

# Opposing roles of the nucleus accumbens and anterior lateral hypothalamic area in the control of sexual behaviour in the male rat

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## Abstract

Opposing roles have been implicated for the nucleus accumbens (NAc) and anterior portion of the lateral hypothalamic area (aLHA) in the regulation of sexual behaviour in male rats based on *in vivo* neurochemical correlates. The present study provides functional evidence supporting this hypothesis by examining the effects of lesions to these structures on copulation, noncontact erection and receptive female preference. Sexually naïve male Long-Evans rats received either bilateral 1.0- $\mu$ L injections of NMDA (10  $\mu$ g/ $\mu$ L/site) or vehicle (shams) into either the aLHA or the NAc. During repeated tests of copulation most of the sham-lesioned males, but few of the aLHA-lesioned and NAc-lesioned males, copulated to ejaculation. Most of the NAc-lesioned males also failed to intromit, whereas the majority of the aLHA-lesioned males intromitted repeatedly. During exposure to an inaccessible receptive female behind a wire-mesh screen, aLHA-lesioned males displayed facilitation of noncontact erections, whereas NAc-lesioned males displayed impaired noncontact erections. Conversely, during simultaneous exposure to inaccessible receptive and nonreceptive females in different compartments, all males spent more time in the proximity of the receptive female. These findings indicate that the aLHA plays an inhibitory role in the regulation of sexual arousal and an excitatory role in the regulation of ejaculation. Conversely, the NAc plays an excitatory role in the regulation in sexual arousal.

## Introduction

The lateral hypothalamic area (LHA) is an integrator for the limbic forebrain and autonomic nervous system and receives diverse and complex input from many brain regions via the medial forebrain bundle (for reviews see Nieuwenhuys *et al.*, 1982; Veening *et al.*, 1982; Geeraedts *et al.*, 1990a,b; Bernardis & Bellinger, 1993). Extensive evidence has demonstrated a role of the LHA in the regulation of body weight (see Bernardis & Bellinger, 1993, 1996) and reward processes (see, e.g. Shizgal, 1989; Wise & Hoffman, 1992). Of relevance to sexual behaviour, the LHA has interconnections with the medial preoptic area and ventromedial hypothalamus, the autonomic nervous system, brainstem monoamine systems, olfactory bulb, extended amygdala and nucleus accumbens (NAc). Both the LHA and the NAc receive rich innervation from brainstem monoamine systems that have been implicated in sexual function. Dopamine (DA) release in the NAc increases throughout copulation and decreases following ejaculation (Phillips *et al.*, 1991; Blackburn *et al.*, 1992; Fiorino & Phillips, 1999; Lorrain *et al.*, 1999). Serotonin (5-HT) release in the anterior portion of the LHA (aLHA) remains at basal levels during copulation but increases near the point of ejaculation (Lorrain *et al.*, 1997). Further, infusion of 5-HT into the aLHA blocks the increase in NAc DA during copulation (Lorrain *et al.*, 1999). These findings prompted Lorrain and colleagues to propose that NAc DA and aLHA

5-HT have opposing functions in the regulation of male sexual arousal: DA release in the NAc facilitates male sexual arousal, whereas 5-HT release in the aLHA opposes this effect.

Functional studies support the idea that the NAc facilitates sexual arousal but do not provide strong evidence that the aLHA inhibits sexual arousal. Electrolytic and dopamine-depleting lesions of the NAc decreased noncontact erections (NCEs) induced by exposing males to inaccessible estrous females (Liu *et al.*, 1998). Blockade of DA receptors in the NAc by haloperidol infusions diminish conditioned locomotion induced by sexual reward in male rats (Pfaus & Phillips, 1991). Infusions of the 5-HT reuptake inhibitor, alaproclate, into the aLHA of male rats delayed the initiation of copulation and ejaculation (Lorrain *et al.*, 1997). Reports of the effects of lesions of the LHA on sexual behaviour have yielded conflicting results. Large excitotoxic lesions of the LHA, which included the posterior LHA and zona incerta, disrupt copulatory behaviour (Edwards & Maillard, 1990; Maillard & Edwards, 1991). Conversely, copulatory behaviour is not affected by small excitotoxic lesions of the aLHA (Hansen *et al.*, 1982); however, these lesions also do not decrease food intake and body weight, suggesting largely intact LHA function. Thus, the role of the aLHA in sexual behaviour remains unclear.

In the present study, we examined the effects of excitotoxic lesions of the aLHA and the NAc on a battery of tests to assess specific, dissociable aspects of sexual behaviour (for reviews see Pfaus, 1996, 1999; Sachs, 2000). In addition to repeated tests of copulation, we measured noncontact erections (Sachs *et al.*, 1994) to assess the effects of lesions on sexual arousal (i.e. autonomic control of erection), preferences for estrous over nonestrous females (e.g. Agmo, 2003) to assess the effects of lesions on unconditioned appetitive sexual

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motivation (i.e. response to incentive cues), and the acquisition of conditioned sexual excitement (Mendleson & Pfau, 1989) to assess the effects of lesions on sexual reinforcement. Our findings indicate that the aLHA plays an inhibitory role in sexual arousal and a facilitatory, perhaps essential, role in ejaculation, whereas the NAc plays a facilitatory role in sexual arousal.

## Methods

### Subjects

#### Males

The 65 Long-Evans rats that served as subjects in this experiment were obtained from Charles River Canada (St Constant, QC). All subjects were sexually naïve males that were  $\approx$ 3 months of age at surgery (weighing 300–350 g) and 4–6 months of age at the start of behavioural tests. They were housed in pairs in Plexiglas cages (36 × 26 × 19 cm) with *ad lib* access to food (Purina Rat Chow) and water. All rats were kept in a 12-h reversed light–dark cycle colony room (lights off at 08.00 h) maintained at 21 °C. All experimental protocols were approved by the Concordia University Animal Care Committee, in accordance with the guidelines of the Canadian Council on Animal Care.

#### Females

Female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions under ketamine (75 mg/kg) and xylazine (10 mg/kg) anaesthesia at least 2 months prior to the start of the experiment and had extensive sexual experience. Females were housed under the same conditions as males. Sexual receptivity was induced by subcutaneous administration of estradiol (10  $\mu$ g) 48 h, and progesterone (500  $\mu$ g) 4–6 h, prior to each test. Nonreceptive females received no hormone treatments for a period of at least 30 days.

#### NMDA lesions

Bilateral microinjections of 10  $\mu$ g of *N*-methyl-D-aspartic acid (NMDA; Research Biochemicals) or vehicle were directed at either the NAc or the aLHA under ketamine–xylazine anaesthesia. NMDA was injected in 1.0  $\mu$ L of vehicle (phosphate-buffered saline, pH 7.2) per side over a 10-min period. Lesion coordinates were derived from the atlas of Paxinos & Watson (1986). aLHA coordinates were AP –1.3, ML  $\pm$ 2.0 and DV –8.5 mm relative to bregma. NAc coordinates were AP +1.0, ML  $\pm$ 1.5 and DV –7.5 mm relative to bregma. Lesioned rats were allowed to recover from surgery until their body weights stabilized and returned to at least 80% of their presurgery weights (1–3 months for aLHA-lesioned rats) during which time they were fed meal replacement formula (Boost, Mead Johnson Nutritionals) as necessary. Rats whose body weight did not stabilize were killed with a pentobarbital overdose. Sham-lesioned males were left undisturbed until lesioned rats had recovered and were tested at the same time; however, time between surgery and testing did not alter any measure of sexual behaviour (data not shown). Following behavioural testing, all rats were killed with a pentobarbital overdose and perfused with saline and formaldehyde, and their brains were removed and sliced on a cryostat (50- $\mu$ m sections). The extent of the lesions was verified by microscopic analysis of Cresyl Violet-stained tissue.

#### Apparatus

Copulatory tests took place in pacing chambers constructed from a standard laboratory Plexiglas cage (36 × 26 × 19 cm) with a Plexiglas obstacle-insert (partially bisecting the cage along its longest dimen-

sions with 3-cm openings at either end), which allowed the female to pace the copulatory behaviour of the male by moving around the obstacle. The obstacle-insert was made by attaching a Plexiglas divider (30 × 20 × 0.5 cm) lengthwise to the centre of a Plexiglas base (35 × 18 × 0.5 cm). The insert was then placed into the Plexiglas cage and the base was covered with bedding material. A piece of wire mesh (0.25-cm grid, 35 × 18 cm) with a groove cut into the centre to fit snugly around the divider was placed on top of the bedding. A cover constructed of wire mesh (0.5-cm grid, 36 × 20 cm) was placed over the chamber. All copulatory tests were recorded on video and scored subsequently using a PC-based program. Noncontact erection tests took place in a Plexiglas chamber (30 × 40 × 50 cm) which was divided into two equal-sized compartments by wire mesh (0.5-cm grid). The floor of the chamber was constructed of wire mesh (0.5-cm grid) elevated 10 cm above the bottom of the chamber and a mirror placed below the floor on a 45° angle to allow ventral viewing. NCEs were scored at the time of testing. Receptive female preference tests took place in a large open field (123 × 123 × 46 cm) with a thin layer of Beta Chip bedding material over the floor. Stimulus females were confined to a corner by placing them under an inverted standard stainless-steel home cage (24 × 20 × 18 cm) with the mesh portion facing away from the corner, such that the males could not interact directly with the females.

### Procedure

#### Copulation tests

Following recovery from the lesions, each male received three tests of copulation 4–6 days apart. At the start of each test, each male was placed in a pacing chamber for 5 min, after which a receptive female was placed in the chamber for 120 min on the first test and for 30 min on the second and third tests. The criteria of Meisel & Sachs (1994) were used to score latency and frequency measures for mounts, intromissions and ejaculations. In addition to copulation measures, the amount of anticipatory locomotion (side changes) was recorded as an index of conditioned sexual excitement (Mendelson & Pfau, 1989).

#### Noncontact erection test

Four to six days following the final copulation test, each male received a noncontact erection test. Males were placed on one side of a divided chamber for 5 min, then a receptive female was placed on the opposite side of the chamber for 20 min, and penile erection and grooming were measured directly, as described by Sachs and colleagues (Sachs *et al.*, 1994; Sachs, 1996). Some males displayed continuous erections not previously described in the literature; in such instances, the NCE were scored as a single erection in order to provide a conservative estimate.

#### Receptive female preference (RFP) test

Four to six days following the noncontact erection test, each male received a receptive female preference test. Males were placed in an open field that had one receptive and one nonreceptive female confined in opposite corners for 15 min. The females were inaccessible during this test. The tests were videotaped and scored by visually dividing the open field into four quadrants. The amount of time spent in each quadrant (containing receptive female or nonreceptive female, or empty) was measured.

#### Statistical analyses

Rats receiving sham surgeries for the NAc and the aLHA lesions did not differ from each other on any measures examined and were combined for statistical analyses. For copulation tests, the proportion of rats in each group that mounted, intromitted and ejaculated on each test was analysed using  $\chi^2$  analyses; copulation latencies and frequen-

cies were analysed using mixed-design (group  $\times$  test session) ANOVAs with significant  $F$ -values followed by *post hoc* comparisons between individual means using the Tukey HSD method. For noncontact erections, the proportions of rats in each group displaying noncontact erection were analysed with  $\chi^2$  tests; the latency to display and the frequency of noncontact erections were analysed using one-way ANOVAs with significant  $F$ -values followed by *post hoc* comparisons between individual means using the Tukey HSD method. For receptive female preference tests, the times spent in the receptive female quadrant, the nonreceptive female quadrant and the empty quadrants were analysed using a two-way between-within (group  $\times$  quadrant type) ANOVA with significant  $F$ -values followed by *post hoc* comparisons between individual means using the Tukey HSD method. The level of significance for all comparisons was  $P < 0.05$ .

## Results

### Histology

Of the 34 males that received lesions of the aLHA, two died prior to completion of the surgery and 12 died or were killed because they failed to maintain a stable body weight following surgery; the remaining aLHA males lost up to 40% of their body weight following surgery

but were subsequently able to maintain their body weight at or above a criterion minimum of 80% of their presurgery weight. These remaining 20 aLHA-lesioned males completed behavioural testing, but six were excluded from data analyses because their lesions did not damage the aLHA or included portions of the medial preoptic area. Thus, the final aLHA-lesioned group consisted of 14 males. All of the 15 NAc-lesioned males recovered following surgery, but one male was excluded because his lesion did not include bilateral damage to the NAc. Thus, the NAc-lesion group consisted of 14 males. The males that were included in the analyses had sustained either partial or complete bilateral damage to the target structure and schematic representation of the largest and smallest lesions for the aLHA-lesioned and the NAc-lesioned groups is displayed in Fig. 1. The sham-lesioned group consisted of 16 males (eight NAc-sham and eight aLHA-sham).

### Copulation tests

To assess the effects of aLHA and NAc lesions on copulation, males that had received sham surgeries or lesions received three copulation tests. All males in all groups approached and engaged in anogenital investigations with the females. Most of the sham (either aLHA or NAc) males copulated to ejaculation on each test and developed

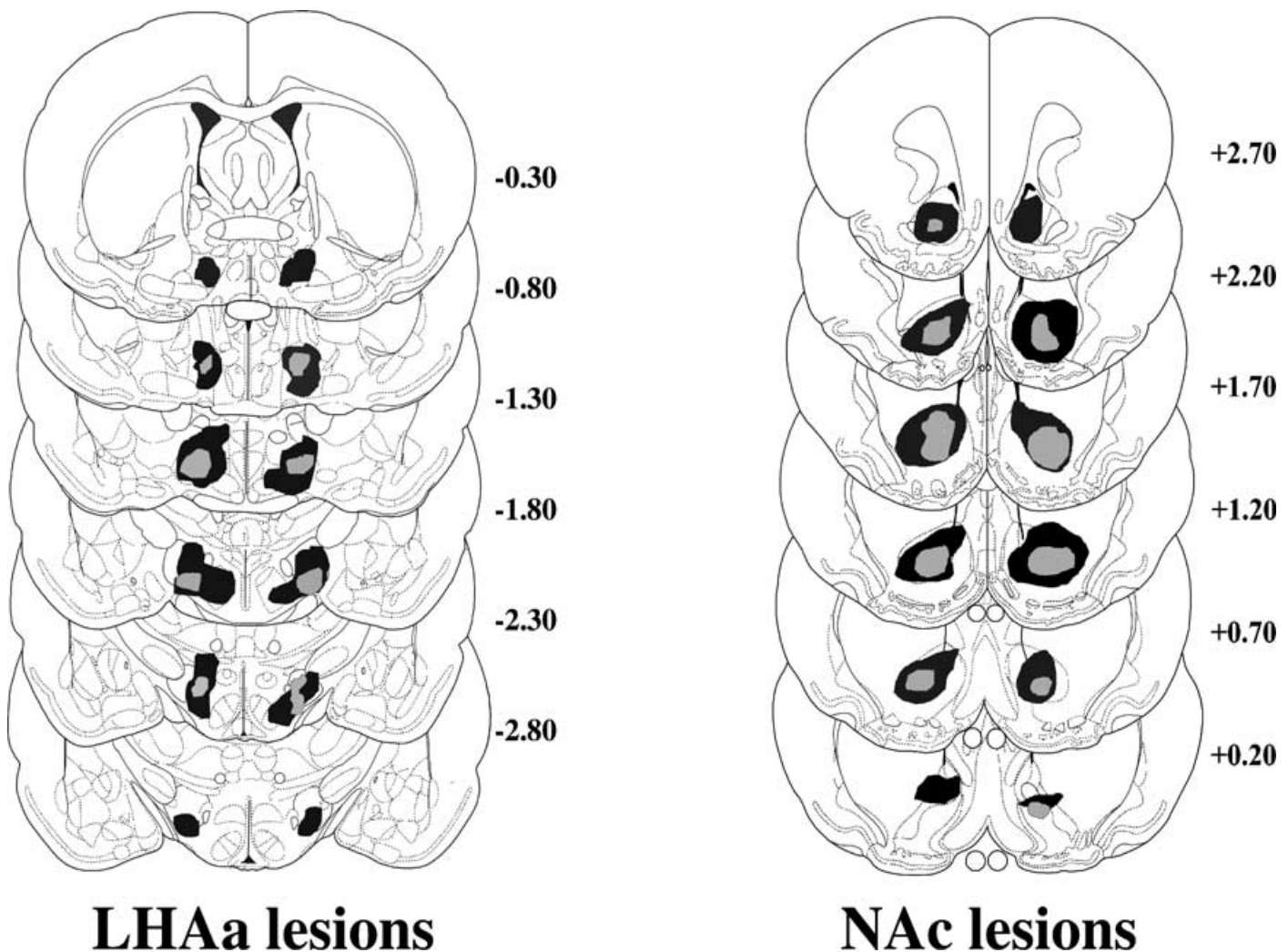


FIG. 1. Schematic representation of largest (black) and smallest (grey) aLHA lesions (left panel) and NAc lesions (right panel) mapped on atlas of Paxinos & Watson (1986). Numbers indicate anterior–posterior coordinates relative to bregma in mm.

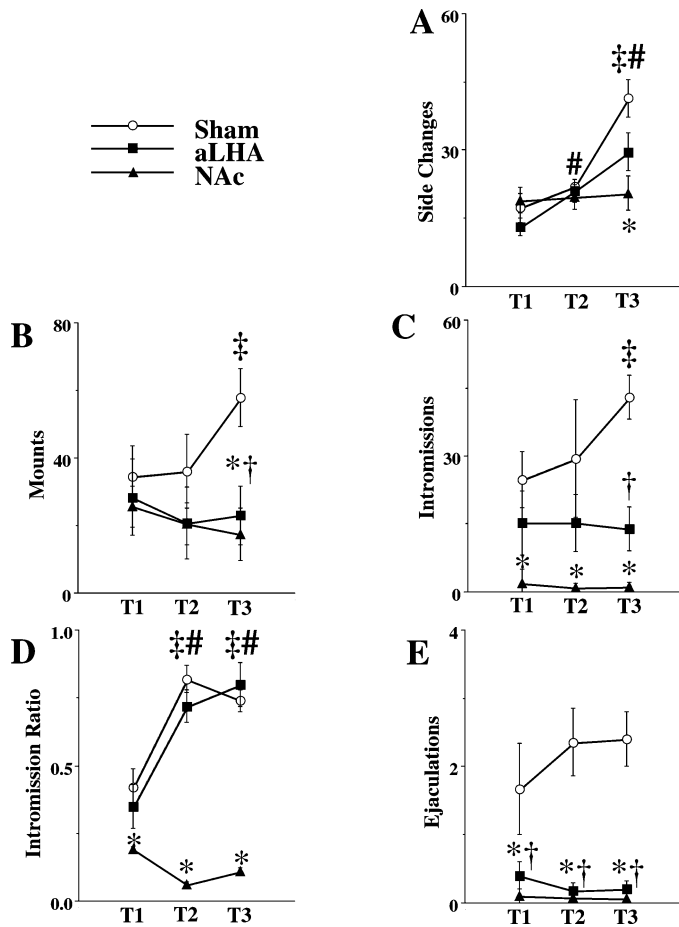


Fig. 2. Effects of aLHA and NAc lesions on conditioned sexual excitement and copulatory behaviour. For sham-, aLHA- and NAc-lesioned males, mean  $\pm$  SEM (A) frequencies of anticipatory side changes, (B) frequencies of mounts, (C) frequencies of intromissions, (D) intromission ratios and (E) frequencies of ejaculation on each of the three copulation tests. \* $P < 0.05$  (see text for details) for comparison between sham- and NAc-lesioned males, † $P < 0.05$  for comparison between sham- and aLHA-lesioned males, ‡ $P < 0.05$  for comparison between tests for sham-lesioned males; and # $P < 0.05$  for comparison between tests for aLHA-lesioned males.

conditioned sexual excitement. Most of the aLHA-lesioned males failed to ejaculate but did mount and intromit and develop conditioned sexual excitement. In contrast, although most of the NAc-lesioned males mounted, they failed to intromit and ejaculate and did not develop conditioned sexual excitement. The mean numbers of anticipatory side changes, mounts, intromissions, intromission ratios and ejaculations are displayed in Fig. 2.

Sham- and aLHA-lesioned, but not NAc-lesioned, males displayed increased frequency of anticipatory side changes prior to the introduction of the receptive female across the copulation tests (Fig. 2A). The statistical significance of these observations was confirmed by a significant group  $\times$  test interaction ( $F_{4,58} = 3.27$ ,  $P < 0.05$ ). Between-group *post hoc* analyses revealed that the sham-lesioned males displayed significantly higher side change frequencies than the NAc-lesioned males on test 3 ( $P < 0.05$ ); no other comparisons between groups differed on individual tests. For the sham-lesioned males, *post hoc* analyses revealed that side change frequencies were significantly lower on tests 1 and 2 than on test 3 (all  $P < 0.05$ ). For the aLHA-lesioned males, *post hoc* analyses revealed that side change frequencies were significantly lower on test 1 than on tests 2 and 3 and on test 2

than on test 3 (all  $P < 0.05$ ). For the NAc-lesioned males, *post hoc* analyses revealed that side change frequencies did not differ significantly between any of the tests (all  $P > 0.05$ ).

Almost all males in each group mounted on each test and the proportions of males that mounted on at least one test did not differ significantly between any of the groups (data not shown; all  $P > 0.05$ ). However, sham-lesioned males displayed an increase in the frequency of mounts across tests, whereas the aLHA- and NAc-lesioned males did not (Fig. 2B). The statistical significance of these observations was confirmed by a significant group  $\times$  test interaction ( $F_{4,58} = 4.88$ ,  $P < 0.05$ ). Between-group *post hoc* analyses revealed that the sham-lesioned males displayed significantly more mounts than either the aLHA- or NAc-lesioned males on test 3 ( $P < 0.05$ ). For the sham-lesioned males, *post hoc* analyses revealed that the number of mounts was significantly lower on tests 1 and 2 than on test 3 ( $P < 0.05$ ), but no other comparisons differed significantly. For the aLHA- or NAc-lesioned males, *post hoc* analyses revealed no significant differences between the numbers of mounts between any of the tests (all  $P > 0.05$ ).

Although most of the sham- (88%) and aLHA-lesioned (77%) males intromitted on at least one test, few of the NAc-lesioned males (14%) intromitted on at least one test. The statistical significance of these observations was confirmed by  $\chi^2$  analysis of the proportion of males that intromitted in all three groups:  $\chi_{44}^2 = 8.50$ ,  $P < 0.05$ ; follow-up comparisons revealed that the NAc-lesioned males differed significantly from the sham- ( $\chi_{30}^2 = 6.24$ ,  $P < 0.05$ ) and from the aLHA-lesioned males ( $\chi_{28}^2 = 4.65$ ,  $P < 0.05$ ), but the sham- and aLHA-lesioned males did not differ significantly from each other ( $\chi_{30}^2 = 0.59$ ,  $P > 0.05$ ). Similarly, the frequency of intromissions displayed by the NAc-lesioned males was substantially lower than for the sham- or aLHA-lesioned males (Fig. 2C). The statistical significance of these observations was confirmed by significant effect of group ( $F_{2,58} = 10.64$ ,  $P < 0.05$ ). *Post hoc* analyses revealed that the NAc-lesioned males intromitted significantly fewer times than the males in the other two groups ( $P < 0.05$ ). No significant interaction ( $F_{4,58} = 1.60$ ,  $P > 0.05$ ) or test ( $F_{2,58} = 1.30$ ,  $P > 0.05$ ) effects were detected. Further, the intromission ratios were also substantially reduced in the NAc-lesioned males relative to the sham- or aLHA-lesioned males on all tests and the intromission ratios of the latter groups increased across tests (Fig. 2D). The statistical significance of these observations was confirmed by a significant interaction effect of group and test ( $F_{4,58} = 6.63$ ,  $P < 0.05$ ). Between-group *post hoc* analyses revealed that the NAc-lesioned males displayed intromission ratios significantly lower than the males in the other two groups on all tests (all  $P < 0.05$ ). For the sham-lesioned males, follow-up analyses revealed that intromission ratios were significantly lower on test 1 than on tests 2 and 3 ( $P < 0.05$ ), but that no other comparisons differed significantly. For the aLHA-lesioned males, *post hoc* analyses revealed that intromission ratios were significantly lower on test 1 than on tests 2 and 3, but no other comparisons differed significantly. For the NAc-lesioned males, *post hoc* analyses revealed that intromission ratios did not differ significantly between any of the tests (all  $P > 0.05$ ). Further inspection of histological results revealed that the two NAc-lesioned males that intromitted (and ejaculated; see below) had lesions of similar size to other NAc-lesioned males but had either unilateral or bilateral sparing of the lateral portions of the NAc (i.e. core), suggesting that this region is important for intromission.

Few of the aLHA-lesioned males (14%) or NAc-lesioned males (14%) ejaculated on any of the copulation tests, whereas the majority of the sham-lesioned males (88%) ejaculated on at least one copulation test. The statistical significance of the failure of most of the NAc-lesioned males and aLHA-lesioned males to ejaculate on at least one test was confirmed by  $\chi^2$  analysis between all three groups:

$\chi_{44}^2 = 9.32$ ,  $P < 0.05$ ; follow-up comparisons revealed that the sham-lesioned males differed significantly from the aLHA-lesioned males ( $\chi_{30}^2 = 16.77$ ,  $P < 0.05$ ) and from the NAc-lesioned males ( $\chi_{30}^2 = 9.07$ ,  $P < 0.05$ ), but the aLHA-lesioned and the NAc-lesioned males did not differ significantly from each other ( $\chi_{30}^2 = 0.13$ ,  $P > 0.05$ ). Similarly, the frequencies of ejaculations displayed by the aLHA-lesioned and the NAc-lesioned males was substantially lower than the sham-lesioned males (Fig. 2E). The statistical significance of these observations was confirmed by significant effect of group ( $F_{2,58} = 17.99$ ,  $P < 0.05$ ); *post hoc* analyses revealed that the sham-lesioned males ejaculated significantly more than the males in the other two groups ( $P < 0.05$ ). No significant interaction ( $F_{4,58} = 1.60$ ,  $P > 0.05$ ) or test ( $F_{2,58} = 1.30$ ,  $P > 0.05$ ) effects were detected. Further inspection of histological results revealed that both of the two aLHA-lesioned males that ejaculated had lesions that were smaller than the other aLHA-lesioned males and that had bilateral sparing of the dorsal portion of the aLHA, suggesting that this region is important for ejaculation.

Mount latencies did not differ between any of the groups of males, and intromission latencies did not differ between the sham- and aLHA-lesioned males. An insufficient number of NAc-lesioned males intromitted to permit analysis of intromission latency, and insufficient numbers of NAc-lesioned males or aLHA-lesioned males ejaculated to permit analysis of ejaculation latencies (all  $P > 0.05$ ; data not shown).

#### Noncontact erection test

The proportion of males in each group displaying noncontact erection, the mean latency to display noncontact erection and the mean frequency of noncontact erections for each group are shown in Table 1. The majority of sham- and aLHA-lesioned males displayed noncontact erections, whereas less than half of the NAc-lesioned males displayed noncontact erections. The statistical significance of these observations was confirmed by  $\chi^2$  analyses:  $\chi_{44}^2 = 7.62$ ,  $P < 0.05$ ; follow-up analyses revealed that the NAc-lesioned group differed significantly from both the sham- group ( $\chi_{30}^2 = 8.44$ ,  $P < 0.05$ ) and aLHA-lesioned group ( $\chi_{28}^2 = 5.14$ ,  $P < 0.05$ ). For the males that displayed noncontact erections, the latency to display noncontact erection was lower in the aLHA-lesioned males than sham- or NAc-lesioned males. The statistical significance of these observations was confirmed by one-way ANOVA ( $F_{2,23} = 5.41$ ,  $P < 0.05$ ). *Post hoc* analyses revealed that the aLHA-lesioned males differed significantly from the other two groups ( $P < 0.05$ ). Conversely, for the males that displayed noncontact erections, the frequency of noncontact erections was lowest in the NAc-lesioned males and highest in the aLHA-lesioned males. The statistical significance of these observations was confirmed by one-way ANOVA ( $F_{2,23} = 4.65$ ,  $P < 0.05$ ); *post hoc* analyses revealed that the NAc-lesioned males differed significantly from the other two groups ( $P < 0.05$ ), and there was a trend toward significance between the sham- and aLHA-lesioned males ( $P < 0.10$ ). In addition, six of the aLHA-lesioned males displayed continuous partial-to-full erections

TABLE 1. The proportion of males in each group displaying NCE, the latency to display NCE and the frequency of NCE for males in each group

Lesion	Proportion	Latency (s)	Frequency
Sham	13/16	428 ± 55	3.27 ± 0.75
aLHA	10/14	191 ± 56*	4.10 ± 1.08
NAc	4/14*	454 ± 116	1.50 ± 0.25*

NCE, noncontact erection. Mean values latency and frequency are ±SEM. \* $P < 0.05$  for comparison to sham; see text for details.

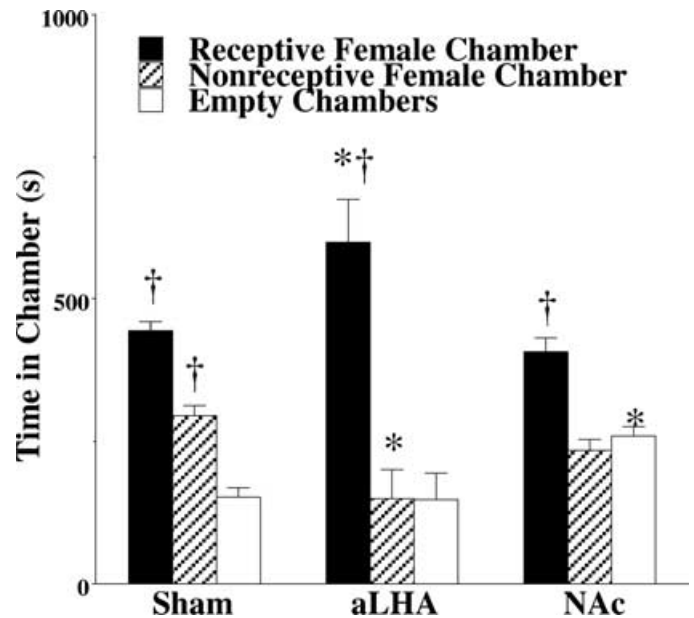


FIG. 3. Effects of aLHA and NAc lesions on receptive female preference. The mean ± SEM time spent in the receptive female quadrant (chamber), the nonreceptive female quadrant and the empty quadrants. \* $P < 0.05$  (see text for details) for comparison between sham-lesioned males and either the aLHA- or NAc-lesioned males, † $P < 0.05$  for comparison between quadrants within groups of males.

throughout the duration of the test; this phenomenon was not observed in either the sham- or NAc-lesioned males.

#### Receptive female preference test

The mean time spent in the receptive female quadrant, the nonreceptive female quadrant and the empty quadrants for each group are displayed in Fig. 3. Males in all groups displayed a preference to remain in the proximity of the receptive female compared to the nonreceptive female. Further, the aLHA-lesioned males spent more time in the proximity of the receptive female and less time in the proximity of the nonreceptive female than males in the other two groups, whereas the NAc-lesioned males spent more time in the empty quadrants than the other two groups. The statistical significance of these observations were confirmed by a two-way ANOVA (group × quadrant interaction,  $F_{4,82} = 7.4$ ,  $P < 0.05$ ). *Post hoc* analyses revealed that the sham-lesioned males spent significantly more time in the receptive quadrant than in the nonreceptive or empty quadrants, and more time in the nonreceptive quadrant than in the empty quadrants (all  $P < 0.05$ ). Both the aLHA-lesioned and the NAc-lesioned males spent significantly more time in the receptive quadrant than in the nonreceptive or empty quadrants (all  $P < 0.05$ ). Additionally, the aLHA-lesioned males spent significantly more time in the receptive quadrant than did the other two groups ( $P < 0.05$ ), the sham-lesioned males spent significantly more time in the nonreceptive quadrant than did the other two groups ( $P < 0.05$ ) and the NAc-lesioned males spent significantly more time in the empty quadrant than did the other two groups ( $P < 0.05$ ).

#### Discussion

The present findings provide functional evidence for an inhibitory role of the aLHA and an excitatory role of the NAc in the regulation of sexual arousal (i.e. penile erection) as measured by copulation and

noncontact erection. In addition, the present findings demonstrate that the aLHA is necessary for ejaculation, at least in sexually naïve males. In contrast, neither NAc nor aLHA lesions disrupted appetitive motivation for an unconditioned sexual incentive as revealed by receptive female preferences. These results are consistent with the hypothesis that the aLHA and the NAc have opposing roles in the regulation of sexual arousal (Lorrain *et al.*, 1999) but not other aspects of sexual behaviour.

The disruptions of copulatory behaviour caused by aLHA or NAc lesions in the present study appear to be inconsistent with the results of previous studies. However, at least two methodological differences exist that may account for the discrepancies. First, the males employed in the present study were sexually naïve at the time of surgery, whereas previous reports that failed to find disruptions of copulation following aLHA and NAc lesions used sexually experienced males (e.g. Hansen *et al.*, 1982; Liu *et al.*, 1998). As discussed in Pfaus *et al.* (2001), sexual experience generally protects sexual behaviour from disruptive influences of castration (S. Centeno and J. G. Pfaus, unpublished observations), anosmia (Thor & Flannelly, 1977), and brain stem lesions (Kippin & van der Kooy, 2003), thus sexual experience may similarly protect against the disruptive effect of NAc and aLHA damage on sexual behaviour. Second, the lesions produced in the present and previous studies differ in a number of aspects which may account for observed differences in sexual behaviour. Compared to the NAc lesions produced by Liu *et al.* (1998) the present lesions were larger and more rostral. Compared to the lesions produced by Hansen *et al.* (1982) the present aLHA lesions were larger and slightly more rostral. Further, the present lesions resulted in substantial weight loss indicating disruption of LHA function, whereas the lesions in Hansen *et al.* (1982) failed to alter body weight, indicating substantial retention of LHA function.

Conversely, Edwards & Maillard (1990) and Maillard & Edwards (1991) found that relatively large lesions that included posterior regions of the LHA disrupted mounting, intromitting and ejaculating in male rats, and that LHA damage correlated positively with disruption in all aspects of copulatory behaviour. Compared to the LHA lesions produced by Edwards & Maillard (1990) and Maillard & Edwards (1991), the present LHA lesions were substantially more rostral and specifically prevented ejaculation without preventing other aspects of copulation. Thus differences in lesion size and placement may account for the differences in sexual behaviour observed between studies and suggest that regions of the LHA may serve different functions in the regulation of male sexual behaviour. The posterior LHA appears to be necessary for mounting and intromitting behaviour and the anterior LHA appears to be essential only for ejaculation.

The effects of lesions of the aLHA and the NAc on NCEs in the present study are consistent with the proposed roles of these structures in sexual arousal described by Lorrain *et al.* (1999), namely that increased DA transmission in the NAc facilitates, whereas increased 5-HT transmission in the aLHA inhibits, sexual arousal. In the present study, aLHA lesions facilitated and NAc lesions inhibited sexual arousal as measured by NCEs. The inability of males with NAc lesions to become sexually aroused (i.e. gain erection) in the presence of a receptive female probably underlies their failure to gain intromissions during copulation tests. Moreover, these opposite effects of aLHA and NAc lesions on sexual arousal are consistent with delayed initiation of copulation following infusion of either a 5-HT reuptake inhibitor into the aLHA (Lorrain *et al.*, 1997) or a dopamine receptor antagonist into the NAc (Pfaus & Phillips, 1991). To date, no studies have addressed the effects of serotonergic manipulations in the aLHA or dopaminergic manipulations in the NAc on tests assessing sexual arousal (e.g. NCE

test) or appetitive sexual motivation (e.g. receptive female preference test) independent of copulation. Thus, it would be of interest to more fully examine the effects of neurochemical manipulations of these structures on a battery of tests assessing specific aspects of sexual behaviour.

In addition to the excitatory effects of aLHA lesions on NCEs, the present study also found that aLHA lesions specifically prevented ejaculation without preventing other aspects of copulatory behaviour or sexual motivation. Importantly, the prevention of ejaculation does not appear to be due to males with aLHA lesions achieving insufficient numbers of intromissions (see Fig. 2C) or changes in intromission ratio (see Fig. 2D). These findings demonstrate that the aLHA plays two distinct roles in copulation: first, an inhibitory role in the control of sexual arousal and, second, a facilitatory role in the control of ejaculation. Consistent with the latter finding, electrical stimulation of the aLHA has been shown to facilitate ejaculation (Singh *et al.*, 1996). However, increased 5-HT levels in the aLHA delay the initiation of both copulation and ejaculation (Lorrain *et al.*, 1997). Thus, manipulation of the aLHA does not necessarily have opposing effects on sexual arousal and ejaculation and suggests independent functions of neurons in the aLHA in the control of sexual arousal and ejaculation.

In contrast to the effects on copulatory measures, appetitive sexual motivation does not appear to be directly affected by lesions of the aLHA or the NAc. Receptive female preferences were not disrupted following lesions of the aLHA and the NAc, indicating an intact approach to a sexual incentive. Similarly, initiation of copulation (i.e. mount latency) on each test was not changed following lesions of either the aLHA or NAc, indicating intact pursuit of a sexual incentive. In contrast, males with lesions of the NAc failed to acquire conditioned sexual excitement, reflecting disruption of sexual reinforcement possibly due to disruption of intromission (i.e. preventing experience of the reinforcing stimulus). Although lesions of either the aLHA or NAc failed to disrupt receptive female preference, both lesions did alter the amount of time spent in the nonreceptive female and empty areas of the test apparatus. Males with aLHA lesions increased the amount of time spent near the receptive female and decreased the amount of time spent near the nonreceptive female, suggesting that these males may have higher sexual motivation than sham-lesioned males. This finding is particularly interesting in light of extensive evidence that similar lesions appear to reduce motivation for other forms of rewards, such as food (see Bernardis & Bellinger, 1996) or electrical brain stimulation (Yeomans *et al.*, 1988; Stellar *et al.*, 1991). However, the present finding may also be interpreted as a reduced appetitive motivation for non-sexual social interactions. Accordingly, it is difficult to fully interpret these results, although motivation for unconditioned sexual incentives is intact following aLHA lesions. Conversely, lesions of the NAc increased the amount of time that the males spent in the empty quadrants of the open field, suggesting that these males have intact motivation for sexual incentives but perhaps reduced social motivation or increased social aversion. Regardless, the finding that both lesions failed to disrupt receptive female preference demonstrates that appetitive approach behaviour to a receptive female is not dependent on either the aLHA or the NAc. Moreover, the findings of intact receptive female preferences demonstrate that the roles of the aLHA and NAc in sexual arousal and ejaculation are dissociable from appetitive approach behaviour.

## Abbreviations

5-HT, serotonin; DA, dopamine; LHA, lateral hypothalamic area; aLHA, anterior portion of the LHA; NAc, nucleus accumbens; NCE, noncontact erection; NMDA, *N*-methyl-D-aspartic acid.

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