent at either dose. The lower dose (7.5 mg/kg) is sufficient to reduce seizure activity (Frye and Reed, 1998), and to enhance aggressive behavior (Martinez-Sanchis et al., 1998) and social conflict (Barreto-Estrada et al., 2004). Furthermore, the high dose sc T (37.5 mg/kg) is much higher than those shown to have significant physiological and behavioral effects. The lack of DA release at either dose suggests subcutaneously administered testosterone does not induce Acb DA release. In contrast, subcutaneous amphetamine dose-dependently increased DA release in hamster Acb. The magnitude of amphetamine-stimulated DA increase appears to be somewhat lower in hamsters than in rats (Di Chiara and Imperato, 1988). We observed approximately 250% increase in Acb DA with 1 mg/kg amphetamine, while >800% increase has been reported in rats (Di Chiara and Imperato, 1988). The amphetamine-induced Acb DA release demonstrates that hamster Acb is similar to that in rats not only anatomically (Johnson and Wood, 1999), but functionally as well.

Most of our T self-administration studies have utilized ICV as route of administration (Wood et al., 2004, for example). Therefore, we examined Acb DA changes induced by ICV T infusion. We used a dose slightly higher than average T intake during self-administration (27 μg/4 hrs (Wood et al., 2004). We also tested 80 μg T, a dose much higher than typically self-administered. In both cases, Acb DA decreased, and animals appeared to be heavily sedated at the end of the 4 hr session. We have previously demonstrated that 40 μg T ICV produces significant depression of locomotion, respiration, and body temperature (Peters and Wood, 2005). Therefore, we suspect the observed Acb DA decrease was a byproduct of autonomic depressant effects of T, rather than direct effects on the mesolimbic DA system. In these drug naive animals, it is possible that autonomic depressant effects of T have masked any DA increase. Since hamsters develop tolerance to autonomic depressant effects of T within 15 days (Peters and Wood, 2005), we infused 1 μg/infusion T daily for 14 days, and examined the DA response on the 15$^{th}$ day. ICV T still failed to stimulate Acb DA release, further supporting the lack stimulant-like effects of T on Acb DA. Furthermore, the profound sedation with 80 μg ICV T suggests that higher doses cannot be tested. Therefore, it is highly unlikely that the doses used in this study were insufficient to induce Acb DA increase.

Currently, the pharmacological mechanism of AAS reinforcement is unknown. There are numerous AAS available, each with slightly different pharmacological profile. Furthermore, AAS are administered through various routes using various vehicles, thus pharmacokinetics

of AAS differ significantly. AAS may even exert opposing effects, via their estrogenic and androgenic metabolites. For example, the facilitative effects of estrogens on Acb DA (Becker and Rudick, 1999) may have been masked by inhibitory effects elsewhere in the brain. Nonetheless, the results of this study have clinical relevance, because T is the most prevalent AAS (WorldAnti-DopingAgency, 2006).

The importance of the mesolimbic DA system in drug abuse is well-established (Berridge and Robinson, 1998; Koob and Nestler, 1997). Most drugs of abuse are known to increase Acb DA (Di Chiara and Imperato, 1988). Since AAS are also recognized as drugs of abuse (DSM-IV-TR, 2000), we expected to see an increase in Acb DA. Behavioral and neurochemical evidence also suggested involvement of the mesolimbic DA system in AAS abuse. For example, T-induced CPP is blocked by a DA antagonist in Acb (Packard et al., 1998). In addition, chronic AAS-treatment is known to alter monoaminergic activity in several brain structures, including Acb (Kindlundh et al., 2004; Thiblin et al., 1999). Thus, the lack of Acb DA increase with both ICV and sc T administration was unexpected. However, Birgner and colleagues (2007) have recently demonstrated an attenuated Acb DA response to amphetamine in rats treated with chronic AAS. In light of the results of these data, we suspect that the relationship between the mesolimbic DA system and AAS abuse may be indirect. In fact, the relative paucity of nuclear androgen receptors in Acb or the VTA is well-documented (Kritzer, 1997; Simerly et al., 1990; Wood and Newman, 1995). Therefore, the influence of AAS on the mesolimbic DA system is likely to be mediated by androgen-sensitive afferents and/or non-classical androgen receptors (Simoncini and Genazzani, 2003).

### AAS is another drug of abuse with DA-independent effects

Based on this and previous studies, it is clear that AAS do not resemble stimulants. Instead, AAS appear to be another class of non-stimulant drugs, with at least some of their behavioral effects mediated by DA-independent pathways. Self-administration of opiates (Pettit et al., 1984), ethanol (EtOH; Rassnick et al., 1993), and phencyclidine (Carlezon and Wise, 1996) are not dependent on mesolimbic DA. In particular, although benzodiazepines reduce Acb DA (Finlay et al., 1992; Invernizzi et al., 1991; Zetterstrom and Fillenz, 1990), they are nonetheless self-administered (Naruse and Asami, 1987; Szostak et al., 1987) and produce CPP (File, 1986; Spyraki and Fibiger, 1988; Spyraki et al., 1985). Likewise, EtOH-induced CPP is not DA-dependent (Cunningham et al., 1992; Risinger et al., 1992). Even drug-induced locomotion, which is closely linked to Acb DA, may not be always dependent on Acb DA for opiates (Kalivas et al., 1983; Pert and Sivit, 1977; Pettit et al., 1984; Vaccarino et al., 1986). These studies demonstrate that some drugs of abuse may be reinforcing and may produce significant behavioral effects through DA-independent pathways. AAS may fall into this category.

### AAS may act through pathways utilized by other non-stimulant drugs of abuse

While AAS do not act as stimulants, AAS share some properties with other drugs of abuse, particularly opiates. AAS induce autonomic depression quite similar to that induced by opiates (Peters and Wood, 2005). Interestingly, autonomic depressant effects of T, including the development of tolerance to locomotor, respiration, and body temperature suppression, can be blocked by naloxone-pretreatment. Furthermore, AAS self-administration is blocked by naltrexone-pretreatment. With chronic treatment, AAS can also alter endogenous opioid levels (Celerier et al., 2003; Johansson et al., 2000a; Johansson et al., 1997; Menard et al., 1995). It is tempting to speculate on how endogenous opioids may mediate the reinforcing properties of AAS. Nyberg and colleagues (Johansson et al., 2000a) hypothesized that AAS cause an imbalance in activation of μ-opioid receptors (MORs) in the VTA and κ-opioid receptors (KORs) in Acb, two prominent pathways through which endogenous opioids regulate mesolimbic DA system (Spanagel, 1995). While brains stem MORs likely mediate autonomic

depressant effects of AAS, Acb KORs may play a central role in Acb DA and AAS self-administration. κ-agonists, like AAS, are mild reinforcers that reduce Acb DA (Marinelli et al., 1998) and known to reduce drug self-administration (Glick et al., 1995; Schenk et al., 1999). Thus, it is plausible that κ-opioids mediate Acb DA and AAS self-administration.

In addition to opiates, AAS also resemble GABAergic drugs of abuse. Both AAS and benzodiazepines do not increase Acb DA levels (Finlay et al., 1992; Invernizzi et al., 1991; Zetterstrom and Fillenz, 1990), despite their abuse potential. In addition, AAS have been shown to be anxiolytic (Agren et al., 1999; Aikey et al., 2002; Berbos et al., 2002; Frye and Seliga, 2001). Some of these acute behavioral effects may be mediated by T and its metabolites binding to the steroid binding site of the GABAa receptor (Masonis and McCarthy, 1995, 1996). In addition to their acute benzodiazepine-like effects on behavior, AAS have been shown to alter GABAa receptor (Clark and Henderson, 2003; Penatti et al., 2005), and glutamic acid decarboxylase 65 (Grimes et al., 2003) expression. It appears that AAS can modulate opioidergic and GABAergic systems, both acutely and chronically.

The possibility that AAS modulate pathways that mediate the effects of other drugs of abuse raises a concern that AAS abuse may alter vulnerability for the abuse of other drugs. In fact, there are reports suggesting that AAS abusers are more likely to use tobacco, alcohol, and illicit drugs than non-users (see Yesalis et al., 1993 for example). The evidence from animal studies suggest that AAS alter sensitivity to the rewarding effects of amphetamine (Clark et al., 1996) and increase EtOH intake (Johansson et al., 2000b). In addition to their recreational uses, it is not difficult to imagine use of AAS with other potentially addictive drugs for non-recreational purposes. For example, amphetamines and AAS may be taken by the same athletes in order to enhance their athletic performance. Also, AAS and opiates may be taken together following injuries, the former to hasten recovery (Ferry et al., 1999) and the latter for analgesia. This combination of opiates and AAS is particularly worrisome, since progestins, T, and related substances also have analgesic/anesthetic properties (Belelli et al., 2006; Frye et al., 2007; Frye et al., 1996; Weir et al., 2004). In fact, AAS may exacerbate morphine withdrawal symptoms (Celerier et al., 2003). Therefore, the underlying neural mechanism of AAS abuse and their interaction with other drugs of abuse warrant further investigation.

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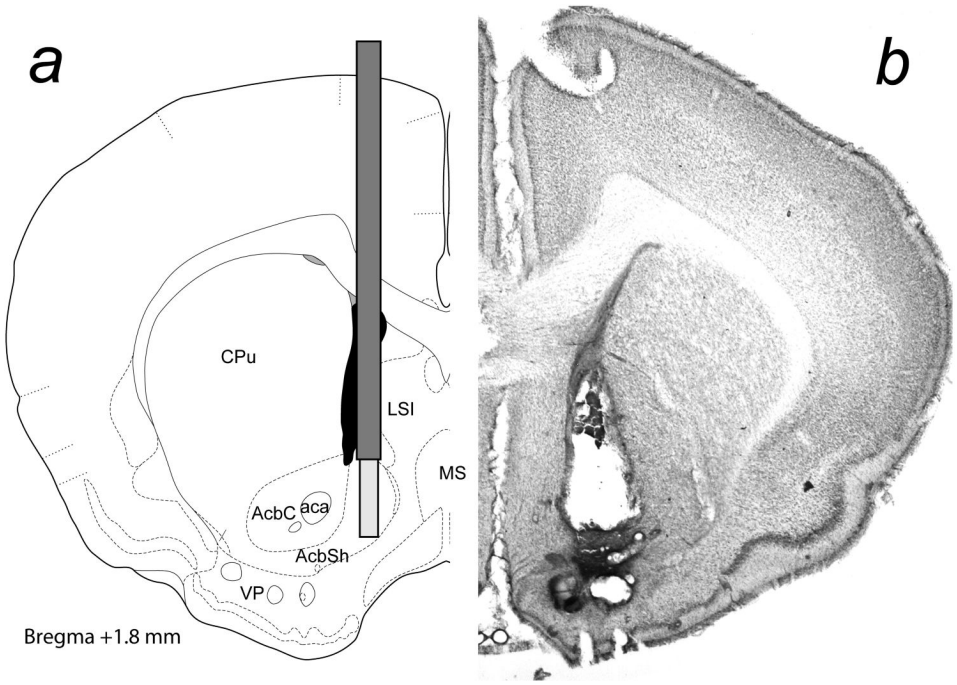


**Fig. 1.**

The location of a) microdialysis probe, and b) the photomicrograph of a representative microdialysis probe placement. Coordinates are based on a stereotaxic atlas of the golden hamster brain (Morin and Wood, 2001).

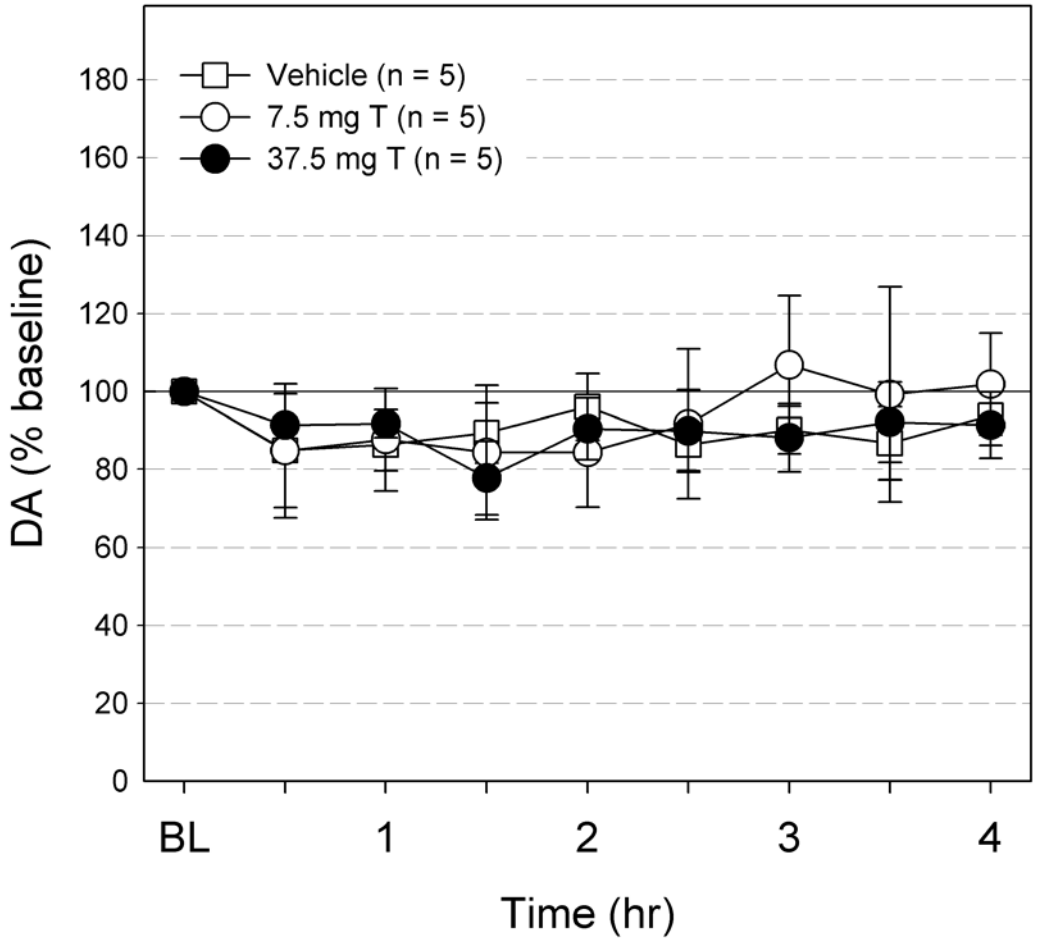


**Fig. 2.**

The effects of subcutaneous injection of vehicle (n =5), 7.5 mg/kg T, and 37.5 mg/kg T on Acb DA levels. The DA levels are expressed as % baseline ± S.E.M. The DA levels did not change with any of the treatments.

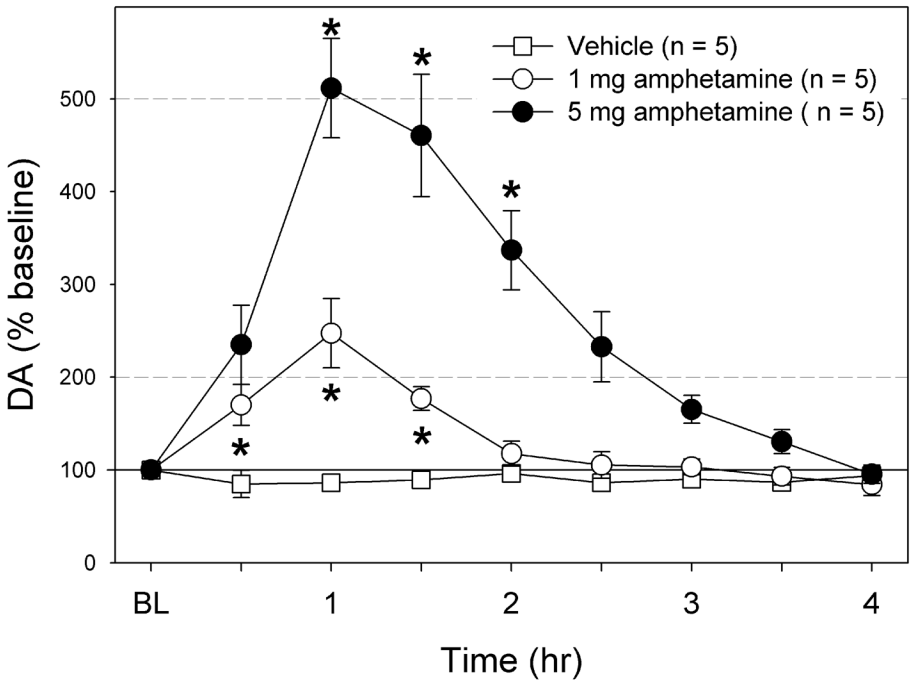


**Fig. 3.**

The effects of subcutaneous injection of vehicle (n =5), 1 mg/kg amphetamine, and 5 mg/kg amphetamine on Acb DA levels. The DA levels are expressed as % baseline ± S.E.M. The DA levels did not change with vehicle, but significantly increased with both doses of amphetamine. * Significantly different from baseline.

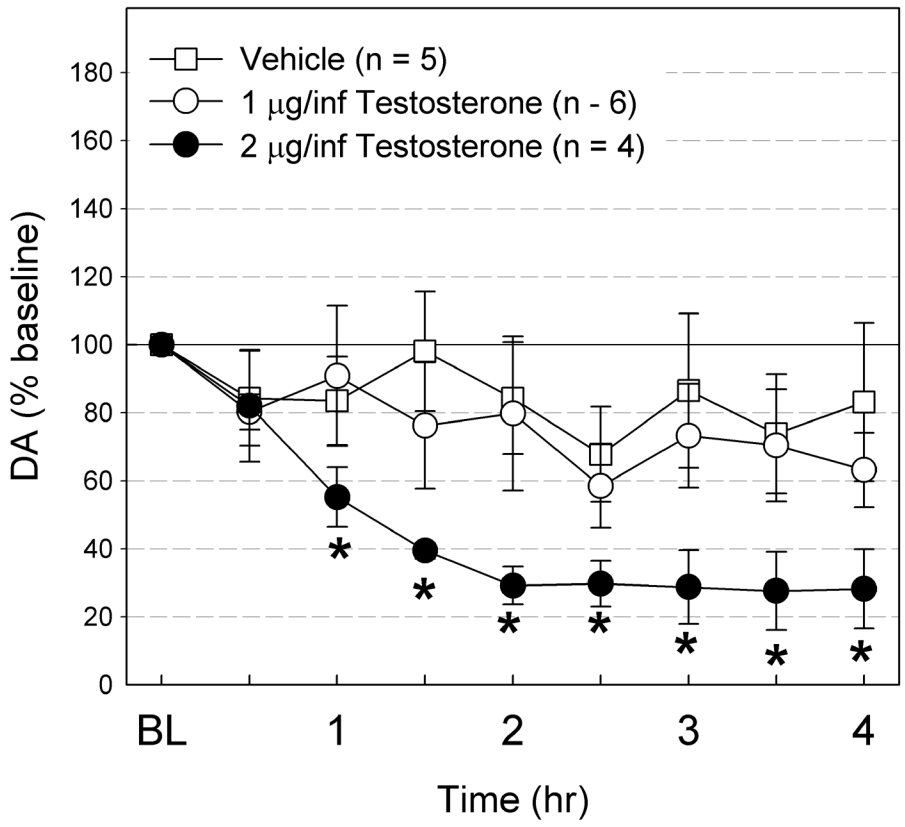


**Fig. 4.**

The effects of ICV infusion of vehicle (n = 5), 1 μg/infusion T (40 μg total, n = 6), and 2 μg/infusion T (80 μg total, n = 4) on Acb DA levels. The DA levels are expressed as % baseline ± S.E.M. The DA levels did not change with vehicle or T at 1 μg/infusion, but significantly declined with T at 2 μg/infusion. * Significantly different from baseline.

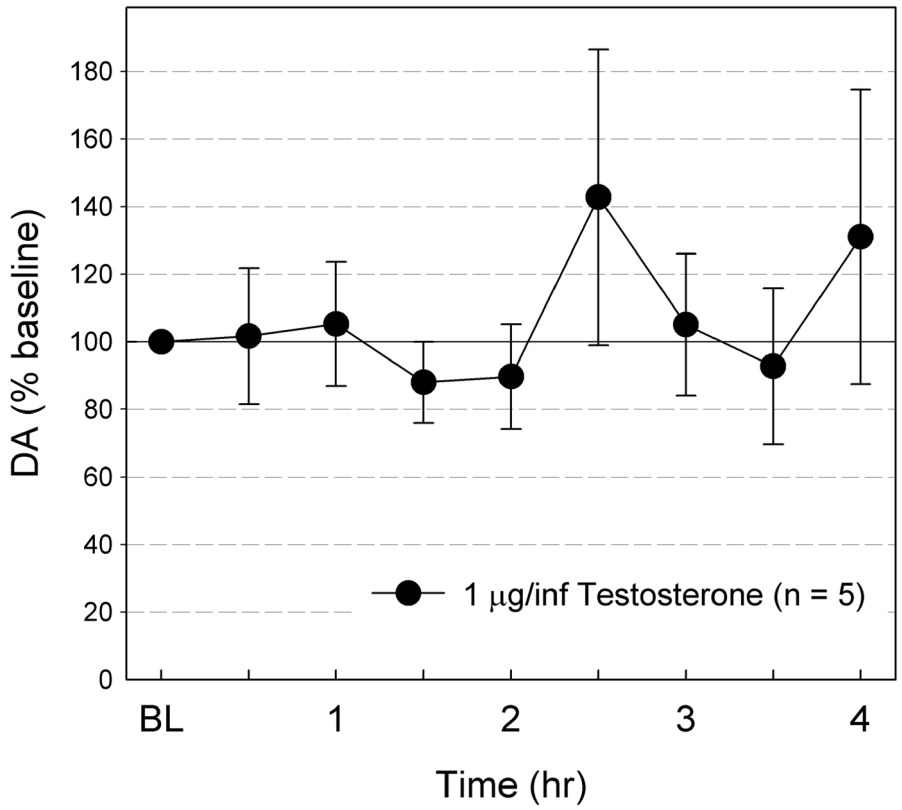


**Fig. 5.**

The effects of ICV T (40 μg, n = 5) infusion on Acb DA levels following 14 days of repeated ICV T infusion. The DA levels are expressed as % baseline ± S.E.M. The DA levels did not significantly change from baseline.