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The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5α-reduced metabolites

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Abstract

Testosterone (T)'s positive hedonic effects may be mediated by actions of its metabolites, dihydrotestosterone (DHT) or 3α-androstanediol (3α-diol), in the nucleus accumbens (NA). In Experiment 1, adult, intact, male rats were systemically administered 1 mg of T, DHT, 3α-diol or vehicle, at different time points to examine concentrations of androgens in the NA. Rats administered 3α-diol had significantly increased concentrations of 3α-diol in the region of the brain encompassing the NA. These data are consistent with previous data from our laboratory demonstrating that 3α-diol elicits a conditioned place preference (CPP) more effectively than either T or DHT, when administered systemically. In Experiment 2, rats received implants of T, DHT or 3α-diol to the NA immediately prior to placement in the CPP apparatus on conditioning days. Implants of T, DHT or 3α-diol, but not vehicle, significantly increased time spent on the non-preferred side of the chamber on the test day. This effect was only produced by androgenic stimulation of the shell of the NA and not the core of the NA. Thus, androgen regimens we have previously found to enhance CPP produced the greatest increases in 3α-diol concentrations in the NA region and direct implants of T, DHT or 3α-diol to the shell, but not the core, of the NA enhanced CPP. These data are consistent with the hypothesis that the hedonic effects of T may be due to actions of its metabolites in the NA.
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1. Introduction

The abuse of anabolic–androgenic steroid (AS), the synthetic variants of the primary masculinizing androgen testosterone (T), is a growing problem. Like other drugs of abuse, illicit use of AS has spread from elite Olympic, professional, college or high school athletes to the general population. Estimates indicate that approximately 375,000 adolescent boys and 175,000 adolescent girls are steroid users. Out of the 6.6% of 12th grade male students that admitted to having used AS, 27% of the user group listed appearance as the main reason (Buckley et al., 1988). Accompanying the enhancing physical effects of AS use, AS users self-report positive changes in mood, behavior and somatic perceptions (Bahrke et al., 1990, 1996; Wilson, 1988) that are consistent with reports that T was used to treat depression in the 1930s (Altschule and Tilletson, 1948). The positive physical and mental sequelae of AS use may potentiate their use despite adverse health consequences that can include growth retardation, kidney and liver damage, liver cancer, heart disease and hypertension (Haupt and Rovere, 1984; Yesalis and Bahrke, 1995).

Anabolic–androgenic steroid use may lead to addiction, dependence and withdrawal such that use may be continued despite short- and long-term health risks. Dependence may result from prolonged AS abuse (Kashkin and Kleber, 1989; Tennant et al., 1988; Wright, 1980); AS abusers often experience a stimulant-like withdrawal syndrome characterized by depressive symptoms. A number of studies and case reports have documented behavior, perceptions and attitudes in some AS abusers that are also indicative of dependence (Brower et al., 1989, 1990, 1991; Corcoran and Longo,

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1992; Pope and Katz, 1994). For example, a noncompetitive weight lifter reported low self-esteem and AS cravings after AS cessation (Hays et al., 1990). As well three weightlifters report initiating AS use to enhance performance but maintaining use to prevent depression (Tennant et al., 1988). Interestingly, AS use elicits electroencephalographic changes similar to those seen with amphetamines and tricyclic antidepressants (Bahrke et al., 1990). Finally, recent data show that AS abuse may be a gateway to opioid dependence. Men who abuse AS may go on to abuse opioid agonist–antagonists, such as nalbuphine (McBride et al., 1996; Wines et al., 1999) or classic opioids, such as heroin (Arvary and Pope, 2000).

Evidence from animal studies suggests T can have positive hedonic effects. Testosterone (Caggiula, 1970; Campbell, 1970; Olds, 1958), like many drugs of abuse (Kornetsky, 1995), will increase rates of bar pressing for electrical brain stimulation, which is considered an indication of a drugs rewarding effects. In many studies of conditioned place preference (CPP), which is used to examine hedonic effects of drugs (Scoles and Siegel, 1986), a CPP was established by pairing systemic T (Alexander et al., 1994; Arnedo et al., 2000; Caldarone et al., 1996; De Beun et al., 1992; Kashkin and Kleber, 1989; Packard et al., 1997, 1998; Schroeder and Packard, 2000) or T applied centrally to the nucleus accumbens (NA) (Packard et al., 1997) or the medial preoptic area (MPOA) (King et al., 1999), with a distinctive chamber. However, there is considerable variability in this effect. In some studies, by pairing T with a distinctive chamber, CPP resulted with only with very high systemic T dosages and not with lower dosages. In other studies, no effect of T was observed when its effects were compared to that of rigorous controls (Caldarone et al., 1996).

What are the mechanism(s) of action by which T may have its positive hedonic effects? Testosterone is readily metabolized by 5α-reductase to dihydrotestosterone (DHT) and by 3-oxidoreductase to 3α-androstanediol (3α-diol). Testosterone and DHT both bind readily to intracellular androgen receptors (ARs), while 3α-diol is devoid of affinity for ARs (Cunningham et al., 1979; Roselli et al., 1987; Verhoeven et al., 1975), and instead has actions primarily through $GABA_A$/benzodiazepine receptor complexes (GBRs) (Frye et al., 1996a,c,d,e), which T and DHT are not particularly effective at modulating (Gee, 1988). Testosterone's rewarding incentive properties may not be solely due to actions at ARs. Infusions of T into the NA or the MPOA and immediate pairing with the nonpreferred side of the CPP chamber reliably condition a place preference (King et al., 1999; Packard et al., 1997). Typically, actions of steroids at intracellular receptors would require minutes to occur (Pfaff and McEwen, 1983), whereas actions of the T metabolite 3α-diol at GBRs can occur within seconds (Frye, 2001; Frye and Reed, 1998). As well, T to the NA and MPOA similarly conditions a place preference although the NA has very few intracellular ARs and the MPOA has many ARs through which T could have its effects (Stumpf and Sar, 1976). The manner in which T is administered, dosage, bioavailability, route of administration and vehicle, could influence T's metabolism to its active products, and therefore may influence the variability of T's effects on CPP. Hence, interoceptive effects of T may be mediated in part by actions of 3α-diol.

The purpose of the present experiments was to test the hypotheses (1) that if positive hedonic effects of T are in part due to the actions of its metabolite 3α-diol in the NA, then 3α-diol concentrations in the NA region should be elevated at time points that correspond to those previously demonstrated for androgens to result in the formation of a place preference (Experiment 1); and (2) that if T, DHT and 3α-diol have their actions through the NA then these androgens should be effective at conditioning a place preference when applied to this brain region (Experiment 2).

2. Methods

All methods were pre-approved by the Institutional Animal Care and Use Committee.

2.1. Animals and housing

Experimentally naive, adult, gonadally intact male rats (Experiment 1, $n=141$; Experiment 2, $n=121$) from Harlan Laboratories (Indianapolis, Indiana) were housed 1–2 per cage in a temperature-controlled (70–74 °F) room in polypropylene cages (25 × 23 × 20 cm) with wood chip bedding and free-access to food and water.

2.2. Procedure

2.2.1. Experiment 1

Animals were randomly assigned ($n=10$–15 per group) to one of 10 hormone treatments that involved subcutaneous injections of 1.0 mg T, DHT or 3α-diol, 30, 90 or 180 min prior to sacrifice, or vehicle (10% ethanol in propylene glycol). Tissues were collected to ascertain hormone concentrations in the brain.

2.2.2. Experiment 2

Rats received their assigned implants 2–4 min prior to exposure to the CPP chambers. Androgen exposure lasted the duration of the 30-min pairing. Rats received their assigned androgen (T, $n=32$; DHT, $n=36$; 3α-diol, $n=31$; control, $n=22$) on the conditioning days (Days 4–6 and 10–12) and all animals received vehicle implants on Days 7–9 and 13–15, irrespective of the androgen they were assigned to receive. Rats were tested on Day 16; this was followed by perfusion and histology for site analyses.

2.3. Systemic steroids (Experiment 1)

Testosterone, DHT and 3α-diol (Sigma, St. Louis, MO) were dissolved (10 mg/1 ml) in a vehicle solution of 10% ethanol in propylene glycol (v/v). Injection volume was held constant at 1.0 mg in 0.1 ml administered subcutaneously on the dorsum of the neck. These dosages of androgens have previously been demonstrated to produce different effects on CPP (Frye et al., 2001; Rosellini et al., 2001).

2.4. Intracranial implants (Experiment 2)

One week prior to behavioral testing, rats were anesthetized with Rompun (60 mg/kg) and Ketaset (80 mg/kg) and stereotaxically implanted with bilateral guide cannulae aimed at the NA (from bregma AP=+1.7, ML=+1.5, DV=−6.0) (Menard et al., 1995). Cannulae assembly consisted of 23-gauge thin wall guide cannulae and 30-gauge removable inserts (Frye et al., 1993).

Immediately prior to behavioral testing experimental inserts were tamped in crystalline T, DHT or 3α-diol. Inserts were verified with a dissecting microscope to ensure that the bottom of the insert was filled with the androgen and that no androgen was on the outside of the insert. Then the filled insert was placed in the guide cannulae. Control inserts were left empty or were tamped in bovine serum albumin.

2.5. Behavioral testing (Experiment 2)

2.5.1. Apparatus

Eight conditioning chambers were used throughout these studies. These chambers were closely modeled after those employed by Reid et al. (1989) (Olds, 1958). Three walls of the chambers and the ceiling were constructed of clear Plexiglas and the floor was constructed from plastic panels. Each chamber measured 57 × 28 × 20 cm. The walls on the left half of the chamber consisted of 2.5-cm wide horizontal black and white stripes and those on the right half were uniform white. The floor on the left side was black and white striped with a smooth surface and the floor on the right was clear Plexiglas with a jagged surface. Each chamber could be divided into two equal halves by means of a Plexiglas partition painted with the appropriate stimulus for each side. The chambers were dimly illuminated by means of two 40-W lights (Sylvania, Model #40A1 5/FAN/RP) each of which was located 5 cm above and behind the rear corner of each side of the chamber. Each chamber was suspended in a sound- and light-attenuating container by means of an axel system that allowed the chamber to pivot and therefore tip when the animal placed the majority of its weight on one side. The location of the animal in the chamber was monitored by a microswitch system. Control of the apparatus and data collection were accomplished by a Zenith XT computer.

2.5.2. Procedure

This study consisted of four phases: (1) habituation to the laboratory and apparatus, (2) baseline preference assessment, (3) place preference conditioning and (4) place preference test.

Days 1 and 2—*Habituation*—After a 1-week acclimation to the laboratory, rats had access to both sides of the CCP chamber for 30 min/per day, for 2 days.

Day 3—*Baseline*—Each animal was again given access to both sides of the chamber for 30 min and the amount of time the rat spent on each side of the chamber was recorded. Subjects were balanced across the experimental conditions based on baseline side preference.

Days 4–9 and 10–15—*Conditioning*—This phase of the study was based on the conditioning procedures employed by Calderone et al. (1996) and Reid et al. (1989). Rats were conditioned to the nonpreferred side of the chamber. In Experiment 2, rats received T, DHT or 3α-diol implants aimed at the NA immediately prior to placement in the nonpreferred side of the chamber on Days 4–6 and 10–12. On the subsequent 3 days (Days 7–9 and 13–15), vehicle implants were given and rats were placed in the originally preferred side of the chamber. The conditioning cycle of 3 days of androgen administration followed by 3 days of vehicle administration was repeated for a total of six androgen and six vehicle administrations. Previous research in our laboratory has demonstrated that the order of injection (3 days of androgens followed by 3 days of vehicle or vice versa) does not affect CPP conditioning (Frye et al., 2001; Rosellini et al., 2001). Control rats received vehicle implants on all days.

Day 16—*Place preference test*—On the day following the last day of pairing, rats were again put in the chamber for thirty min, with access to both sides. Rats were not administered steroid or vehicle on test day. The animal's preference for each side of the chamber was assessed in a 30-min test.

2.6. Necropsy

2.6.1. Experiment 1

Rats were rapidly decapitated 30, 90 or 180 min following androgen or vehicle administration and the ventral portion of the brain anterior to the optic chiasm that contains the NA and MPOA was dissected out on ice and frozen for later measurement of T, DHT and 3α-diol according to previously published radioimmunoassay methods (Erskine et al., 1992; Frye et al., 1996b).

2.6.2. Experiment 2

Following 16 days of testing, rats were perfused with 0.9% saline and 10% formalin, brains were collected, frozen and sliced at 40 μm, and stained with cresyl violet to examine implant location. Site analysis revealed that 32 rats received implants to the shell of the NA (T, $n=10$; DHT, $n=7$; 3α-diol, $n=7$; control, $n=8$). The majority of

the rats with incorrect implants were located in the core of the NA (see Fig. 1), a few had implants to the lateral hypothalamus (not shown). Androgens to sites other than the shell of the NA were behaviorally ineffective.

2.7. Statistical analyses

2.7.1. Experiment 1

Steroid measurement data were analyzed with analyses of variance (ANOVA). Alpha level for determination of statistical significance was $P < .05$.

2.7.2. Experiment 2

A mixed within (baseline vs. test preference) between (androgen administration) subject ANOVA was utilized to determine the effects of androgen administered on time spent on the conditioned (originally nonpreferred) side of the chamber. Where appropriate, ANOVAs were followed by Fisher's least significant difference *post-hoc* tests to determine differences among groups. Alpha level for the determination of statistical significance was $P < .05$.

3. Results

3.1. Experiment 1

Notably, there were differences in 3α-diol concentrations in the region of the brain encompassing the NA and MPOA, 30 [$F(2,33) = 116.5$, $P = .001$], 90 [$F(2,31) = 7.259$, $P = .01$] and 180 [$F(2,35) = 8.018$, $P = .01$], min following androgen administration (see Fig. 2, bottom). Rats administered 3α-diol 30 min prior to tissue collection had increased 3α-diol that was not seen in the T or DHT administered groups. Notably, our previous findings indicate that this 3α-diol regimen more reliably elicits CPP, than does the same T or DHT regimen (Frye et al., 2001; Rosellini et al., 2001). Ninety and one hundred eighty minutes following 3α-diol administration, 3α-diol concentrations were slightly greater than those seen following T or DHT administration, which were different from vehicle. These data are consistent with our previous data demonstrating that administration of 3α-diol 30 min prior to placement in the CPP apparatus reliably elicits a CPP, and that given adequate time for metabolism to 3α-diol (i.e., 90 or 180 min) T or DHT administration can also produce a place preference (Rosellini et al., 2001).

Concentrations of T or DHT in the region of the brain encompassing the NA cannot account for androgens' enhancing effects on CPP. Although T levels were significantly increased 30 [$F(3,36) = 33.908$, $P = .001$], 90 [$F(3,34) = 40.074$, $P = .001$] and 180 [$F(3,38) = 43.645$, $P = .001$] min following T, but not DHT, 3α-diol or vehicle administration (see Fig. 2, top), our previous data demonstrated that T was only effective at producing a CPP when administered 90 or 180 min prior to placement in the conditioning apparatus. As well, DHT was significantly increased 30 [$F(3,36) = 40.452$, $P = .001$], 90 [$F(3,34) = 25.10$, $P = .001$] and 180 min [$F(3,38) = 27.002$, $P = .001$] following DHT and 3α-diol, but not vehicle administration and in our previous report DHT only produced a CPP when administered 180 min prior to placement in the conditioning apparatus. DHT concen-

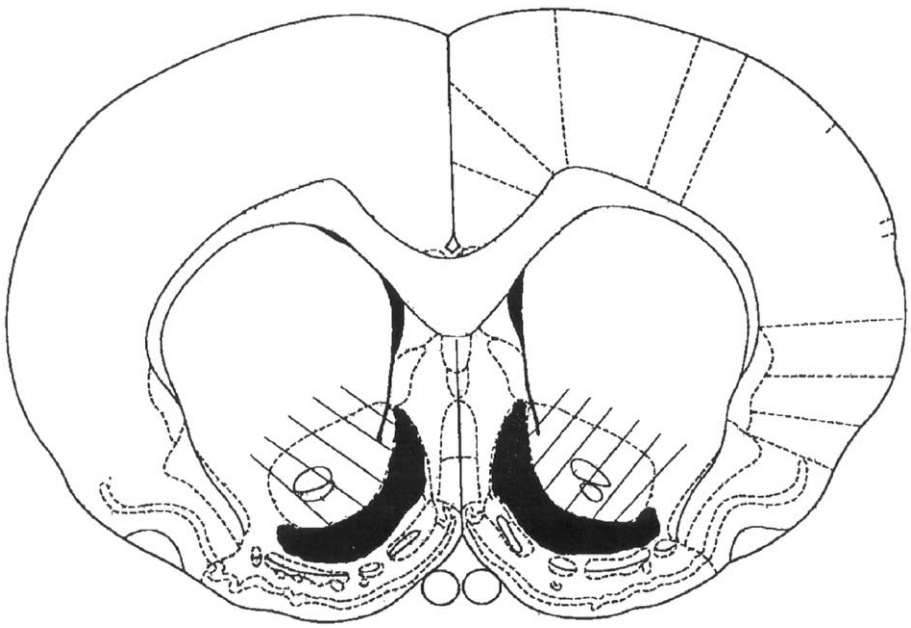

Fig. 1. Schematic represents the areas in which androgen implants were localized. Implants that produced a place preference were localized to the shell of the NA (shaded). Implants were not in the shell of the accumbens, e.g., core or some other missed site were not effective at conditioning a place preference (hatched area). The diagram depicted is from The Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 1997). The AP coordinate is 1.2 from bregma; most implant sites were localized in this area.

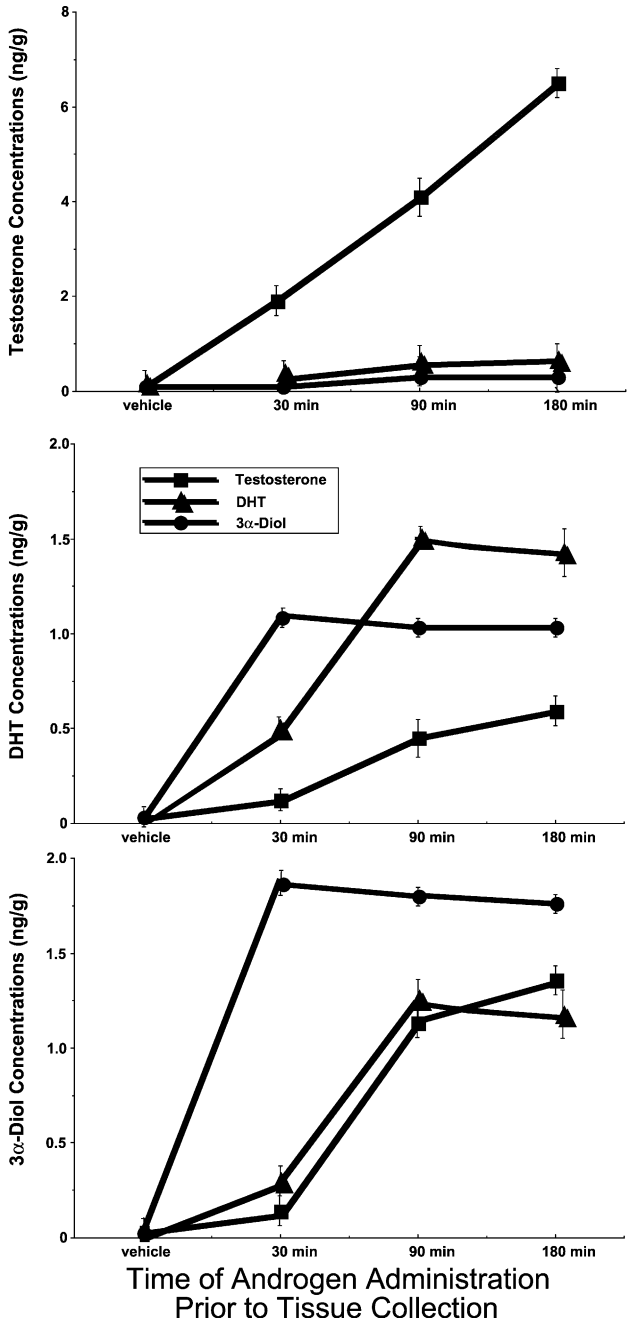

Fig. 2. Top panel represents T concentrations (ng/g) 30, 90 or 180 min after systemic administration of T (squares), DHT (triangles) or 3α-diol (circles). Middle panel represents DHT concentrations (ng/g) 30, 90 or 180 min after systemic administration of T (squares), DHT (triangles) or 3α-diol (circles). Bottom panel represents 3α-diol concentrations (ng/g) 30, 90 or 180 min after systemic administration of T (squares), DHT (triangles) or 3α-diol (circles). All data points represent 10–15 observations per group.

trations were also increased 90 and 180, but not 30 min, following T administration (see Fig. 2, middle).

3.2. Experiment 2

There was no significant effect of androgens on CPP when data from all rats tested for CPP were analyzed [$F(3,116) = 0.845$, $P = .47$]; however, after rats were grouped according to site of implant administration, analyses revealed that T, DHT and 3α-diol implants administered to the shell of the NA (see below), but not the core [$F(3,78) = 1.572$, $P = .20$; see Table 1], conditioned a place preference to the nonpreferred side of the testing chamber and vehicle administration had no effect.

Table 1
Time spent on side of putative conditioning (s) on baseline and test days of rats administered T, DHT, 3α-diol or vehicle implants to brain areas other than the shell of the NA

Group	Baseline day	Test day
T	199 ± 33	572 ± 98
DHT	205 ± 26	395 ± 84
3α-diol	151 ± 30	418 ± 91
Vehicle	226 ± 55	419 ± 116

For those rats with implants to the shell of the NA, there was a main effect of type of implant [$F(3,28) = 7.795$, $P = .0006$] on the duration of time spent on the nonpreferred side of the CPP chamber. Rats administered T (892 ± 101 s)>DHT (767 ± 127 s)>3α-diol (668 ± 109 s) spent more time on the nonpreferred side of the chamber than did rats which received control implants (363 ± 125 s).

There was also a main effect of test time [$F(1,28) = 95.432$, $P < .0001$] on the duration of time spent on the nonpreferred side of the CPP chamber for rats that received implants to the shell of the NA. Overall, irrespective of implant type, rats spent longer on the nonpreferred side of the chamber on the test day (1155 ± 171 s) compared to the baseline day (191 ± 60 s).

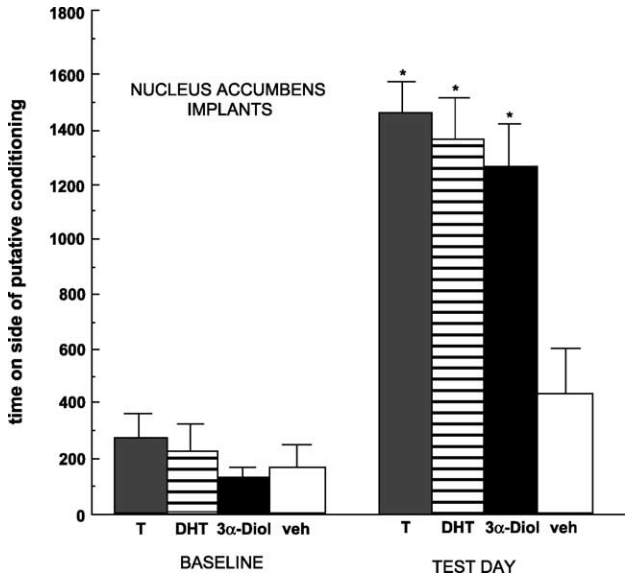

Fig. 3. Represents time spent (seconds) on the nonpreferred side of the chamber on baseline (left side) and test day (right side) of rats administered T (gray bar, $n = 10$), DHT (horizontally striped bar, $n = 7$), 3α-diol (black bar, $n = 7$) or vehicle (open bar, $n = 8$) to the shell of the nucleus accumbens. * Indicates a statistically significant increase compared to vehicle control and baseline ($P < .05$, Fisher's least significant difference test).

Table 2
Number of bouts on baseline and test days of rats administered T, DHT, 3α-diol or vehicle implants to the shell of the NA

Group	Bouts on baseline day	Bouts on test day
T	15	21
DHT	22	24
3α-diol	15	21
Vehicle	23	28

There was also an interaction between the type of implant administered and the test time [$F(3,28)=3.433$, $P<.05$], when implants were administered to the shell of the NA. As Fig. 3 illustrates, the interaction is attributable to the androgens increasing the time spent on the nonpreferred side of the chamber on the test day compared to control implants and compared to their respective time spent on the nonpreferred side of the chamber on the day of baseline testing.

Notably, there were no differences in motor behavior of rats administered androgens or vehicle to the shell of the NA on baseline [$F(3,108)=0.873$, $P>.05$] or test day [$F(3,108)=2.407$, $P>.05$] as indicated by the number of bouts (crossings from one side of the chamber to the other) of rats in each group (see Table 2).

4. Discussion

The present findings supported our hypothesis that 3α-diol concentrations in the the NA region were elevated in a manner consistent with our previously published data demonstrating that 3α-diol is the most effective androgen for eliciting a CPP, when administered systemically. Rats administered 3α-diol had the highest concentrations of 3α-diol in the brain at each of the temporal pairings investigated. Testosterone or DHT administration elevated 3α-diol concentrations when administered 90 or 180 min prior to tissue collection, when systemic administration is more effective at producing a CPP. Data from Experiment 2 supported our hypothesis that androgens administered directly to the NA would be effective at conditioning a place preference. Administration of T, DHT or 3α-diol implants to the NA, when applied immediately prior to placement on the nonpreferred side of the chamber on conditioning days, increased time spent on the nonpreferred side of the chamber on the test day. This effect was very specific and only produced by androgenic stimulation of the shell of the NA and not the core. Together, these data suggest that androgen regimens that increase 3α-diol concentrations in the region of the brain encompassing the NA can enhance CPP and direct implants of T, DHT or 3α-diol to the shell of the NA elicit CPP.

These findings confirm and extend previous results that demonstrate that a CPP can be established by pairing T with a distinctive chamber (Alexander et al., 1994; Arnedo et al., 2000; Caldarone et al., 1996; De Beun et al., 1992; King et al., 1999; Packard et al., 1997, 1998; Schroeder and Packard, 2000; Scoles and Siegel, 1986). The present findings in conjunction with previous research from other laboratories suggest T can have variable effects on CPP. Our work suggests that such variability may be due in part to the effects of metabolism of T to 3α-diol. This result expands the list of other factors that are known to influence T's effects upon CPP formation. For example, dosage of T influences the propensity to elicit CPP. Higher dosages (800 μg–1 mg) of T, when administered SC in oil 30 min prior to exposure to the nonpreferred side of the chamber, enhanced CPP but lower dosages (10–500 μg) did not (Alexander et al., 1994; Caldarone et al., 1996; De Beun et al., 1992; Packard et al., 1998; Schroeder and Packard, 2000). The medium in which T is administered influences CPP. When T is administered in a molecular encapsulation vehicle rather than oil 30 min prior to exposure to the nonpreferred side of the chamber, CPP was produced following lower dosages of T (800–1200 μg/kg) (Alexander et al., 1994). The latter regimen produced supraphysiological levels of plasma T suggesting that rewarding effects of T may depend upon high androgen levels that are more readily metabolized (Taylor et al., 1989).

The present findings suggest T's metabolism to 3α-diol in the NA may modulate CPP. Intra-brain infusion of 0.25 or 0.50 μg of T immediately prior to exposure to the nonpreferred side of the chamber enhanced a place preference (Olds, 1958). The enzymes that metabolize T to DHT, 5α-reductase and DHT to 3α-diol, 3-oxidoreductase, have been localized to the NA region of the telencephalon (Melcangi et al., 1998; Mellon, 1994). The present findings indicate that T is metabolized to DHT and 3α-diol in this brain area and that T and 3α-diol are able to have behaviorally relevant actions when applied there. Also, our present data demonstrate that systemic administration of 3α-diol at all time points examined results in the greatest concentrations of 3α-diol, compared to T or DHT administration and this is consistent with our previous data that show that CPP is enhanced most following 3α-diol administration compared to T or DHT (Rosellini et al., 2001). Moreover, because of the relatively short-time course employed here for effects of androgen implants, and the limited opportunity for diffusion to other brain areas, our findings suggest that T's actions on the NA are very site specific. These findings are consistent with the notion that 3α-diol can act in the NA and that T can be rapidly converted to 3α-diol in the NA to produce its effects.

The present results were very specific in that only implants of androgens applied directly to the shell of the NA produced CPP. Implants of T, DHT or 3α-diol to the shell of the NA produced a robust CPP, while implants to the core of the NA were ineffective at eliciting a CPP. These data are consistent with previous reports that the subregions of the NA play different functional roles in reinforcement and reward. Some reports have made distinctions between the core versus the shell as the motor versus the limbic

components of the NA. For example, the core of the NA is generally considered part of the striatal complex, while the shell can be considered a component of the extended amygdala (Alheid and Heimer, 1988; Heimer et al., 1997). Administration of drugs of abuse has been demonstrated to result in an increase in dopamine release selectively in the shell of the NA (Di et al., 1993; Pontieri and Tanda, 1995). Animals will self-administer drugs of abuse into the shell, but not the core, of the NA (Carlezon and Wise, 1996a,b; Carlezon et al., 1995). Further, androgens, when applied to the shell of the NA were effective at producing a CPP, suggests that indeed androgens should be considered a drug of abuse.

These findings have important implications for the mechanism of action of T in mediating CPP. First, because there are few ARs in the NA (Stumpf and Sar, 1976), our findings suggest that T's effects in the NA on CPP may not be due to actions on these hormone receptors. Second, T, DHT and 3α-diol had a common effect on CPP when applied to the NA despite their discrepant affinities for ARs. These data suggest the effects of T and DHT may be a result of metabolism to 3α-diol and subsequent actions at GBRs. The regimens presently employed can alter GBR function as demonstrated by changes in GABA-stimulated chloride influx in cortical tissues and muscimol binding in the hippocampus (Frye et al., 1996b). Other researchers have demonstrated that T, and other AS, may have their actions in part as a result of altering GBR function (Bitran et al., 1993; Masonis and McCarthy, 1996) and can enhance 3α-diol concentrations (Bitran et al., 1996). Third, evidence suggests that substrates other than ARs and GBRs may be important for mediating T's nongenomic effects. The mesolimbic dopamine system is believed to be an important substrate in the reinforcing actions of drugs of abuse (Koob, 1992; Koob and Le Moal, 1997). Castration of rats causes a decrease of mesolimbic dopamine levels and T replacement restores dopamine concentrations in castrated rats (Alderson and Baum, 1981; Mitchell and Stewart, 1989). 6-OHDA lesions to the NA abolish a preference for environments associated with a sexually receptive female rat (Everitt, 1990) or with amphetamine administration (Spyraki et al., 1982). Peripheral and intra-NA administration of the mixed D1/D2 dopamine receptor antagonist, flupenthixol, blocked T-induced CPP (Packard et al., 1998). This suggests that dopamine receptor activation may be necessary for T's effects on CPP through actions in the NA.

Androgens may also have positive reinforcing effects through modulation of other substrates to influence androgens' hedonic effects. Androgens rewarding effects may involve actions at opiate receptors. Injection of the opiate receptor antagonist, naloxone (Mehrara and Baum, 1990; Miller and Baum, 1987) blocks CPP for a sexually receptive female rat and these effects are similar to the blockade of CPP produced by castration (Hughes et al., 1990). Testosterone and other AS can modulate opiate activity in the brain (Limonta et al., 1987; Menard et al., 1995; Tennant et al., 1988). Notably, men who abuse AS often go on to abuse opioid agonists–antagonists (McBride et al., 1996; Wines et al., 1999) or classic opioids (Arvary and Pope, 2000). Androgens may also have effects at membrane steroid receptors (Towle and Sze, 1983) or other substrates to influence androgens hedonic effects.

Our primary interpretation of the present data has been that androgens have positive hedonic effects to mediate CPP. However, since a biased design was used in the present experiments, there is another interpretation that must be considered. It could be possible that the change in preference of sides in the present study is due to androgens reducing the aversive qualities of the originally nonpreferred side of the chamber.

In summary, androgen regimens that increased levels of 3α-diol in the NA region of the brain occurred in a time course consistent with previous data demonstrating androgens effects on formation of a CPP. All rats administered 3α-diol had the highest concentrations of 3α-diol in the NA region at each of the temporal pairings tested, consistent with our previous data that this regimen of 3α-diol is more effective than T or DHT at eliciting a CPP. Testosterone, DHT or 3α-diol implants to the NA, when applied immediately prior to placement on the nonpreferred side of the chamber on conditioning days, increased time spent on the nonpreferred side of the chamber on the test day. This effect was very specific and only produced by androgenic stimulation of the shell of the NA and not the core. Together these data suggest that androgen regimens that increase 3α-diol concentrations in the brain can enhance CPP and direct implants of T, DHT or 3α-diol to the shell of the NA elicits CPP. The present findings are important for elucidating the mechanisms through which androgens may act to produce positive hedonic effects. These results ultimately may help us to understand how AS produces addictive effects as well as how androgens in general may influence/ or affect sexual motivation.

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