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Effects of acute and chronic apomorphine on sex behavior and copulation-induced neural activation in the male rat

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Abstract

Apomorphine is a non-selective dopaminergic receptor agonist. Because of its pro-erectile effects, apomorphine is clinically used for treatment of erectile dysfunction. We investigated the effects of subcutaneous apomorphine administration (0.4 mg/kg rat) on sexual behavior and mating-induced Fos-expression following acute (day 1) or chronic apomorphine treatment (days 8 and 15) in sexually experienced male rats. Consistent facilitatory effects of apomorphine were observed in the reduced numbers of mounts and intromissions over time and an increased ejaculation frequency on day 1. The first post-ejaculatory interval, however, was lengthened, while other behavioral parameters were unaffected. Fos-immunoreactivity induced by acute apomorphine administration (barrel cortex, paraventricular hypothalamic nucleus, central amygdala and locus coeruleus) was strongly reduced after chronic administration. After mating, induction of Fos-immunoreactivity was observed in well-known areas like medial preoptic nucleus and the posterodorsal medial amygdaloid area. Apomorphine, however, reduced mating-induced Fos-immunoreactivity in the nucleus accumbens shell and prevented its occurrence in its core area. This remarkable apomorphine effect was not observed in any other brain area. We conclude that the behavioral (pro-erectile) effects of apomorphine are consistent over time, and that the diminished accumbens-Fos-immunoreactivity and the elongated post-ejaculatory interval may reflect a decreased response to remote cues from the estrus female.
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1. Introduction

Male sexual behavior is a complex phenomenon that depends on intrinsic and extrinsic factors, including hormonal state and olfactory, visual and somatosensory cues. The neural circuitry underlying this behavior has been investigated for many years, especially in the rat. Therefore, many data are available about the involvement of specific areas in the brain and spinal cord and of specific neurotransmitters, neuropeptides or receptors, that play a role in sexual behavior of the male rat (Hull et al., 2004; Meisel, 1994).

Over the last decade, studies using Fos as a marker of activation (Curran and Morgan, 1995; Hoffman and Lyo, 2002; Morgan and Curran, 1991), provided extensive information about the involvement of the brainstem, the caudal thalamus, the amygdala, the bed nucleus of the stria terminalis, and the medial preoptic area in ejaculation, as well as in preceding elements of male sexual

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behavior in the rat (Baum and Everitt, 1992; Baum and Wersinger, 1993; Coolen et al., 1996, 1997a,b, 1998; Heeb and Yahr, 1996; Kollack and Newman, 1992; Pfaus et al., 1993; Veening and Coolen, 1998; Veening et al., 2005). Specific neuropeptides like Galanin, cholecystokinin and Substance P play a role in this neural circuitry (Coolen et al., 2003a,b; Polston and Simerly, 2003). In addition, serotonin and dopamine also play a role in male sexual behavior. A single intraperitoneal injection of the 5-HT_{1A} receptoragonist 8-hydroxy-2-(di-*n*-propylaminotetralin) (8-OH-DPAT) induces an almost instantaneous ejaculation in the male rat (Ahlenius et al., 1981; Coolen et al., 1997b), while administration of selective serotonin reuptake inhibitors may induce a delayed ejaculation, particularly after chronic administration (Mos et al., 1999; Waldinger et al., 2002). Selective serotonin reuptake inhibitors are clinically applied successfully for treatment of premature ejaculation (Waldinger et al., 2003, 1998).

Dopamine is one of the main neurotransmitters involved in the central control of erectile functions, genital reflexes and male sexual behavior (Andersen et al., 2003; Argiolas and Melis, 2005; Giuliano and Rampin, 2000a,b; Giuliano and Allard, 2001; Heaton, 2000; Hull et al., 1986; Martino et al., 2005; Melis and Argiolas, 1995; Melis et al., 2006; Moses et al., 1995; Paredes and Agmo, 2004; Pehek et al., 1989; Succu et al., 2007). Apparently, the paraventricular hypothalamic nucleus, especially its oxytocinergic neurons, play an eminent role in the control of erectile function (Argiolas and Melis, 2004, 2005; Martino et al., 2005; Succu et al., 2007). Extracellular dopamine levels increase in the medial preoptic area (Hull et al., 1993), nucleus accumbens (Pfaus et al., 1990; Pleim et al., 1990) and in the paraventricular hypothalamic nucleus (Melis et al., 2003) during sexual activity. The increased dopaminergic transmission in the nucleus accumbens appears to be controlled by lateral hypothalamic serotonin activity (Hull et al., 2004; Lorrain et al., 1999), and in the medial preoptic nucleus by activity of the medial amygdala (Dominguez et al., 2001). In addition to forebrain control, spinal dopaminergic mechanisms may play a role as well (Giuliano et al., 2002).

Apomorphine is a mixed dopamine receptor agonist ($D_2 > D_1 > D_4$), inducing changes in male sexual behavior in the rat (Bitran and Hull, 1987; Giuliano et al., 2002; Hsieh et al., 2004; Hull et al., 1986; Malmnas, 1973; Paglietti et al., 1978; Pehek et al., 1989; Tagliamonte et al., 1974) and mice (Rampin et al., 2003). In reviewing the available data, Melis and Argiolas (1995), described the changes observed as indicative for a lowered ejaculatory threshold. In a recent review, Paredes and Agmo (2004) suggested that the observed behavioral effects of apomorphine are reliably obtained only in animals with low sexual activity, like castrated or inexperienced animals.

Apomorphine attracts more attention than other dopaminergic agonists, because of its potential clinical applications. A down-regulating effect on neural activity in frontal limbic areas has been shown in a recent functional-Magnetic-Resonance-Imaging study (Montorsi et al., 2003). Subcutaneous injections of low doses of apomorphine (0.25–0.75 mg s.c.) elicited penile erections in healthy subjects without sexual stimulation (Lal et al., 1984). At a higher dose (2–4 mg), apomorphine can induce penile erection in men with erectile dysfunction (Heaton, 2000). Because of this pro-erectile effect, apomorphine, sublingual, has recently been approved and introduced for the treatment of erectile dysfunction in men. Apomorphine is used as on-demand treatment but it is unknown whether repeated treatment affects the efficacy to induce prosexual effects.

In view of these clinical applications, two important questions arose about the effects of apomorphine treatment on male sexual behavior. First: are the observed behavioral effects and the underlying changes in neural activity consistent over time? If not, what are the changes? Second: which brain areas are most likely involved in the effects of apomorphine on sexual behavior? To answer these questions, the effects of acute and chronic administration of apomorphine a): on sexual behavior of the male rat; b): on neural activation of fore- and midbrain areas in these rats, were investigated.

2. Materials and methods

2.1. Experimental animals

Adult male (400–500 g, $n=60$) and female (200–300 g, $n=80$) Wistar rats (Harlan, Horst, The Netherlands) were used. Males were housed individually and females were housed in pairs (macrolon cage 3, with B03 bedding) under a controlled 12-h/12-h reversed light/dark cycle with a dim red light switched on during the dark period (7 AM lights off, 7 PM lights on). Rats had free access to water and food. The animal room was held under controlled temperature (20–22 °C) and humidity (50–60%) conditions. The females were used as stimulus rats and were sterilized by ligation of the oviducts. Their sexual receptivity was reliably induced by a subcutaneous injection of 50 μg estradiol maleate/0.1 ml sesame oil, saturated with lecithin, 36 h prior to each sexual behavior test. Only 'proven' lordotic females served to test the copulatory activities of each experimental group. Occasionally, the same female was used for 2 different males on the experimental days.

The experiment was approved by the animal ethics committee of the Central Animal Laboratory, Radboud University of Nijmegen, The Netherlands, and performed in accordance with the national and NIH-guidelines for animal care and welfare and with the ECC Directive.

2.2. Experimental procedures

All mating sessions were performed between 2 and 8 h after onset of the dark period in a dark room under dim red light. The male rats were placed in the mating cage (a wooden box (60 × 40 × 30 cm) with a Plexiglas front window and soft bedding) and allowed to adapt to the environment for 10 min before a receptive female was introduced. Between the mating sessions, the cage was not cleaned in order to stabilise the test environment, as a familiar area rich with sex-related odors and to minimise variability in behavioral correlates as well as in Fos-induction.

Prior to the experiment, the male rats were handled every day for 4 weeks. In addition, the sexual behavior of all male rats was repeatedly tested to provide sexual experiences and to monitor their performance. During four training sessions of 30 min each, male rats were allowed to copulate with a receptive female.

Male rats that displayed at least two ejaculations per mating session during the last training session were included in the final experiment. Rats with very high (≥4) and very low (≤1) scores of ejaculations during the training sessions were excluded from further participation, because we potentially are interested in the possibility that apomorphine treatment may have both stimulating and inhibitory effects on certain aspects of male sexual behavior and therefore we selected 'normal' performing animals (see Pattij et al., 2005).

A total of 48 male rats were included and randomly allocated in two groups: 24 animals underwent the sexual behavior tests and 24 animals remained always in the home-cage. Each of the two groups (N=24) was further (sub)divided in four treatment groups: acute saline (n=4), chronic saline (n=8), acute apomorphine (n=4) and chronic apomorphine (n=8). See Table 1 for an overview of the experimental design and subdivision in groups. On day 1 of the experiment, all rats received a subcutaneous injection with either apomorphine dissolved in saline with 0.1% vitamin C (apomorphine 0.4 mg/kg (Sigma) or vehicle (saline with 0.1% vitamin C). This dose of 0.4 mg/kg apomorphine was selected on the basis of preceding experience with the behavioral effects of apomorphine on male sexual behavior.

Four rats received an apomorphine-injection prior to each sexual behavior test: two rats were immediately placed in the mating box and allowed to adapt to the environment for 10 min prior to the sexual behavior test, the other two were immediately returned to their home-cage. Individuals from the four treatment groups were injected and/or tested alternatively.

During the ensuing 30-min behavioral test the mounts (M), intromissions (I) and ejaculations (E) of the male rats were scored with a custom-event recording software program (Observer, Noldus BV, Wageningen, The Netherlands). Afterwards the ejaculation frequency (EF, total number of ejaculations) was determined, as well as the mount frequency (MF, number of mounts prior to an ejaculation) and intromission frequency (IF, number of intromissions prior to an ejaculation) for the first two ejaculations. In addition, the ejaculation latency (EL: time from the first M or I until E), the post-ejaculatory interval (PEI: time from an E until the first M or I of the next cycle) and the copulation efficiency (CE: IF/(IF+MF) were calculated for the first two ejaculations. Finally, the total number of mounts and intromissions throughout the 30-min sexual behavior test was determined.

Exactly 1 h and 40 min after the injections (1 h after the sexual behavior test) on day 1, the rats in the acute treatment groups (n=4) that underwent either the sexual behavior test or remained in the home-cage, were sacrificed and processed for immunohistochemical staining. The remaining 'chronic' rats (n=8) were injected with vehicle or apomorphine between 4 PM and 5 PM on days 2 to 7 and days 9 to 14. On day 8 and 15, the rats were injected immediately prior to the sexual behavior test. After the sexual behavior test on day 15, all remaining rats (n=8 per group) including the home-cage controls were sacrificed 1 h and 40 min after the injections (1 h after the sexual behavior test) and processed for immunohistochemical staining.

2.3. Immunohistochemistry

After the final behavioral test, the animals were deeply anesthetized, using sodium pentobarbital (Nembutal®, Ceva Santa Animale BV, Maassluis, NL, 60 mg/ml, 0.2 ml/100 g intraperitoneally). Then they were perfused transcardially with Tyrode calcium-free, pH 7.3, followed by 400 ml 4% paraformaldehyde dissolved in 0.1 M PBS, pH 7.2. Subsequently, the brains were removed from the skull and postfixed overnight at 4 °C. Before sectioning, the brains were cryoprotected with a 30% sucrose solution.

Brain sections were cut on a freezing microtome, thickness 35 μm, and collected in 6 parallel series in 0.1 M PBS

Table 1
Distribution of animals over experimental groups

4 weeks of handling and 4 times sexual performance testing				N=60
Number of experimental animals (after excluding the fastest and the slowest ejaculators)				N=48
'Home-cage animals', n=24			'Sex-performing animals', n=24	
Day	APO: n=12	Vehicle: n=12	APO: n=12	Vehicle: n=12
Day 1	'acute APO': Injection: n=12; +perfusion/Fos: n=4 [a];	'acute VEH': Injection: n=12; +perfusion/Fos: n=4 [a];	'acute APO-SEX': Injection: n=12; SEX+perfusion/Fos: n=4 [a];	'acute VEH-SEX': Injection: n=12; SEX+perfusion/Fos: n=4 [a];
Days 2–7	Daily APO-injections; n=8 [b]	Daily VEH-injections: n=8 [b]	Daily APO-Injections; n=8 [b]	Daily VEH-injections; n=8 [b]
Day 8	'Chronic APO': n=8 [b];	'Chronic VEH': n=8 [b];	'Chronic APO-SEX' +SEX: n=8 [b];	'Chronic VEH-SEX' +SEX: n=8 [b];
Days 9–14	Daily APO-injections; n=8 [b]	Daily VEH-injections; n=8 [b]	Daily APO-injections; n=8 [b]	Daily VEH-injections; n=8 [b]
Day 15	'Chronic APO': n=8 [b]; +perfusion/Fos: n=5 [c];	'Chronic VEH': n=8 [b]; +perfusion/Fos: n=4 [c];	'Chronic APO-SEX': n=8 [b]; SEX+perfusion/Fos: n=5 [c];	'Chronic VEH-SEX': n=8 [b]; SEX+perfusion/Fos: n=5 [c];

[a] From each n=12 group, 4 animals were perfused for Fos-IR on day 1.
[b] Animals in n=8 groups were identical from days 2 to 15.
[c] From each n=8 group, 5 (4) animals were perfused for Fos-IR on day 15.

containing 0.1% azide. One series of each individual was used for the staining. The free-floating sections were washed three times in PBS and preincubated with 1% perhydrol (30% H_2O_2, Merck) for 30 min. The sections were soaked for 30 min in: PBS containing 0.1% bovine serum albumin and 0.5% Triton X-100. Then the sections were incubated overnight at room temperature, on a shaker with a polyclonal anti-Fos antiserum raised in rabbit (cat# SC-052, lot# F277, Santa Cruz Biotechnology), diluted 1:10,000 in the incubation medium. The sections were incubated for 90 min at room temperature in donkey anti-rabbit (1:1500 in incubation medium, Jackson ImmunoResearch Laboratories, West Grove, PA) and for 90 min at room temperature in ABC-elite (Vector elite 1:800 in PBS). In between incubations, sections were rinsed three times with PBS. The Fos-antibody peroxidase complex was visualized by 3,3′-diaminobenzidine tetrahydrochloride (DAB) staining. Sections were incubated for 10 min in a chromogen solution consisting of 0.02% DAB and 0.03% Ni-ammonium sulfate in 0.05 M Tris–buffer (pH 7.6), and subsequently for 10 min in chromogen solution containing 30% hydrogen peroxide. This resulted in a blue-black staining. Following the immunocytochemical staining procedures, the sections were rinsed three times in PBS and mounted on gelatin chrome alum-coated glass slides, dried overnight, cleared in xylene, embedded with Entellan (Merck), and coverslipped.

2.4. Quantification procedures

Fos-immunoreactivity in the forebrain and brainstem was extensively studied in the brain slices of every treatment group, in order to detect brain areas showing changes in Fos-immunoreactivity possibly induced by acute or chronic treatment with apomorphine and/or sexual behavior. Eventually, based on these initial observations and on the current knowledge of the involvement of several brain areas in copulatory behavior, twelve brain areas were selected for quantification and statistical analysis. Immunoreactive cell nuclei were quantified using the software program Neurolucida (Brightfield, USA). A grid overlay was applied in order to count Fos-immunoreactivity nuclei in a fixed number of squares per area, in the center region of the activated brain areas (Coolen et al., 1997a). The number of Fos-positive nuclei were counted in the barrel cortex (3 squares of 70×70 μm), the deep (layers 5+6) and superficial (layers 2+3) layers of the prelimbic cortex (both 200×200 μm), the infralimbic cortex (200×200 μm), the core and the shell of the nucleus accumbens (both 200×200 μm), the medial preoptic area (300×300 μm), the medial parvocellular paraventricular hypothalamic nucleus (200×200 μm), the posterodorsal medial amygdala (3 squares of 100×100 μm), the central amygdala (200×200 μm), the dorsomedial hypothalamic nucleus (400×400 μm) and the locus coeruleus (200×200 μm).

2.5. Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences version 12.0.1 for windows (SPSS Inc., Chicago, IL, USA).

The majority of the behavioral data was not normally distributed and was therefore analyzed with non-parametric tests. To analyze the effect of the treatment (two groups: saline and apomorphine treated) we used the non-parametric Mann–Whitney test. To analyze within one group if there is a difference in the duration of the treatment (three groups: 1, 8 or 15 days of treatment) we used the non-parametric Friedman test, when a significant difference was found ($P<0.05$) data were further analyzed with the non-parametric Wilcoxon test.

The majority of the anatomical data was not normally distributed and was also analyzed with non-parametric tests. Overall significant differences were determined with the Kruskall–Wallis test, differences between treatment groups were further determined with the Mann–Whitney test.

In each test, a level of $P<0.05$ was considered statistically significant.

3. Results

3.1. Sexual behavior

The effects of acute and chronic treatment with apomorphine on sexual behavior of male rats were measured on day 1, day 8, and day 15 of the treatment (Fig. 1 and Tables 2, 3).

Analysis with the Mann–Whitney test showed that apomorphine decreased the total numbers of mounts (Fig. 1A) in the 30-min sexual behavior test both acutely ($Z=-3.587$; $P<0.001$) and after 1 week ($Z=-2.555$; $P<0.011$) and 2 weeks of treatment ($Z=-3.048$; $P<0.002$) compared to saline. The mount frequency prior to the first ejaculation (Fig. 1B) was reduced by acute ($Z=-2.742$; $P<0.006$) and after 1 week ($Z=-2.047$; $P<0.041$), but not after 2 weeks of treatment with apomorphine. In addition, a significant difference was found in the mount frequency within the apomorphine-treated group ($\chi^2=8.000$; $P<0.018$): rats treated acutely with apomorphine showed significant less mounts prior to the first ejaculation compared to rats treated with apomorphine for 8 days ($Z=-2.384$; $P<0.017$).

The total number of intromissions (Fig. 1C) in the 30-min sexual behavior test was not changed after apomorphine treatment. The intromission frequency prior to the first ejaculation (Fig. 1D) was reduced on day 1 ($Z=-2.396$; $P<0.017$) and day 15 ($Z=-2.754$; $P<0.006$).

Acute, but not chronic apomorphine treatment increased the number of ejaculations ($Z=-2.058$; $P<0.04$) in the 30-min sexual behavior test (Fig. 1E).

Treatment with apomorphine lengthened the first post-ejaculatory interval (Fig. 1F) on day 8 ($Z=-2.364$; $P<0.018$) and day 15 ($Z=-3.151$; $P<0.002$), but not on day 1. Differences in the post-ejaculatory interval were found within the saline treated group ($\chi^2=6.000$; $P<0.05$): the post-ejaculatory interval was significantly higher on day 8 ($Z=-2.201$; $P<0.028$) compared to day 15 in the saline treated animals.

In addition, apomorphine had some effects on the second ejaculatory cycle (Table 3). Acute treatment with apomorphine reduced the ejaculation latency ($Z=-2.487$; $P<0.013$) and the mount frequency ($Z=-2.899$; $P<0.004$) and

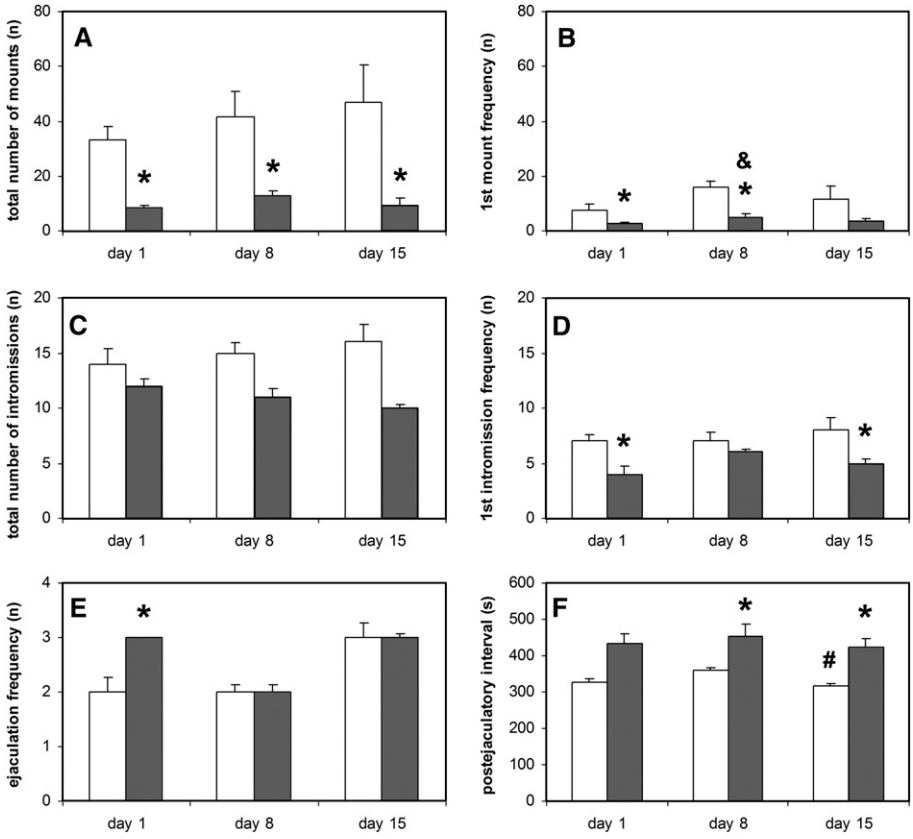

Fig. 1. The effects of acute and chronic treatment with saline (white bars) or apomorphine (0.4 mg/kg s.c., black bars) on the total number of mounts (A) and intromissions (C), the mount (B) and intromission (D) frequency prior to the first ejaculation, the number of ejaculations (E) and the first post-ejaculatory interval (F) of male rats in a 30-min sexual behavior test with a receptive female. Data are medians ± standard error of the median; * = different from saline, & = different from day 1, # = different from day 8 ($P<0.05$). Groupsize: Day 1: $N=12$; Days 8 and 15: $N=8$. Acute = 40 min after the first apomorphine or Saline injection; Chronic = after 7 or 14 preceding days with daily treatment with either apomorphine or saline.

increased the copulation efficiency ($Z=-2.779$; $P<0.005$) in the second ejaculatory cycle compared to saline. Moreover, apomorphine reduced the intromission frequency prior to the second ejaculation after 15 days of treatment ($Z=-2.164$; $P<0.03$).

Table 2
The effects of 1, 8 and 15 days of treatment with apomorphine (0.4 mg/kg sc) or saline on the sexual behavior of male Wistar rats in a 30-min test with a receptive female

Day	Parameter	Saline	Apomorphine
1	M (tot)	33.00±5.12	8.50±0.81 [a]
	I (tot)	14.00±1.35	12.00±0.65
	EF	2.00±0.27	3.00±0.00 [a]
8	M (tot)	41.50±9.43	13.00±1.55 [a]
	I (tot)	15.00±0.92	11.00±0.71
	EF	2.00±0.13	2.00±0.14
15	M (tot)	47.00±13.75	9.50±2.44 [a]
	I (tot)	16.00±1.55	10.00±0.33
	EF	3.00±0.26	3.00±0.07

Data are medians±standard error of the median.
Groupsize: Day 1 (acute) $N=4$; Days 8 and 15 (chronic) $N=8$.
M (tot) = total number of mounts; I (tot) = total number of intromissions; EF = ejaculation frequency.
[a] Different from saline ($P<0.05$).

3.2. Fos-immunoreactivity

The effects of acute and chronic (15-day) treatment with apomorphine compared to saline on the number and pattern of Fos-immunoreactive neurons in the central nervous system were analyzed. All four experimental groups were further divided in two subgroups that either remained in the home-cage or underwent the 30 min sexual behavior test.

The Kruskall–Wallis test revealed overall significant differences in all quantified brain areas: the barrel cortex ($\chi^2=20.005$; $P=0.006$), superficial ($\chi^2=27.503$; $P<0.001$) and deep ($\chi^2=24.939$; $P=0.001$) layers of the prelimbic cortex, infralimbic cortex ($\chi^2=25.970$; $P=0.001$), nucleus accumbens core ($\chi^2=21.901$; $P=0.003$) and shell ($\chi^2=28.009$; $P<0.001$), medial preoptic area ($\chi^2=27.616$; $P<0.001$), medial parvocellular paraventricular hypothalamic nucleus ($\chi^2=28.005$; $P<0.001$), posterodorsal medial amygdala ($\chi^2=26.826$; $P<0.001$), central amygdala ($\chi^2=22.586$; $P=0.002$), dorsomedial hypothalamic nucleus ($\chi^2=24.113$; $P=0.001$) and locus coeruleus ($\chi^2=26.568$; $P<0.001$).

3.2.1. Hypothalamus, amygdala and brainstem

Acute injection with apomorphine in the 'no-sex' group caused a sharp increase in Fos-positive cells compared to saline

Table 3
The effects of 1, 8 and 15 days of treatment with apomorphine (0.4 mg/kg sc) or saline on the sexual behavior of male Wistar rats in a 30-min test with a receptive female

Day	Parameter	1st cycle		2nd cycle	
		Saline	Apomorphine	Saline	Apomorphine
1	MF	7.50±2.05	2.50±0.48[a]	13.50±5.04	2.00±0.54[a]
	IF	7.00±0.59	4.00±0.75[a]	4.00±0.37	3.50±0.22
	CE	46.15±4.73	60.00±3.02	20.49±8.70	66.07±8.21[a]
	EL	302.42±65.89	309.23±48.28	342.43±90.40	210.75±7.43[a]
	PEI	327.33±9.49	432.05±29.47	457.53±21.05	416.91±20.88
8	MF	16.00±2.26	5.00±1.13[a]	10.50±5.33	7.00±2.00
	IF	7.00±0.85	6.00±0.28	4.00±0.23	3.00±0.67
	CE	36.84±5.36	54.55±4.20	29.29±5.92	33.33±4.29
	EL	482.85±78.83	465.99±118.30	289.24±85.85	319.44±32.52
	PEI	360.97±5.27	455.00±33.00[a]	–	–
15	MF	11.50±4.75	3.50±0.73	15.00±5.36	3.00±0.99
	IF	8.00±1.12	5.00±0.33[a]	4.00±0.14	3.00±0.14[a]
	CE	43.36±11.88	52.27±4.66	21.05±14.87	50.00±8.38
	EL	370.36±63.89	300.37±33.67	215.20±38.10	171.76±24.24
	PEI	315.93±7.87	421.97±23.60[a]	417.49±8.27	442.62±20.35

Data are medians±standard error of the median; [a] = different from saline ($P<0.05$).
Groupsize: Day 1 (acute) $N=4$; Days 8 and 15 (chronic) $N=8$.
MF = mount frequency; IF = intromission frequency.
CE = copulation efficiency: IF/(IF+MF).
EL = ejaculation latency (time from the first M or I).
PEI = post-ejaculatory interval (time between E and first M or I of the next cycle).
Each cycle comprises all copulatory activities between the first M or I and ejaculation.
The next cycle starts with the next series of copulatory activities.

in the medial parvocellular paraventricular hypothalamic nucleus ($P=0.021$; Fig. 2A), locus coeruleus ($P=0.018$; Fig. 2B) and the dorsomedial hypothalamic nucleus ($P=0.020$; Fig. 2C), which was similar to the Fos-immunoreactivity induced by sexual behavior in all experimental groups in these areas ($P<0.05$). In the central amygdala (Fig. 2D), acute injection with apomorphine strongly induced Fos-expression in both non-sex ($P=0.020$) and sex groups ($P=0.038$). Although sexual behavior in general did not alter Fos-immunoreactivity in this area, an increase was visible in rats treated chronically with vehicle ($P=0.019$).

In the non-sex groups, chronic apomorphine treatment increased Fos-immunoreactivity in the locus coeruleus that was both different from chronic saline treatment ($P=0.042$) and acute injection with apomorphine ($P=0.008$). Chronic treatment with apomorphine failed to increase the number of Fos-positive cells compared to saline in the dorsal medial hypothalamic nucleus ($P=0.806$), the paraventricular hypothalamic nucleus ($P=0.106$) and central amygdala ($P=0.081$). In the latter two areas, this resulted in a significant difference between acute and chronic apomorphine treatment (paraventricular hypothalamic nucleus: $P=0.008$; central amygdala: $P=0.014$).

In the medial preoptic area (Fig. 2E) and the posterodorsal medial amygdala (Fig. 2F), two areas known to be activated by sexual behavior, Fos-immunoreactivity was indeed higher in the sex-group compared to the non-sex group ($P<0.05$), independent of drug-treatment.

3.2.2. Cortical areas

Acute injection with apomorphine caused a sharp increase in Fos-positive cells compared to saline in the barrel cortex ($P=0.043$, Fig. 3A), and a similar difference was found with chronically treated rats ($P=0.014$).

In the infralimbic cortex (Fig. 3B), chronic treatment with saline caused a reduction in Fos-expression compared to acute injection with saline in home-cage controls ($P=0.032$). In both the infralimbic cortex and the superficial layer of the prelimbic cortex (Fig. 3D), sexual behavior increased Fos-expression in all treatment groups ($P<0.05$) except the acute saline group ($P>0.05$). In the superficial prelimbic cortex, chronic treatment with apomorphine attenuated the sex-induced increase in Fos-inmmunoreactivity compared to both acute injection with apomorphine ($P=0.019$) and chronic saline-treatment ($P=0.012$).

In the deep layer of the prelimbic cortex (Fig. 3C), sexual behavior increased Fos-immunoreactivity compared to home-cage behavior in all treatment groups ($P<0.05$) except the acute apomorphine group ($P=0.149$).

3.2.3. Nucleus accumbens

A very interesting pattern of Fos-immunoreactivity was visible in the nucleus accumbens core (Fig. 3E) and shell (Fig. 3F). In the core, acute ($P=0.020$) and chronic ($P=0.045$) treatment with apomorphine strongly reduced Fos-immunoreactivity in rats that underwent the sexual behavior test, but not in home-cage controls. In the shell, the number of Fos-positive cells was obviously reduced in response to acute ($P=0.041$) and chronic ($P=0.008$) apomorphine treatment in home-cage controls, but not in rats that performed sexual behavior. Furthermore, sexual behavior increased Fos-expression compared to home-cage behavior in the nucleus accumbens shell of rats treated acutely ($P=0.042$) and chronically ($P=0.004$) with apomorphine. Chronic treatment with saline caused a marked increase in Fos-expression compared

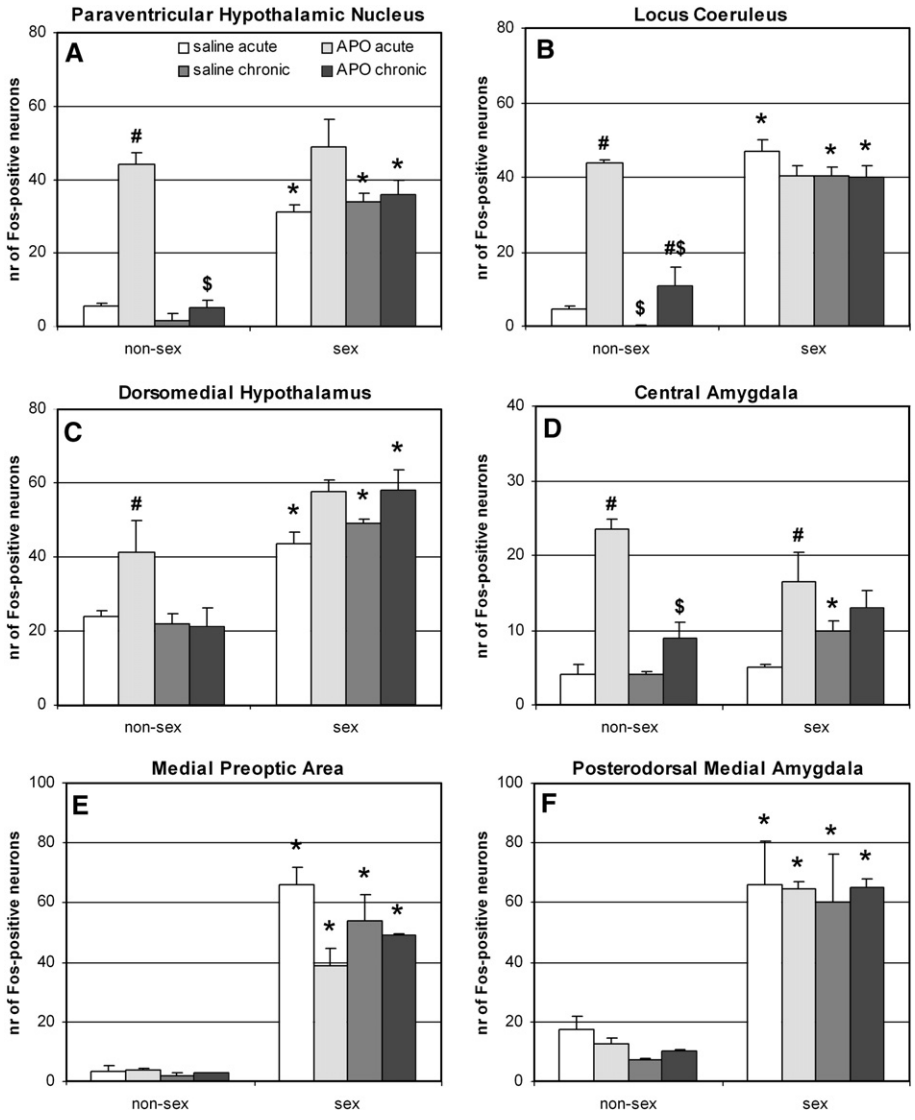

Fig. 2. Effects of acute or chronic treatment of saline or apomorphine (0.4 mg/kg s.c.) on the number of Fos-positive cells in the medial parvocellular paraventricular hypothalamic nucleus (A), locus coeruleus (B), dorsomedial hypothalamic nucleus (C) central amygdala (D), medial preoptic area (E) and posterodorsal medial amygdala (F) of male rats that remained in the home-cage ("non-sex") or underwent a 30-min sexual behavior test with a receptive female ("sex"). Data are medians± standard error of the median; # = different from saline, $ = different from the corresponding acute treatment group and * = different from the corresponding non-sex group ($P<0.05$).

to acute injection with saline ($P=0.014$) as well as chronic treatment with apomorphine ($P=0.009$) in the shell of rats that underwent sexual behavior.

4. Discussion

The possible effects of apomorphine administration on brain activity are complex and may originate from many different parts of the central nervous system. Adding to the complexity, apomorphine, as a non-selective dopaminergic receptor agonist, affects several types of dopaminergic receptors among them D_1, D_2 and D_4 (Brioni and Moreland, 2006; Brioni et al., 2004; Melis et al., 2005, 2006; Schechter and Greer, 1987), whereas chronic treatment with apomorphine may change the balance between dopamine D_1 and D_2 receptors, favoring the D_1 type (Acerbo et al., 2005).

It is, therefore, hardly surprising that the effects of apomorphine on male sexual behavior are complicated as well, because many different areas in the central nervous system, involved in very different functions, varying from erectile effects and appetitive or motivational changes to (post) ejaculatory effects, are affected at the same time. Adding to the complexity are the findings that the effects on dopamine D_1 vs D_2 receptors may change depending on the dose of apomorphine (Castagna et al., 1997; Hull et al., 2004; Moses et al., 1995). The complex role of dopamine in male sexual behavior has been discussed extensively in several recent reviews (Argiolas and Melis, 2005; Giuliano and Allard, 2001; Hull et al., 2004; Melis and Argiolas, 1995; Paredes and Agmo, 2004).

In view of the complexity of the central effects, it is, in fact, surprising to observe such limited and circumscribed behavioral

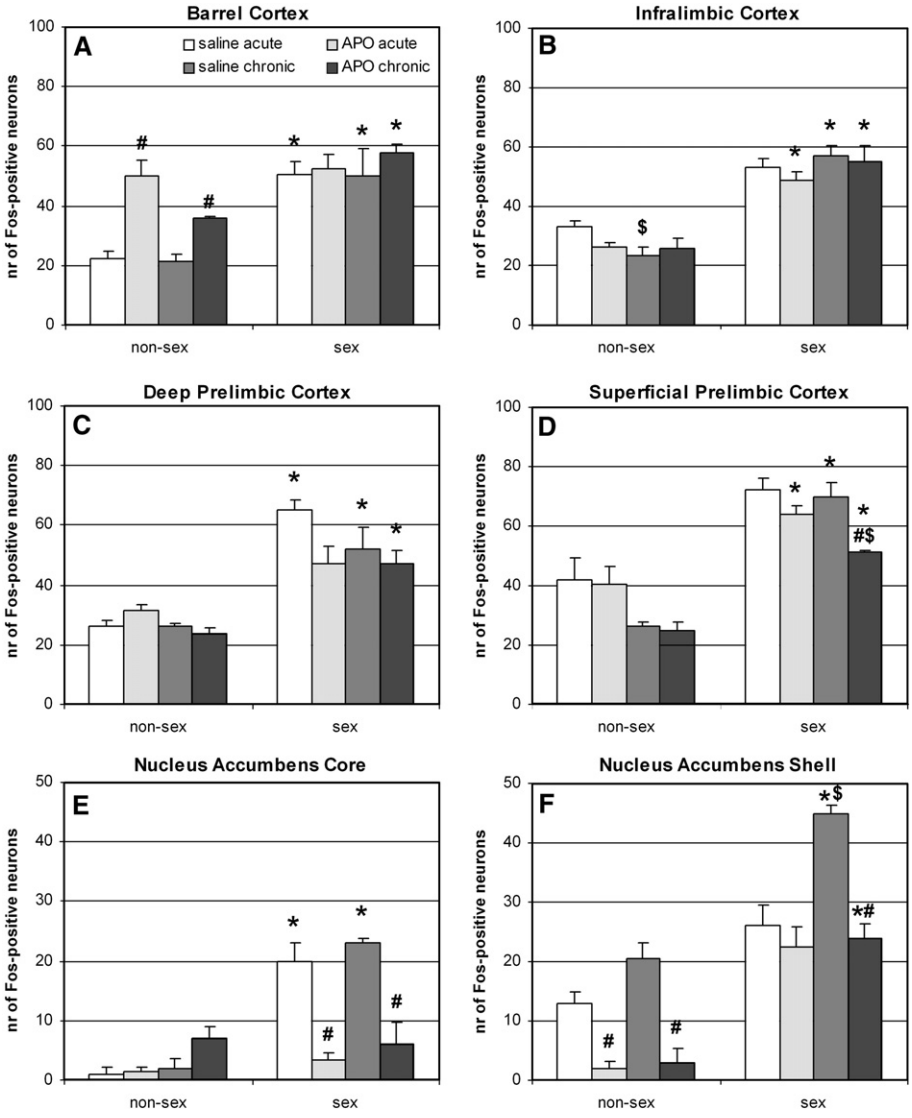

Fig. 3. Effects of acute or chronic treatment of saline or apomorphine (0.4 mg/kg s.c.) on the number of Fos-positive cells in the barrel cortex (A), infralimbic cortex (B), deep and superficial layers of the prelimbic cortex (C and D) and the nucleus accumbens core and shell (E and F) of male rats that remained in the home-cage ("non-sex") or underwent a 30-min sexual behavior test with a receptive female ("sex"). Data are medians ± standard error of the median; # = different from saline, $ = different from the corresponding acute treatment group and * = different from the corresponding non-sex group ($P<0.05$).

effects after apomorphine administration, as observed in the present study. In the following discussion, these behavioral effects will be discussed in relation with the possible involvement of the brain areas affected, showing an increase in neural activity.

4.1. Behavioral effects of apomorphine

In the present study, consistent and sustained changes in sexual behavior were observed: apomorphine-treated animals showed only about 25% of the numbers of mounts compared to vehicle treated controls. The changes in the number of intromissions, preceding the ejaculation, were weaker but showed a similar tendency to about 2/3 of control values. The ejaculation frequency, however, was not permanently influenced, since only the first apomorphine administration induced a significant increase. The elongated first post-ejaculatory interval in apomorphine-treated animals had a duration normally observed for the second and later post-ejaculatory intervals in control animals. The balance between a reduced amount of pre-ejaculatory behavior and an extended succeeding post-ejaculatory interval may have kept the ejaculation frequency basically unchanged.

The present study shows that the observed behavioral changes are consistent over time and do not disappear under conditions of chronic administration of apomorphine, except for the increased ejaculation frequency, that was observed only after acute administration. The size and direction of the acute behavioral changes are in agreement with previous reports, as observed in rats (Bitran and Hull, 1987; Hsieh et al., 2004; Hull et al., 1986; Paglietti et al., 1978; Tagliamonte et al., 1974), mice (Rampin et al., 2003) and men (Hagemann et al., 2003; Montorsi et al., 2003). Melis and Argiolas (1995), reviewing the available data, described the changes observed as an indication

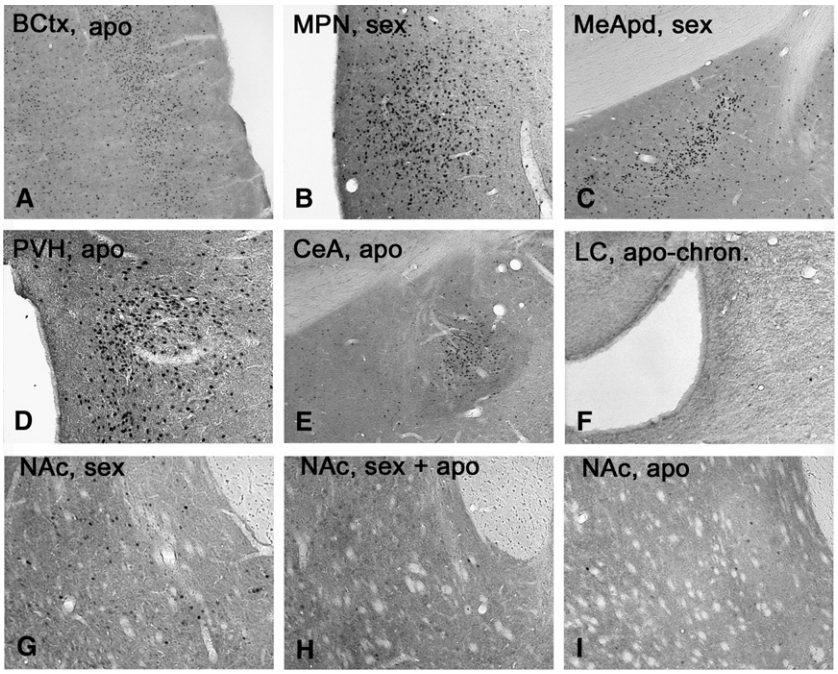

Fig. 4. (A–I) This figure illustrates the amounts of Fos-immunoreactivity, as observed in different brain areas under different conditions. The brain area as well as the condition involved is indicated on each part of the figure. The brain areas shown after sexual activities (B: MPN, medial preoptic nucleus, C: MeApd, posterodorsal medial amygdala) showed no Fos-immunoreactivity in groups where no ejaculations occurred. Acute apomorphine effects are shown in A (BCtx, barrel cortex), D (PVH, paraventricular hypothalamic nucleus) and E (CeA, central amygdaloid nucleus), while the waning Fos-induction by chronic apomorphine treatment is shown in the locus ceruleus (F, LC). G, H and I show the inhibitory effects of apomorphine on sex-induced Fos-immunoreactivity in the nucleus accumbens (NAc).

for a lowered ejaculatory threshold. In a recent review, Paredes and Agmo (2004) suggested that the observed behavioral effects are reliably obtained only in animals with low sexual activity, like castrated or sexually inexperienced animals. Our data do not support their suggestion, since our animals received ample sexual experience during the pre-test sessions and the most rapid and sluggish animals were excluded from the behavioral tests of the present experiment. The data obtained in the present experiment show that apomorphine, rather than having an effect only in low sexually active animals, reduces the pre-ejaculatory behavior (mounts and intromissions) in normal sexually active animals, confirming previously reported data (Bitran and Hull, 1987; Hsieh et al., 2004; Hull et al., 1986; Paglietti et al., 1978; Tagliamonte et al., 1974) and implying dopamine receptors to be involved in pre-ejaculatory behavior.

4.2. Fos-expression and neuronal activation patterns

Fos-immunoreactivity patterns have already been studied extensively after male sexual behavior (Baum and Everitt, 1992; Coolen et al., 1996, 1997a, 1998; Fernandez-Fewell and Meredith, 1994; Heeb and Yahr, 1996; Truitt and Coolen, 2002; Truitt et al., 2003; Veening and Coolen, 1998; Veening et al., 2005). Changes in Fos-immunoreactivity after administration of apomorphine have been described as well (Cenci et al., 1992; Laudrup et al., 1997; Steiner and Gerfen, 1994; Wirtshafter, 2000). However, the effects of chronic administration of apomorphine on male rat sexual behavior, in combination with the changes in Fos-immunoreactivity patterns induced by apomorphine and copulatory behavior, have not been studied thus far.

Especially since apomorphine is currently used as a treatment for erectile dysfunction (Heaton, 2000), knowledge about the location of brain areas involved as well as about the lasting effects of chronic administration is important for the evaluation of the treatment. Obtaining such information was the main goal of the present experiment.

In the present study, changes in Fos-immunoreactivity quantified in 12 different brain regions could be classified in three groups:

- 5 brain areas showing a strong response on (especially acute) apomorphine administration; 4 of these were also activated by copulatory behavior; (Figs. 2 and 3)
- 4 brain areas showing a strong response after copulatory behavior; 3 of these without any response to apomorphine administration; (Figs. 2 and 3)
- 2 brain areas (the core and shell of the nucleus accumbens) showing a complicated pattern of mixed apomorphine and copulatory effects; (Fig. 3)

4.3. Apomorphine reactive brain areas

This group comprises five brain regions in forebrain and brainstem: barrel cortex (Fig. 3A), paraventricular hypothalamic nucleus (Fig. 2A), central amygdala (Fig. 2D), dorsomedial hypothalamic nucleus (Fig. 2C) and locus coeruleus (Fig. 2B). They show some common characteristics in the changes in

neuronal activation induced by apomorphine and male sexual behavior:

- the first administration of apomorphine induces a strong reaction in Fos-immunoreactivity;
- after chronic administration of apomorphine this initial response is either strongly diminished or completely eliminated (except in the barrel cortex, showing less decrease over time);
- male sexual behavior induces about the same amount of Fos-immunoreactivity as the acute administration of apomorphine (except in the central amygdala, where sexual activities hardly influence Fos-immunoreactivity).

Dopamine receptors have been described in each of these brain areas, suggesting possible direct effects of apomorphine followed by desensitization effects upon chronic administration. Other mechanisms involving indirect influences by brain areas participating in the neuronal circuitry cannot be excluded, however.

Except in the central amygdalar nucleus, each of the brain areas also shows activation after copulatory activities. This activation does not depend on the presence or absence of apomorphine administration.

4.3.1. Barrel cortex

The Barrel cortex is the part of the somatosensory cortex, where the mystacial vibrissae are represented (Filipkowski et al., 2001; Steiner and Kitai, 2000). The Barrel cortex contains D_1 receptors and its activation, as shown by an increase in immediate-early gene expression, is dependent on both sensory input from the vibrissae, as well as on dopaminergic receptor activation (Filipkowski et al., 2001; Steiner and Gerfen, 1994; Steiner and Kitai, 2000). The increase in Fos-immunoreactivity is most prominent in the deeper layers (4, 5 and 6) (Filipkowski et al., 2001) and concurs with the increased sniffing/whisking behavior, induced by apomorphine administration as well as by copulatory activities (Figs. 3A and 4A).

Here we show that Fos-immunoreactivity after chronic apomorphine treatment remained elevated in the Barrel cortex, compared to saline. In that respect the Barrel cortex reacted different from the other brain areas in this group. If this reaction remains constant over time, it suggests that desensitization does not occur in this particular cortical area.

4.3.2. Paraventricular hypothalamus (Figs. 2A and 4D)

The paraventricular nucleus of the hypothalamus (PVH) contains dopamine D_1 as well as D_4 receptors (Chocyk et al., 2000; Melis et al., 2005). Exploring the systemic effects of apomorphine in the rat, Buller et al. (2003) have observed a considerable induction of Fos-immunoreactivity in the paraventricular nucleus of the hypothalamus. Our data, as induced by acute subcutaneous apomorphine administration, are in agreement with these findings, and with the induction of c-*fos* mRNA described by Drolet et al. (Drolet et al., 1996). This induction disappeared, however, under chronic apomorphine conditions, in agreement with our present findings.

Concerning copulatory behavior, the paraventricular nucleus of the hypothalamus appears to have a specific function in penile erection and seminal emission mechanisms (Arezki et al., 1985; Argiolas et al., 1987; Giuliano and Allard, 2001; Lowry et al., 2003; Melis et al., 1992b, 1996, 2001, 2003; Pehek et al., 1989; Tian et al., 1991). The effects of apomorphine administration, applied systemically, intracerebroventricularly or locally, have shown convincingly that dopaminergic receptors in the paraventricular nucleus of the hypothalamus, probably of the D_4-type located on oxytocinergic neurons (Melis et al., 1992a, 2005), are crucial, although with peripheral administration a direct pro-erectile effect on the sacral parasympathetic nucleus may play an additional role (Giuliano et al., 2002).

Our findings, however, of diminishing amounts of Fos-immunoreactivity after chronic apomorphine administration, while the behavioral effects were consistent over time, suggest that the paraventricular nucleus of the hypothalamus is not primarily responsible for the observed changes in sexual behavior.

4.3.3. Central amygdaloid nucleus (Figs. 2D and 4E)

The central nucleus of the amygdala contains dopaminergic afferents and mainly dopamine D_2 receptors (Weiner et al., 1991). Buller et al. (2003) have reported a clear increase in Fos-immunoreactivity after apomorphine administration, similar to the effects of the indirect dopamine D_2 agonist amphetamine (Day et al., 2001). Interaction effects with other neurotransmitters like noradrenalin (Pucilowski et al., 1987) and serotonin (Carter and Pycock, 1980) at the level of the central amygdaloid nucleus contribute to the complexity of the behavioral effects of apomorphine administration. Therefore, our observation that the activation of the central amygdaloid nucleus after chronic apomorphine administration is strongly reduced, compared to the acute effects, may have several causes and additional experiments are necessary to explain the observed effects.

There are no indications for a specific involvement of the central amygdaloid nucleus in copulatory behavior, and in agreement with previous studies (Baum and Everitt, 1992; Coolen et al., 1998; Veening and Coolen, 1998; Veening et al., 2005), no Fos-immunoreactivity related to copulatory activities was observed in the central amygdaloid nucleus. In that respect the central amygdaloid nucleus forms an exception in the present group.

4.3.4. Dorsomedial hypothalamus (Fig. 2C)

Despite the existence of dopaminergic afferents to the dorsomedial hypothalamic nucleus (Arezki et al., 1985; Lookingland and Moore, 1984; Lowry et al., 2003; Tian et al., 1991), and the presence of functional D2 receptors (Eaton et al., 1994) no induction of Fos-immunoreactivity has been described so far after administration of dopaminergic agonists. In the present experiments, induction of Fos-immunoreactivity occurred in the dorsomedial hypothalamic nucleus after acute apomorphine administration but this effect disappeared completely with chronic administration and probably reflects some autonomic and behaviorally stimulating effects related to our behavioral testing conditions.

Evidence for a direct involvement of the dorsomedial hypothalamic nucleus in sexual activities is virtually lacking.

4.3.5. Locus coeruleus (Fig. 2B)

Despite the presence of dopaminergic terminals in the locus coeruleus (McRae-Degueurce et al., 1988), no indications have been found in the literature about Fos-immunoreactivity induced in the locus coeruleus after administration of apomorphine. Our results, however, showed a clear increase in Fos-expression after acute administration of apomorphine. This induction almost disappeared with chronic administration (Fig. 2B). Concerning the role of the locus coeruleus in sexual activities, the locus coeruleus proper receives only a sparse projection from the medial preoptic area, but, as shown by Rizvi et al. (Rizvi et al., 1998), extensive Fos-expression occurs in locus coeruleus after activation of the medial preoptic area. Thus, our finding of increased numbers of Fos-immunoreactive locus coeruleus neurons after copulatory activities may reflect the excitatory influence of medial preoptic regions, regulating locus coeruleus neuronal activity during reproductive behaviors.

Evidence concerning the direct involvement of the locus coeruleus in aspects of copulatory is scarce, despite the noradrenergic mechanisms controlling penile erection (Giuliano and Rampin, 2000c), possibly via the hippocampal formation (Chang et al., 2001) and the duration of the post-ejaculatory refractory period (McIntosh and Barfield, 1984).

4.4. SEX- (but not apomorphine-) reactive brain areas

This group comprises 4 cortical and subcortical forebrain brain areas (prelimbic cortex (Fig. 3C, D) (with deeper and superficial layers analysed separately), infralimbic cortex (Fig. 3B), medial preoptic nucleus (Fig. 2E) and posterodorsal medial amygdala) (Fig. 2F). These areas shared the following characteristics:

- all areas showed a consistent activation induced by copulatory behavior;
- this activation was not influenced by the presence or absence of apomorphine (with the exception of the superficial layers of the prelimbic cortex);
- neither acute nor chronic apomorphine induced Fos-immunoreactivity in these areas.

4.4.1. Prelimbic cortex, deep (Fig. 3C) and superficial layers (Fig. 3D) and infralimbic cortex (Fig. 3B)

From the wealth of available literature data, it is immediately obvious that in the medial prefrontal cortical areas several types of dopaminergic receptors (from D_1 to D_4, Feldpausch et al., 1998; Glickstein et al., 2005; Wall et al., 2003, 2004) are involved in responding to the dopaminergic 'mesocortical' afferents as well as in the communication with the basolateral amygdala (Rosenkranz and Grace, 2002), hippocampus (Seamans et al., 1998) and especially the shell of the nucleus accumbens (Feldpausch et al., 1998).

In view of the important role of dopamine in the functioning of the medial prefrontal cortical areas, it was somewhat surprising, but in agreement with earlier findings, to find that the numbers of Fos-immunoreactivity neurons did not change after acute or chronic administration of apomorphine (see Fig. 3). Maybe due to a mix of excitatory and inhibitory effects, directly and indirectly working on the medial prefrontal corticaneurons, we detected no changes in Fos-immunoreactivity as a result of the apomorphine administration.

Performance of sexual activities, however, induced a clear and consistent increase in Fos-immunoreactivity in each of the quantified subareas. To our knowledge, this induction of Fos-immunoreactivity by copulatory activities in both the prelimbic and the infralimbic areas has not been reported before. Interestingly, the administration of apomorphine, either acute or chronically, did not seem to influence this Fos-immunoreactivity-induction, except in the superficial layers of the prelimbic cortex (Fig. 3D), where the chronic apomorphine administration significantly depressed the induced amount of Fos-immunoreactivity, compared to saline. Since this effect is reminiscent of the effects observed in the nucleus accumbens (see below) and because of the extensive relationships between this region and the medial prefrontal areas (Balfour et al., 2006), this effect will be discussed below, in relation with the observed effects in the nucleus accumbens.

4.4.2. Medial preoptic nucleus (Fig. 2E)

Since several decades, it is known that dopamine plays a complex role in the control of sexual behavior (Hull et al., 1986; Melis and Argiolas, 1995; Paredes and Agmo, 2004) with different dopaminergic systems involved in different behavioral phases (Melis and Argiolas, 1995). Extracellular dopamine levels are increased in the medial preoptic area of male rats immediately before and during copulation (Hull et al., 1993). However, the role of dopamine D_1 vs D_2 receptors is complex (Hull et al., 1992) and hard to define, because of conflicting findings (Paredes and Agmo, 2004). The possible involvement of dopamine D_4 receptors in the pro-erectile effects of apomorphine (Argiolas and Melis, 2005; Brioni et al., 2004; Hsieh et al., 2004) may shed new light on the existing confusing situation.

Increases in c-*fos* mRNA in the medial preoptic nucleus have been reported only once (after subcutaneous administration of apomorphine (Drolet et al., 1996)), but we observed no Fos-induction during the present experiments.

The medial preoptic area has a long standing history as a critical brain area for the regulation of male copulatory behavior (for review, see Meisel, 1994), and the consistent increase in Fos-immunoreactivity after copulatory activities (Fig. 2E) is in complete agreement with this role. Especially in the medial preoptic nucleus, olfactory (pheromonal), hormonal (testosterone) and genito-sensory cues, essential for the control of male rodent sexual behavior, appear to be integrated (Coolen et al., 1996, 1997a, 1998; Simerly and Swanson, 1986, 1988; Simerly et al., 1990; Wood and Newman, 1995). From the earliest Fos-studies on, activation of the medial preoptic nucleus after copulatory activities has been reported (Robertson et al., 1991; Veening and Coolen, 1998) as confirmed in the present study. The medial preoptic nucleus has extensive and mostly reciprocal relationships with other brain areas involved in

copulation (Coolen et al., 1998, 2003a,b; Robertson et al., 1991; Simerly and Swanson, 1986, 1988).

4.4.3. Medial amygdaloid nucleus, posterodorsal part (Fig. 2F)

Despite high densities of dopamine D_1-receptors in the medial amygdala (Dawson et al., 1986), induction of Fos-immunoreactivity by administration of apomorphine has never been reported. Day et al. (2001) showed that the indirect D_2 agonist amphetamine did not increase the levels of c-Fos mRNA in the medial amygdala. Confirming these data, our results show that apomorphine has no effect on Fos-expression in this brain area.

After copulatory activities, part of the medial amygdala-activation may be related to olfactory and hormonal input and becomes obvious already in the appetitive phase of sexual behavior (Coolen et al., 1997a). However, a specific small lateral zone in the posterodorsal part becomes activated by genito-sensory stimulation, ascending from the spinal cord, as occurring during ejaculation (Coolen et al., 1996, 1997a, 1998). This pattern of activation may signal a kind of sexual "satiety" (Parfitt and Newman, 1998).

In line with all available data, our results also show a strong induction of Fos-immunoreactivity after copulation. Acute or chronic apomorphine administration did not have any effect on the neural activation patterns induced by copulation (Fig. 2F).

4.5. APO-SEX-interactive brain areas

This group comprises two brain areas (the core (Fig. 3E) and shell (Fig. 3F) of the nucleus accumbens) which showed a complicated pattern of mixed apomorphine and copulatory effects.

4.5.1. Nucleus accumbens core and shell (Figs. 3E,F and 4G,H,I)

In the core region, apomorphine administration did not induce Fos-immunoreactivity, whereas the Fos-immunoreactivity induced by copulatory activities was completely inhibited by acute or chronic administration of apomorphine (Fig. 3E).

In the shell region, the basal Fos-immunoreactivity levels, not related to sexual activities, were also strongly inhibited by acute or chronic apomorphine administration. Copulatory activities, with or without apomorphine administration, did not induce additional Fos-immunoreactivity in the shell region. However, the elevated levels eventually reached after chronic vehicle treatment were again inhibited by chronic apomorphine treatment. This inhibited level was similar again to 'control levels' of Fos-immunoreactivity in the shell region, and still considerably higher than the inhibited amounts of Fos-immunoreactivity observed without sexual activities (Fig. 3F).

This inhibitory effect of apomorphine administration is reminiscent of and may be causally related to the effect observed in the superficial layers of the prelimbic cortex, where chronic apomorphine administration also reduced the sex-induced neural activity (see above, Fig. 3D).

The accumbens region contains many types of dopamine receptors and receives a dense dopaminergic innervation. This 'mesolimbic' dopaminergic projection, arising from the ventral tegmental area, has been demonstrated to be involved in many functions, including motivation (Mogenson et al., 1980), reward (Wise and Rompre, 1989), as an interface between sensory input and locomotory output (Weissenborn and Winn, 1992) and in 'cue-elicited drug seeking' (Miller and Marshall, 2005). This projection is extensively controlled by a variety of regulatory mechanisms. Recently it has been shown that infralimbic and prelimbic subareas of the medial prefrontal cortex extensively project into the ventral tegmental area, in addition to their projections into both the shell and the core of the nucleus accumbens, and to the medial preoptic nucleus and other 'ejaculation-related' areas (Balfour et al., 2006).

Effects of accumbens-manipulations on male sexual behavior have been described in the rat, but they are not specific for sexual behavior and may be related to reward and arousal processes induced by sex-associated environmental cues (Giuliano and Allard, 2001; Hull et al., 1986; Liu et al., 1998; Melis and Argiolas, 1995; Moses et al., 1995; Paredes and Agmo, 2004). However, the mesolimbic system becomes activated in male rats by mating and sex-associated environmental cues (Balfour et al., 2004; Pfaus et al., 1990).

Induction of Fos-immunoreactivity by copulatory behavior has been shown before in shell and core parts of the nucleus accumbens (Balfour et al., 2004; Robertson et al., 1991). Our data are in agreement with these earlier findings.

Induction of Fos-immunoreactivity by apomorphine has been described only once, in the dorsomedial shell of the nucleus accumbens, in response to a very high (5 mg/kg s.c.) dose of apomorphine (Dilts et al., 1993). Other reports (Cole et al., 1992; Laudrup et al., 1997; Paul et al., 1995; Saka et al., 1999) have shown no such induction. Our present findings suggest even an opposite effect in the shell region: apomorphine may induce a decrease in Fos-immunoreactivity, compared to control situations.

In the core region, an interesting interaction-effect became visible in our experiments: the normally occurring increased Fos-immunoreactivity after mating was absent when apomorphine was administered. A similar effect was observed after chronic treatment in the shell region and in the superficial layers of the prelimbic cortex, where the elevated mating-induced Fos-immunoreactivity levels were reduced by apomorphine administration. This finding shows that apomorphine may affect activation patterns, normally induced by mating or mating-related cues. The questions about direct "priming" (Pollack et al., 1997) and/or "sensitization" effects (Laudrup et al., 1997; Saka et al., 1999) of apomorphine playing a role here, remain to be addressed in more specific experiments. In our view, indirect effects on reward and arousal processes induced by sex-associated environmental cues (Balfour et al., 2004; Sachs, 2000) most probably play an important role.

4.6. Concluding remarks

The effects observed in the accumbens regions are complicated and hard to explain, because of the complex functions and intricate prefrontal–accumbens–ventral tegmental area relationships. Even the question whether local and direct accumbens effects or indirect effects, via neural circuitries influencing

accumbens activity, are responsible for the observed interactions of sex and apomorphine on neural activation in the nucleus accumbens, are hard to answer and additional specific experiments are urgently needed. We assume that the diminished accumbens-Fos-immunoreactivity and the elongated post-ejaculatory interval may reflect a decreased response to remote cues from the estrus female.

Overviewing the Fos-induction in the brain areas described in our present paper, it may be worthwhile to pay attention not only to each of the brain areas separately, but also to compare the changes in Fos-immunoreactivity, because common patterns appear in several brain areas together. For instance, the locus coeruleus, paraventricular nucleus of the hypothalamus, central amygdaloid nucleus and barrel cortex together reacted strongly upon the first administration of apomorphine, but less or hardly any more after chronic treatment without sex. Except the central amygdaloid nucleus, these areas showed an increased level of activation after copulatory activities as well. On the other hand, the prelimbic cortex, medial preoptic nucleus, posterodorsal medial amygdala, and nucleus accumbens core were specifically and consistently activated after copulatory activities. These copulation-induced activation patterns were hardly influenced by the apomorphine administration with a single important exception: the nucleus accumbens core.

In each of these cases, the groups of brain areas reacting in an identical way are extensively and mostly reciprocally connected, neuroanatomically. Paying attention to this kind of common changes in Fos-immunoreactivity is certainly helpful in understanding how larger circuitries work. In other words, a correlation analysis of changes in Fos-immunoreactivity may suggest functional relationships, when the brain is studied at the system-approach level. The common or completely opposite changes induced in Fos-immunoreactivity, as observed in the present study, may be indicative for their mutual relationships, and deserve more attention.

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