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Short communication

Stimulation of the medial amygdala enhances medial preoptic dopamine release: implications for male rat sexual behavior

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Abstract

Increased dopamine (DA) in the medial preoptic area (MPOA) facilitates male sexual behavior. A major source of innervation to the MPOA is the medial amygdala (MeA). We now report that chemical stimulation of the MeA enhanced levels of extracellular MPOA DA in anesthetized male rats. These results suggest that DA activity in the MPOA can be regulated by input from the MeA to the MPOA.

Theme: Neural basis of behavior

Topic: Monoamines and behavior

Keywords: Medial preoptic area; Medial amygdala; Dopamine; Sexual behavior; Microdialysis; HPLC–EC

Both the medial preoptic area (MPOA) and the medial amygdala (MeA) play important roles in the regulation of male sexual behavior [18,30]. Chemosensory input from the olfactory bulbs and vomeronasal organ projects to the MeA, which processes the information and relays it to the MPOA and other sites [18,46].

Increased dopamine (DA) in the MPOA facilitates male sexual behavior [15]. When microinjected into the MPOA, DA agonists facilitate copulation [16,27,39] and genital reflexes [33], whereas antagonists impair copulation, genital reflexes, and sexual motivation [34,36,44]. Moreover, levels of extracellular MPOA DA increase in the presence of a sexually exciting stimulus and during copulation [3,9,17].

Recently, we reported that MeA lesions impaired copulation and blocked the mating-induced increase in MPOA DA, seen in animals with sham lesions; microinjections of apomorphine, a DA agonist, into the MPOA of animals with MeA lesions restored measures of copulation [9]. These findings suggest that both copulatory ability and the mating-induced MPOA DA response during exposure to an estrous female and during copulation are mediated in part by input from the MeA into the MPOA. To further elucidate the interaction between the MeA and extracellular DA in the MPOA, the present experiment tested whether chemical stimulation of the MeA would increase extracellular DA in the MPOA of male rats.

Before surgery, 14 adult male Long-Evans Blue Spruce rats (Harlan Sprague–Dawley, Indianapolis, IN; ~350 g at the time of surgery) were anesthetized with intraperitoneal injections of urethane in saline solution (1.5 g/kg; Sigma Aldrich, St. Louis, MO) and were placed into a stereotaxic apparatus. They then received guide cannulae implants, made of 23-gauge thin-wall stainless steel tubing, ending unilaterally above the MeA and the MPOA. For microinjections, guide cannulae ended 2 mm above the MeA (anteriorposterior (AP), −0.8 mm; mediolateral (ML), +3.5 mm; dorsoventral (DV), −6.5 mm; interaural +5.0; according to Ref. [35]), and for microdialysis, guide cannulae ended 2 mm above the MPOA (AP, 2.3 mm; ML, 0.3 mm; DV, −6.2 mm; interaural +5.0; according to Ref. [35]). Both cannulae were secured to the skull and skull screws with dental acrylic. Obturators, cut the same length as the guide cannulae, were then inserted into the guide cannulae until the experiment began. Aseptic techniques were used throughout all surgical procedures.

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Immediately after surgery, while still anesthetized, the animal was taken to a separate test room and attached to our standard microdialysis apparatus. Concentric microdialysis probes were used; the dialysis membrane had an outer diameter of 170 μm, an inner diameter of 150 μm, an active dialyzing length of 1 mm, and an 18 000 molecular weight cut-off. Dead volume in the outflow capillary tubing was 0.4 μl. Probes were implanted in the guide cannula aimed at the MPOA; the perfusion line was attached, and Dulbecco's PBS (in mM: 138 NaCl, 2.7 KCl, 0.5 $MgCl_2$, 1.5 KH_2PO_4, and 1.2 $CaCl_2$, pH 6.8, filtered and degassed prior to use; Sigma, St. Louis, MO) was perfused at a constant rate of 0.5 μl/min with a Harvard Apparatus (South Natick, MA) infusion pump, using a 1-ml gas-tight syringe.

Then, 4 h after probes were implanted, four baseline samples were collected at 6-min intervals. After collecting the last baseline sample, with the probe still in place, animals received chemical stimulation of the MeA ($n=7$), or microinjections of saline vehicle ($n=7$), into the MeA. Chemical stimulation was achieved by microinjecting 2.7 nmol of L-glutamic acid (Sigma, St. Louis, MO) and 3.1 nmol of L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC; Tocris Cookson, Ballwin, MO), a potent and selective glutamate uptake inhibitor [4], in 0.5 μl of saline. Microinjections were performed using a 1-ml gas-tight syringe on an infusion pump. PE-20 tubing was attached, on opposite ends, to the syringe and a 27-gauge injection cannula. The injection cannula and the portion of tubing attached to it were filled with drug solution or saline vehicle, depending on treatment; the syringe and the portion of tubing attached to it were filled with water, an air bubble separating the two. Before injecting the solution, the metal obturator was removed and replaced with the injection cannula. Microinjections were administered over a 1-min interval at a flow of 0.5 μl/min, followed by an additional 1 min with the injection cannula left in place; the injection cannula was then replaced with the obturator. The microinjection procedure lasted ~3 min (1 min for microinjection, 1 min after injection, and ~1 min for handling); the sample designated as post-injection sample 1 (P1) included analytes collected during these 3 min. For each test, a total of ten samples were collected, which included four baseline samples (BL1–BL4) and six post-injection samples (P1–P6). Samples were immediately frozen (−80°C) and later assayed using high performance liquid chromatography with electrochemical detection (HPLC–EC).

The chromatography system consisted of a Valco (Houston, TX) injector with a 500-nl sample loop, and an Antec (Leiden, the Netherlands) microelectrochemical detector, equipped with a microflow cell (11-nl cell volume), with a glassy carbon working electrode and an Ag/AgCl reference electrode. The analytical column was an LC Packings Fusica reversed-phase capillary column (300 μm inner diameter, 5 cm long, packed with 3 μm C-18 particles). The working electrode was maintained at an applied potential of 0.8 V relative to the reference electrode. The mobile phase (in mM: 32 citric acid, 54.3 sodium acetate, 0.074 EDTA, 0.215 octyl sulfonic acid (Fluka, Milwaukee, WI) and 4% methanol (v/v); pH 3.45) was delivered through the system at 0.5 ml/min; however after the tee splitter, flow through the analytical column was ~7 μl/min. A more thorough description of our standard microdialysis and chromatography systems has been previously published [9].

At the end of each test, cannula placements were verified histologically. While the animal was still anesthetized, a dye solution was perfused through the probe to verify MPOA placement. To verify MeA placement, using the same procedure as that used for drug microinjection, 0.5 μl dye solution was injected into the MeA. The animals were immediately sacrificed, and their brains were removed, frozen, and sliced (40 μm), using a cryostat. Brain slices were mounted on slides and examined for cannula placement using a projection magnifier. All procedures were approved by the local IACUC and were in accord with the guidelines of the Society for Neuroscience.

Analysis of DA concentrations in dialysis samples collected during baseline revealed no significant differences between animals receiving MeA stimulation versus animals receiving vehicle microinjections (stimulation, 0.80 ± 0.28 pg/μl; vehicle, 0.86 ± 0.22 pg/μl; $t(7)=-0.38$; $P=0.71$). Analyses of percent changes from baseline, with a two-factor RM-ANOVA, revealed significant differences attributable to treatment ($F(1,42)=6.11$; $P<0.05$; Fig. 1). A Newman–Keuls test revealed that animals receiving MeA stimulation had larger increases in extracellular DA than did animals with vehicle microinjections ($P<0.05$). A one-factor RM-ANOVA for the MeA-stimulation group revealed significant differences attributable to sample ($F(6,24)=2.51$; $P=0.05$); for this group, Dunnet's test revealed significant increases in extracellular DA during P1 and P2 ($P<0.05$), compared to baseline. Analyses of percent changes from baseline for homovanillic acid (HVA), a DA metabolite, revealed no significant differences between animals receiving MeA stimulation or vehicle microinjection ($F(1,42)=1.45$; $P>0.05$).

Urethane, a long-acting anesthetic [45], at a dose of 1.5 g/kg, the dose used in this experiment, has a high mortality rate [10]; of the 14 animals receiving cannulae implants, two died during the microdialysis procedure, and their samples were not analyzed. Histological analysis revealed that nine of the 12 remaining animals received cannulae placements in the MPOA and the MeA (Fig. 2); microdialysis samples collected from only these nine animals were analyzed statistically, leaving five animals in the MeA-stimulation group and four in the vehicle-microinjection group. Of the three animals with missed placements, one was dorsal to the MPOA, and the other two were dorsal to the MeA; one animal with a misplaced MeA cannula received chemical stimulation and the other

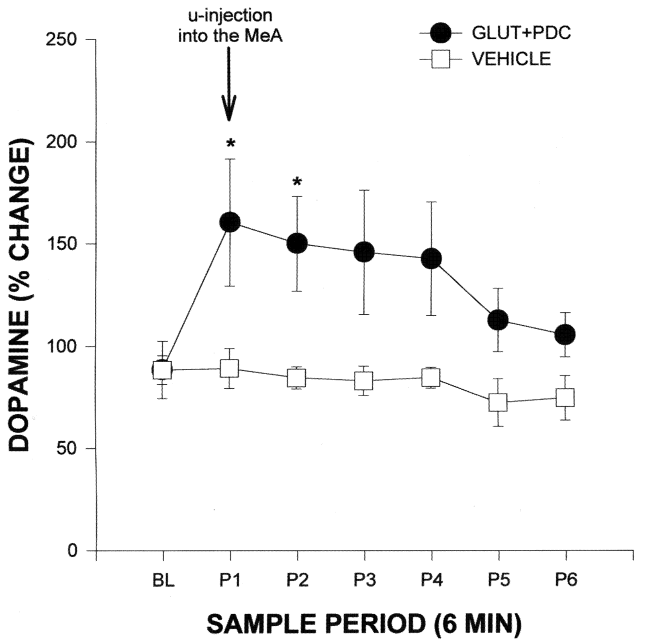

Fig. 1. Levels of DA in dialysate from the MPOA of animals receiving MeA stimulation or vehicle microinjection. Levels represent percent changes from baseline (BL) in response to MeA stimulation or vehicle microinjection; samples collected after microinjections into the MeA are post-injection samples 1–6 (P1–P6). Levels of extracellular DA significantly increased after MeA microinjections for animals receiving MeA stimulation but not for animals receiving vehicle microinjections. The baseline value used for computation was obtained by dividing the value of the last baseline by the mean of the last three baselines. Values are expressed as mean±S.E.M. (*$P<0.05$).

received vehicle. Analysis of samples collected from these animals did not show changes after microinjection; however, they were too few to analyze statistically.

In the present experiment, chemical stimulation of the MeA enhanced DA release in the MPOA. Analyses of microdialysis samples collected from the MPOA of animals receiving chemical stimulation of the MeA showed a significant increase in levels of extracellular DA after stimulation, compared to baseline, and to samples collected from animals receiving vehicle microinjections. Animals receiving vehicle microinjections showed no significant changes, compared to baseline.

Several studies have emphasized the importance of the MeA, the MPOA, and DA in the MPOA, in male sexual behavior. For example, electrical stimulation of the MPOA enhances copulation [25,31,38], whereas damage to the MPOA [7,19,24,32] or the MeA [9,12,14,20,22,23,28] impairs male sexual behavior. Damage to the MeA also inhibits non-contact erections, observed during exposure to an inaccessible female [21], and attenuates the enhancement of copulation, observed after precoital exposure to a female [8]; thus, the MeA is important for the assimilation of sexually relevant stimuli. After sexual activity, cellular activity, measured by Fos immunoreactivity, increases in the MeA and MPOA of male rodents [1,6,13,37,43]. A portion of androgen sensitive cells in the MeA that become activated after copulation also project to the MPOA [11]. Indeed, the MeA is a major site for the integration of sensory and hormonal influences [18,30,46].

Previously, we reported that MeA lesions impaired the mating-induced MPOA DA response and that microinjections of a DA agonist into the MPOA reversed the copulatory impairment caused by MeA lesions [9]. The present experiment showed that stimulation of the MeA increased DA release in the MPOA of male rats. Because there are no DA cells in the amygdala of rats [2], the increase in MPOA DA observed in this experiment likely results from stimulation of either cell bodies or axon terminals of periventricular DA cells that innervate the MPOA [26].

Extrinsic projections from the MeA are reported to be largely GABAergic [42]. However, in male gerbils, 20% of posterodorsal MeA neurons that were activated by ejaculation were glutamatergic, and a similar percentage of posterodorsal MeA neurons project to the MPOA [41]. Therefore, one possible pathway by which stimulation of the MeA might enhance MPOA DA is through direct glutamatergic innervation. Alternatively, because the MeA contains neurons that produce a variety of neurotransmitters and neuromodulators [30], and the MeA sends input to numerous regions other than the MPOA [5], it is possible that chemical stimulation of the MeA indirectly altered MPOA DA by acting on another region that also projects to the MPOA [40].

In this experiment, chemical stimulation was achieved by injecting glutamate+PDC into the MeA. It is possible that injections into the MeA spread to adjacent regions; however, examination of dye injected at the end of the experiment did not reveal spread beyond the intended region. Furthermore, earlier studies showed that 1-μl volume injections remained within ~1 mm of the injection site [29]. Because the present experiment used 0.5-μl volume injections, it is unlikely that the chemicals spread significantly beyond the MeA.

In summary, these results suggest that the MeA plays a role in the regulation of MPOA DA, whether through direct or indirect input. Furthermore, these results support the conclusion of an earlier study [9] that the female-stimulated increase in levels of MPOA DA is regulated by activity in the MeA.

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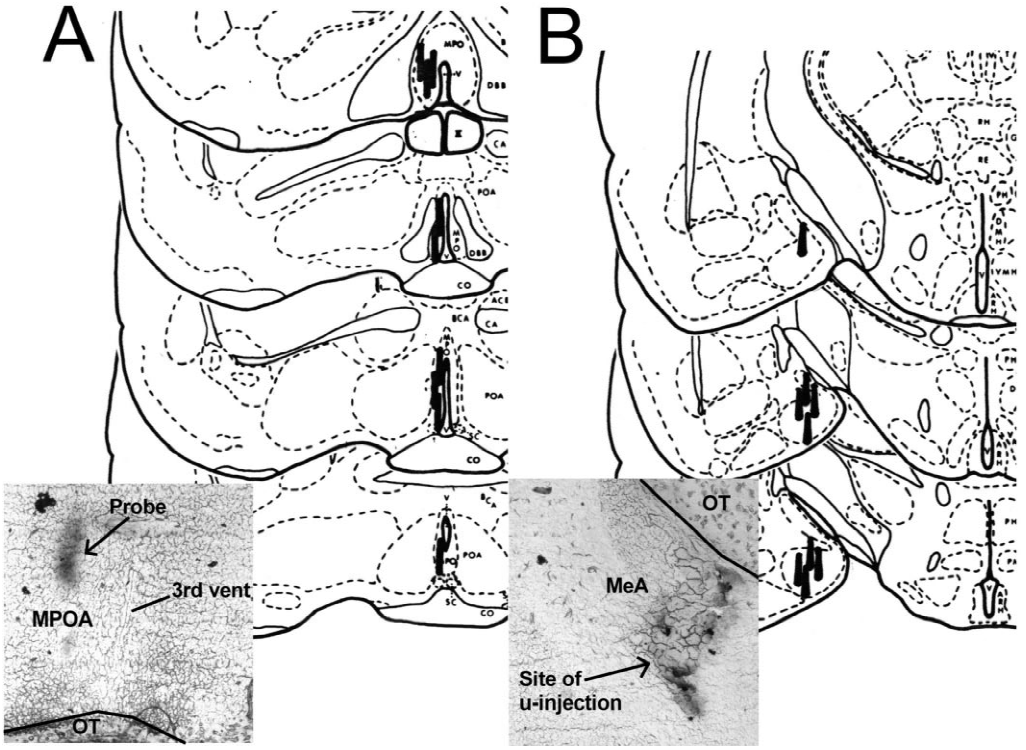

Fig. 2. (A,B) Representative marks of probe placements in the MPOA (left) and microinjection placement in the MeA (right). From bregma, coordinates for the MPOA schematic are (top to bottom) 2.4, 2.2, 2.0, 1.8 mm, and coordinates for the MeA schematic are −0.6, −0.8, −1.0 mm; sections were reproduced from Ref. [35].

References

[1] M.J. Baum, B.J. Everitt, Increased expression of c-fos in the medial preoptic area after mating in male rats: role of afferent inputs from the medial amygdala and midbrain central tegmental field, Neuroscience 50 (1992) 627–646.

[2] A. Bjorklund, O. Lindvall, Dopamine-containing systems in the CNS, in: A. Bjorklund, T. Hokfelt (Eds.), Classical Transmitters in the CNS, Part I, Handbook of Chemical Neuroanatomy, Elsevier, Amsterdam, 1984, pp. 55–122.

[3] J.R. Blackburn, J.G. Pfaus, A.G. Phillips, Dopamine functions in appetitive and defensive behaviors, Prog. Neurobiol. 39 (1992) 247–279.

[4] R.J. Bridges, M.S. Stanley, M.W. Anderson, C.W. Cotman, A.R. Chamberlin, Conformationally defined neurotransmitter analogues. Selective inhibition of glutamate uptake by one pyrrolidine-2,4-dicarboxylate diastereomer, J. Med. Chem. 34 (1991) 717–725.

[5] N.S. Canteras, R.B. Simerly, L.W. Swanson, Organization of projections from the medial nucleus of the amygdala – a PHAL study in the rat, J. Comp. Neurol. 360 (1995) 213–245.

[6] L.M. Coolen, H.J. Peters, J.G. Veening, Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison, Brain Res. 738 (1996) 67–82.

[7] F.H. de Jonge, A.L. Louwerse, M.P. Ooms, P. Evers, E. Endert, N.E. Van de Poll, Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats, Brain Res. Bull. 23 (1989) 483–492.

[8] F.H. de Jonge, W.P. Oldenburger, A.L. Louwerse, N.E. Van de Poll, Changes in male copulatory behavior after sexual exciting stimuli: effects of medial amygdala lesions, Physiol. Behav. 52 (1992) 327–332.

[9] J. Dominguez, J.V. Riolo, Z. Xu, E.M. Hull, Regulation by the medial amygdala of copulation and medial preoptic dopamine release, J. Neurosci. 21 (2001) 349–355.

[10] K.J. Field, W.J. White, C.M. Lang, Anaesthetic effects of chloral hydrate, pentobarbitone and urethane in adult male rats, Lab. Anim. 27 (1993) 258–269.

[11] B. Greco, D.A. Edwards, D. Zumpe, A.N. Clancy, Androgen receptor and mating-induced fos immunoreactivity are co-localized in limbic and midbrain neurons that project to the male rat medial preoptic area, Brain Res. 781 (1998) 15–24.

[12] V.S. Harris, B.D. Sachs, Copulatory behavior in male rats following amygdaloid lesions, Brain Res. 86 (1975) 514–518.

[13] M.M. Heeb, P. Yahr, c-Fos immunoreactivity in the sexually dimorphic area of the hypothalamus and related brain regions of male gerbils after exposure to sex-related stimuli or performance of specific sexual behaviors, Neuroscience 72 (1996) 1049–1071.

[14] M.M. Heeb, P. Yahr, Cell-body lesions of the posterodorsal preoptic nucleus or posterodorsal medial amygdala, but not the parvicellular subparafascicular thalamus, disrupt mating in male gerbils, Physiol. Behav. 68 (2000) 317–331.

[15] E.M. Hull, Dopaminergic influences on male rat sexual behavior, in: P.E. Micevych, R.P. Hammer Jr. (Eds.), Neurobiological Effects of Sex Steroid Hormones, Cambridge University Press, Cambridge, UK, 1995, pp. 234–253.

[16] E.M. Hull, D. Bitran, E.A. Pehek, R.K. Warner, L.C. Band, G.M. Holmes, Dopaminergic control of male sex behavior in rats: effects of an intracerebrally-infused agonist, Brain Res. 370 (1986) 73–81.

[17] E.M. Hull, J. Du, D.S. Lorrain, L. Matuszewich, Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation, J. Neurosci. 15 (1995) 7465–7471.

[18] E.M. Hull, R.L. Meisel, B.D. Sachs, Male sexual behavior, in: D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fahrbach, R.T. Rubin (Eds.), Hormones, Brain and Behavior, Academic Press, San Diego (in press).

[19] J.S. Klaric, S.E. Hendricks, Effects of two-stage lesions of the

medial preoptic area on sexual behavior of male rats, Physiol. Behav. 37 (1986) 539–542.
[20] Y. Kondo, Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats, Physiol. Behav. 51 (1992) 939–943.
[21] Y. Kondo, P.G. Jordan, B.D. Sachs, Small medial amygdala lesions prevent non-contact erection in rats without impairing copulation or partner preference, Soc. Neurosci. (Abstr.) 25 (1999) 345.
[22] Y. Kondo, K. Yamanouchi, The possible involvement of the non-strial pathway of the amygdala in neural control of sexual behavior in male rats, Brain Res. Bull. 38 (1995) 37–40.
[23] M.N. Lehman, S.S. Winans, J.B. Powers, Medial nucleus of the amygdala mediates chemosensory control of male hamster sexual behavior, Science 210 (1980) 557–560.
[24] Y.C. Liu, J.D. Salamone, B.D. Sachs, Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and non-contact erection in male rats, J. Neurosci. 17 (1997) 5245–5253.
[25] C.W. Malsbury, Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area, Physiol. Behav. 7 (1971) 797–805.
[26] K.E. Moore, K.J. Lookingland, Dopaminergic neuronal systems in the hypothalamus, in: F.E. Bloom, D.J. Kupfer (Eds.), Psychopharmacology: The Fourth Generation of Progress, Raven Press, New York, 1995, pp. 245–256.
[27] V.P. Markowski, R.C. Eaton, L.A. Lumley, J. Moses, E.M. Hull, A D1 agonist in the MPOA facilitates copulation in male rats, Pharmacol. Biochem. Behav. 47 (1994) 483–486.
[28] A. McGregor, J. Herbert, Differential effects of excitotoxic basolateral and corticomedial lesions of the amygdala on the behavioural and endocrine responses to either sexual or aggression-promoting stimuli in the male rat, Brain Res. 574 (1992) 9–20.
[29] R.D. Myers, in: Handbook of Drug and Chemical Stimulation of the Brain: Behavioral, Pharmacological and Physiological Aspects, Van Nostrand Reinhold, New York, 1974, pp. 59–63.
[30] S.W. Newman, The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network, Ann. NY Acad. Sci. 877 (1999) 242–257.
[31] R. Paredes, A.E. Haller, M.C. Manero, R. Alvarado, A. Agmo, Medial preoptic area kindling induces sexual behavior in sexually inactive male rats, Brain Res. 515 (1990) 20–26.
[32] R.G. Paredes, T. Tzschentke, N. Nakach, Lesions of the medial preoptic area/anterior hypothalamus (MPOA/AH) modify partner preference in male rats, Brain Res. 813 (1998) 1–8.
[33] E.A. Pehek, J.T. Thompson, E.M. Hull, The effects of intracranial administration of the dopamine agonist apomorphine on penile reflexes and seminal emission in the rat, Brain Res. 500 (1989) 325–332.
[34] E.A. Pehek, R.K. Warner, T.J. Bazzett, D. Bitran, L.C. Band, R.C. Eaton, E.M. Hull, Microinjection of cis-flupenthixol, a dopamine antagonist, into the medial preoptic area impairs sexual behavior of male rats, Brain Res. 443 (1988) 70–76.
[35] L.J. Pellegrino, A.S. Pellegrino, A.J. Cushman, A Stereotaxic Atlas of the Rat Brain, Plenum, New York, 1979.
[36] J.G. Pfaus, A.G. Phillips, Role of dopamine in anticipatory and consummatory aspects of sexual-behavior in the male rat, Behav. Neurosci. 105 (1991) 727–743.
[37] G.S. Robertson, J.G. Pfaus, L.J. Atkinson, H. Matsumura, A.G. Phillips, H.C. Fibiger, Sexual behavior increases c-fos expression in the forebrain of the male rat, Brain Res. 564 (1991) 352–357.
[38] G. Rodriguez-Manzo, F. Pellicer, K. Larsson, A. Fernandez-Guasti, Stimulation of the medial preoptic area facilitates sexual behavior but does not reverse sexual satiation, Behav. Neurosci. 114 (2000) 553–560.
[39] L.L. Scaletta, E.M. Hull, Systemic or intracranial apomorphine increases copulation in long-term castrated male rats, Pharmacol. Biochem. Behav. 37 (1990) 471–475.
[40] R.B. Simerly, L.W. Swanson, The organization of neural inputs to the medial preoptic nucleus of the rat, J. Comp. Neurol. 246 (1986) 312–342.
[41] D.A. Simmons, P. Yahr, Neurotransmitters/modulators and connections of cells in the posterodorsal preoptic nucleus (PdPN) and posterodorsal medial amygdala (MeApd) in relation to cells activated with ejaculation in male gerbils, Soc. Neurosci. (Abstr.) 25 (1999) 345.
[42] L.W. Swanson, G.D. Petrovich, What is the amygdala?, Trends Neurosci. 21 (1998) 323–331.
[43] J.G. Veening, L.M. Coolen, Neural activation following sexual behavior in the male and female rat brain, Behav. Brain Res. 92 (1998) 181–193.
[44] R.K. Warner, J.T. Thompson, V.P. Markowski, J.A. Loucks, T.J. Bazzett, R.C. Eaton, E.M. Hull, Microinjection of the dopamine antagonist cis-flupenthixol into the MPOA impairs copulation, penile reflexes and sexual motivation in male rats, Brain Res. 540 (1991) 177–182.
[45] H.B. Waynforth, P.A. Flecknell, in: Experimental and Surgical Technique in the Rat, Academic Press, New York, 1992, p. 132.
[46] R.I. Wood, Thinking about networks in the control of male hamster sexual behavior, Horm. Behav. 32 (1997) 40–45.