

RESEARCH PAPER

Brain oxytocin receptors mediate ejaculation elicited by 7-hydroxy-2-(di-*N*-propylamino) tetralin (7-OH-DPAT) in anaesthetized rats

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Background and purpose: The involvement of the neuropeptide oxytocin in the control of male sexual responses is documented although its exact mechanisms of action, and especially the site(s) of action, are not fully delineated. In order to clarify this issue, we tested the effects of a peptide oxytocin antagonist delivered through different routes on sexual responses elicited, in anaesthetized male rats, by i.c.v. 7-hydroxy-2-(di-*N*-propylamino) tetralin (7-OH-DPAT), a dopamine agonist, preferentially active on D₃ receptors.

Experimental approach: Seminal vesicle pressure (SVP) and bulbospongiosus muscle (BS) electromyograms were recorded as physiological markers of emission and expulsion phases of ejaculation respectively and intracavernosal pressure (ICP) was monitored as a physiological marker of erection.

Key results: When injected i.v., the oxytocin antagonist did not impair 7-OH-DPAT-induced SVP and ICP responses while BS burst frequency was diminished. When delivered i.c.v., the oxytocin antagonist dose-dependently inhibited occurrence of 7-OH-DPAT-induced sexual responses. When delivered intrathecally (i.t.) at the level of the 6th lumbar (L6) segment, but not the 13th thoracic (T13) segment, the oxytocin antagonist reduced the duration of BS responses and the occurrence of ejaculation without impairing ICP responses.

Conclusions and implications: Brain oxytocin receptors mediate male sexual responses elicited by i.c.v. 7-OH-DPAT in anaesthetized rats whereas L6 spinal oxytocin receptors only impair the occurrence of ejaculation. Peripheral oxytocin receptors are marginally involved in 7-OH-DPAT-induced sexual responses. These findings should be considered for the development of potential pharmacological treatment of premature ejaculation in man.

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Abbreviations: 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; BS, bulbospongiosus muscle; ICP, intracavernosal pressure; PVN, paraventricular nucleus of the hypothalamus; SVP, seminal vesicle pressure

Introduction

Ejaculation comprises two successive phases, emission and expulsion, that involve different pelvi-perineal anatomical structures. The emission phase consists of secretion of the various components of semen by seminal vesicles (SVs), prostate and through release of ampullary vasa deferentia content into the prostatic urethra. The expulsion phase corresponds to forceful propulsion of sperm from the

prostatic urethra to the urethral meatus through rhythmic contractions of perineal striated muscles, with a primary role for the bulbospongiosus muscle (BS). A tight coordination between sympathetic, parasympathetic and somatic divisions of the nervous system is necessary for normal anterograde ejaculation. As a centrally integrated and highly coordinated process, ejaculation involves sensory receptors and areas, afferent neural pathways, cerebral sensory and motor areas, spinal motor centres, as well as multiple efferent pathways (reviewed by Giuliano and Clement, 2005). A variety of neurotransmitters distributed in supraspinal and spinal nuclei are important for control of ejaculation. Animal studies have provided insights into the central neurochemicals involved in the control of the ejaculatory

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process by demonstrating the primary importance of dopamine and 5-HT (serotonin), as well as other factors such as oxytocin (for review, see Hull *et al.*, 2004; Peeters and Giuliano, 2008).

Behavioural investigations have shown that the dopamine D₃ receptor-preferring agonist, 7-hydroxy-2-(di-*N*-propylamino) tetralin (7-OH-DPAT), decreases the number of intromissions preceding ejaculation and the latency of ejaculation in rats (Ahlenius and Larsson, 1995; Ferrari and Giuliani, 1996). Furthermore, 7-OH-DPAT has recently been reported to trigger rhythmic contractions of BS and ejaculation when delivered centrally in anaesthetized rats (Clement *et al.*, 2007; Kitrey *et al.*, 2007).

The involvement of oxytocin in male sexual functions is well documented, and notably this neuropeptide was found to be one of the most potent agents to induce penile erection in various species (reviewed by Argiolas and Melis, 1995). Supraspinal and spinal sites have been found to mediate the pro-erectile effect of oxytocin (Argiolas *et al.*, 1985; Giuliano *et al.*, 2001). A key role for oxytocin in the control of male sexual behaviour, including ejaculation, has also been demonstrated. Infused into the cerebral ventricle of male rats free to copulate with a receptive female, oxytocin facilitates ejaculatory behaviour by shortening ejaculation latency and post-ejaculatory refractory period (Arletti *et al.*, 1985). Administered via intracerebroventricular (i.c.v.) route, oxytocin was also found to significantly increase latencies of mount and intromission (Stoneham *et al.*, 1985). Furthermore, in the mating test a selective oxytocin-receptor antagonist delivered via i.c.v. route to sexually vigorous male rats was reported to inhibit sexual behaviour, including ejaculation, and to reverse the pro-sexual effects of the non-selective dopamine-receptor agonist, apomorphine (Argiolas *et al.*, 1988b). Systemic, similarly to central, administration of oxytocin was reported to shorten ejaculation latency and post-ejaculatory interval in sexually active male rats (Arletti *et al.*, 1985; Stoneham *et al.*, 1985).

To further understand the role of oxytocin in the expression of sexual response, the effects of a peptide antagonist of the oxytocin receptor, administered via different routes, on the pro-sexual activity of 7-OH-DPAT were investigated in anaesthetized male rats. Intracavernosal pressure, SV pressure (SVP) and BS electromyogram were monitored as physiological markers, respectively, of the erection, emission and expulsion phases of ejaculation.

Methods

Animals

All animal experiments were carried out in accordance with the European Community Council Directive (86/609/EEC) on the use of laboratory animals. All efforts were undertaken to minimize the number of animals used and their suffering. A total of 110 sexually naïve, adult, male Wistar rats (Charles-River, L'Arbresle, France) weighing 250–300 g were used in this study. Animals were housed in groups of four at 20 ± 2 °C under a 12-h light/dark cycle, with access to food and water *ad libitum*. Animals were maintained in these conditions for at least 8 days prior to testing.

Surgical preparation

Rats were anaesthetized with urethane (1.2 g kg⁻¹) and absence of hind-paw pinch reflex was checked. Their body temperature was maintained at 37 °C using a homeothermic blanket. The carotid artery was catheterized with polyethylene tubing (PE50) filled with heparinized saline (50 IU mL⁻¹) to record blood pressure with a pressure transducer (EM750; Elcomatic, Glasgow, UK). For injecting via intravenous (i.v.) route, the jugular vein was catheterized with a polyethylene tubing (PE10) filled with 0.9% (w/v) NaCl. SV pressure (SVP) was measured with a catheter filled with mineral oil and inserted in the right SV through the apex and connected to a pressure transducer. For intracavernous pressure (ICP) monitoring, the penis was denuded of skin and a 25-G needle connected to a catheter was inserted into a corpus cavernosum. A pressure transducer was used for recording ICP variations. The BS was exposed with a perineal incision. Electrical activity of BS was recorded by passing a Teflon-insulated stainless steel wire laterally throughout the muscle, with two 1–2 mm pieces (separated by 1–2 mm) of insulation stripped off. Electrical signal from the BS was amplified (DP-301; Warner Instrument Corp., Hamden, CT, USA; gain, 10 000; low pass, 1 kHz; high pass, 10 Hz) before being digitized. At the end of the experiments, rats were killed with an overdose of urethane.

Intracerebroventricular cannula implantation

A guide cannula (22 G) was stereotaxically placed above the cerebral ventricle (coordinates according to Paxinos and Watson rat brain atlas: Paxinos and Watson (1998), 0.5 mm posterior to bregma, 1 mm lateral to midline and 4 mm below the skull). The internal cannula (with 0.5-mm projection below the guide cannula) was connected to a Hamilton syringe placed in a micropump allowing delivery of microvolumes. At the end of the experimental session, methylene blue dye was injected through the cannula and the brain, removed and grossly dissected, was inspected for presence of blue dye in the ventricles.

Intrathecal catheter implantation

For catheter insertion via intrathecal (i.t.) route, the rat's head was placed in a stereotaxic frame and was rotated nose downwards. The catheter was a polyethylene tubing (PE10) stretched to 150% of its original length in hot water and cut to the required length so that its distal opening reached the targeted levels of the spinal cord; that is, the thirteenth thoracic (T13) or the sixth lumbar (L6) segment. The skin and neck muscles were incised and retracted. The atlanto-occipital membrane was opened and the catheter, filled with 0.9% (w/v) NaCl, was carefully advanced in the caudal direction. The rostral free end of the catheter was secured with ligatures that closed the neck muscles and skin layers. The exact location of the caudal tip of the catheter was checked at the end of each experiment after death of the animal and exposure of the spinal cord. Only rats with a catheter tip located at the T13 or L6 spinal level were considered for the results.

Experimental design

Intravenous injection of $d(\text{CH}_2)_5^1, \text{Tyr}(\text{Me})^2, \text{Orn}^8$ -oxytocin, the oxytocin antagonist. Two doses of oxytocin antagonist (0.25 and $1 \mu\text{g kg}^{-1}$) were tested by delivery i.v. route in separate groups of 10 rats. After a 5-min period to obtain baseline values, the oxytocin antagonist or its vehicle was given via i.v. route (volume 1 mL kg^{-1}), followed, 15 min later, by i.c.v. 7-OH-DPAT ($10 \mu\text{g}$ in $5 \mu\text{L}$) delivery.

Intracerebroventricular injection of $d(\text{CH}_2)_5^1, \text{Tyr}(\text{Me})^2, \text{Orn}^8$ -oxytocin. Three doses of oxytocin antagonist (0.001, 0.01 and $0.1 \mu\text{g}$) were tested by injection via i.c.v. route in separate groups of 10 rats. After a 5-min baseline period, i.c.v. delivery of the oxytocin antagonist or its vehicle was performed (volume $5 \mu\text{L}$), followed, 15 min later, by i.c.v. 7-OH-DPAT ($10 \mu\text{g}$ in $5 \mu\text{L}$) delivery. Each injection was given at a flow rate of $1 \mu\text{L min}^{-1}$.

Intrathecal injection of $d(\text{CH}_2)_5^1, \text{Tyr}(\text{Me})^2, \text{Orn}^8$ -oxytocin. One dose of the oxytocin antagonist ($0.1 \mu\text{g}$) was tested by injection via i.t. route either at T13 level, where pre-ganglionic sympathetic neurones sending projections to the anatomical structures involved in sexual response are located, or at L6 level, where pre-ganglionic parasympathetic neurones innervating the same anatomical structures and motoneurones destined to pelvi-perineal striated muscles are located, in separate groups of 10 rats. After the 5-min baseline values were obtained, i.t. delivery of oxytocin antagonist or its vehicle was performed (volume $10 \mu\text{L}$), followed, 15 min later, by i.c.v. 7-OH-DPAT ($10 \mu\text{g}$ in $5 \mu\text{L}$) delivery.

Data analysis

Sexual responses (that is, ejaculation; ICP and SVP rises and BS rhythmic contractile responses) were counted during the recording period. The duration of the BS contractile responses and the frequency of burst within each BS contractile response, the duration and amplitude of SVP and ICP rises, were calculated for each rat displaying such responses and was averaged for each treatment group. The amplitude of ICP increases corresponded to the difference between basal ICP and ICP value at the plateau reached during the erectile response. As there is a correlation between ICP and arterial pressure, the amplitude of the ICP increase was expressed relative to the mean arterial pressure (MAP) as already described (Giuliano *et al.*, 1993). This corresponded to the ratio of the maximal value of ICP increase reached during an erectile response and the MAP calculated for the duration of the same erectile response, and was expressed as a percentage of MAP.

Statistical procedures

Inter-group statistical comparisons of the number of sexual responses were performed using Mann–Whitney test (i.t. oxytocin antagonist) or Kruskal–Wallis test, followed, whenever $P < 0.05$, by Dunn's *post hoc* test (i.v. and i.c.v. oxytocin antagonist delivery). Non-parametric tests were used, as in most of the treatment groups the values did not

follow a Gaussian distribution. Statistical comparisons of the quantitative parameters characterizing sexual responses were performed between treatment groups using Student's *t*-test (i.t. oxytocin antagonist) or one-way analysis of variance (ANOVA), followed, whenever $P < 0.05$, by Newman–Keuls' *post hoc* test (i.v. and i.c.v. oxytocin antagonist). Parametric tests were used here as, in most of the treatment groups, the values followed a Gaussian distribution.

Drugs

R(+)-7-hydroxy-2-(di-*N*-propylamino) tetralin (7-OH-DPAT; Sigma-Aldrich, Saint Quentin Fallavier, France) was dissolved in 0.9% (w/v) NaCl. This solution was prepared weekly and stored at -20°C . $d(\text{CH}_2)_5^1, \text{Tyr}(\text{Me})^2, \text{Orn}^8$ -oxytocin (Bachem, Weil am Rhein, Germany), a potent oxytocin-receptor antagonist, was dissolved in 0.9% (w/v) NaCl. This solution was prepared weekly and stored at -20°C .

Results

Effects of i.c.v. 7-OH-DPAT on the physiological markers of sexual responses

Twenty-five out of the 40 rats (63%) receiving i.c.v. 7-OH-DPAT and the vehicle of the oxytocin antagonist (NaCl (0.9%, w/v)) via i.c.v., i.t. or i.v. route (thereafter control rats) displayed at least one ejaculation (that is, expulsion of a seminal plug out of the urethra) during the 30-min period after 7-OH-DPAT delivery. Ejaculation always occurred concomitantly to coordinated increases in SVP, BS activity and ICP following a typical sequence (Figure 1). A steep rise in SVP was first followed by intense rhythmic BS contractions (on average 5.9 ± 1.3 s after SVP rise has started, $n = 33$), and then by a more progressive increase in ICP (on average 8.6 ± 1.4 s after SVP rise has started, $n = 33$). Two to four peaks were noticed during the SVP response (Figure 1, lower panel). In control rats ($n = 33$), the maximal amplitude of the increase in SVP was 4.1 ± 0.6 mm Hg and the mean duration was 18.5 ± 1.3 s. BS responses ($n = 35$) occurred in the form of burst of rhythmic contractions with a mean duration and burst frequencies, as shown in Figure 2. Increases in ICP were characterized by a plateau with superimposed brief supra-systolic peaks (Figure 1, lower panel). For control rats ($n = 37$), the mean duration of ICP rises and the mean amplitude (as % MAP) are shown in Figure 2.

Effects of i.v. oxytocin antagonist on 7-OH-DPAT-induced sexual responses.

There was no sexual response during the 15-min period separating oxytocin i.v. antagonist delivery and 7-OH-DPAT infusion (data not shown). Pretreatment with the oxytocin antagonist had no effects on the occurrence of sexual responses elicited by 7-OH-DPAT (Tables 1 and 2). The quantitative parameters characterizing SVP and ICP increases were not significantly modified by i.v. oxytocin antagonist delivery (Figure 2). The burst frequency of BS responses, but not their duration, was significantly reduced (one-way ANOVA + Newman–Keul's *post hoc* test, $P < 0.05$) in rats pretreated via i.v. delivery of oxytocin antagonist at both doses when compared with i.v. control rats (Figure 2).

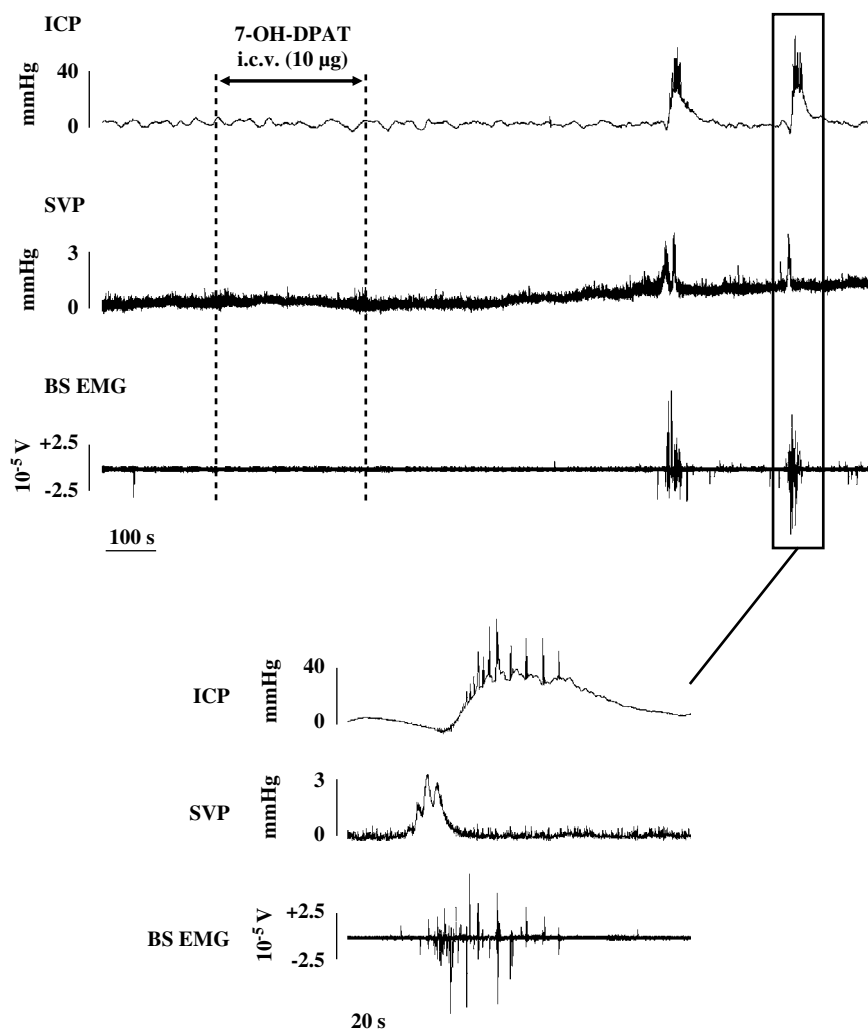


Figure 1 Recording of ICP, SVP and BS electromyogram (BS EMG) obtained in anesthetized rats after i.c.v. delivery of 7-OH-DPAT (10 µg). The tracing is displayed on an expanded time scale to show the temporal organization of a sexual response. BS, bulbospongiosus muscle; ICP, intracavernosal pressure; i.c.v., intracerebroventricular; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; SVP, seminal vesicle pressure.

Effects of i.c.v. oxytocin antagonist on 7-OH-DPAT-induced sexual responses. No sexual response was observed during the 15-min period following oxytocin antagonist i.c.v. injection and preceding 7-OH-DPAT delivery (data not shown). Ejaculation induced by 7-OH-DPAT was abolished in all rats pretreated with the oxytocin antagonist (0.1 µg, i.c.v.), whereas only one rat displayed one ejaculation in the group administered the oxytocin antagonist at 0.01 µg per rat (Table 3). At the highest i.c.v. dose, oxytocin antagonist produced a dose-dependent decrease in the number of 7-OH-DPAT-induced SVP, BS and ICP responses when compared with the decrease by the lowest oxytocin antagonist dose and the vehicle (Tables 2 and 3; Kruskal–Wallis + Dunn's *post hoc* test, $P < 0.05$). In rats pretreated via i.c.v. route with 0.01 and 0.1 µg of oxytocin antagonist, there was a significant increase in the latency of the first SVP, BS and ICP responses after 7-OH-DPAT delivery when compared with the effects of the lowest dose of oxytocin antagonist (0.001 µg) or the vehicle (one-way ANOVA + Newman–Keuls' *post hoc* test, $P < 0.01$; Tables 2 and 3). No appreciable modification of the quantitative parameters of SVP, BS and ICP responses elicited

by 7-OH-DPAT was observed in rats pretreated with the i.c.v. oxytocin antagonist (Figure 3).

Effects of i.t. oxytocin antagonist on 7-OH-DPAT-induced sexual responses. The most effective i.c.v. oxytocin antagonist dose (0.1 µg) in inhibiting ejaculation was selected for i.t. administration. There was no sexual response over the 15-min period following oxytocin antagonist i.t. (either T13 or L6 spinal level) injection and preceding 7-OH-DPAT delivery (data not shown). Occurrence of ejaculation was significantly impaired in rats i.t. delivered with oxytocin antagonist at the L6 level (Mann–Whitney, $P < 0.05$) whereas the latency of the first ejaculation was comparable to that of the L6 i.t. control group (Table 4). In rats receiving i.t. oxytocin antagonist at the L6 level, the number of SVP responses was half that of L6 i.t. vehicle-pretreated animals, although statistical significance was not reached (Mann–Whitney, $P = 0.20$; Table 4). There was no noticeable effect of L6 i.t. administration of oxytocin antagonist on the quantitative parameters of SVP responses (Figure 4). Neither number of BS responses nor latency of the first BS

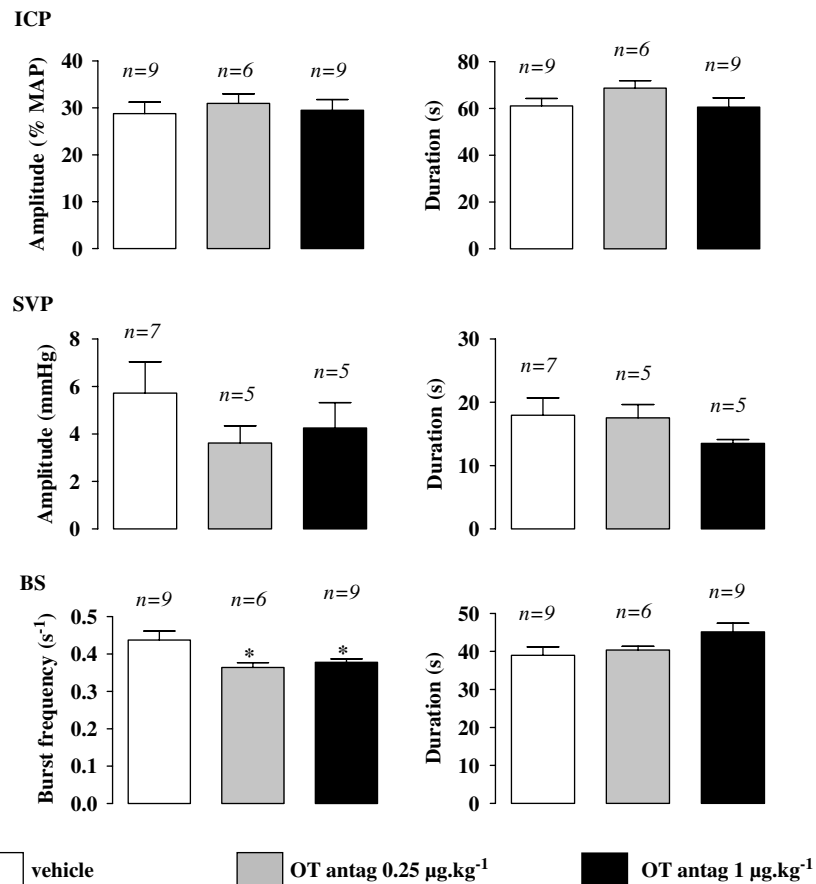


Figure 2 Effects of i.v. injection of oxytocin antagonist (OT antagon) on the quantitative parameters of sexual responses induced by 7-OH-DPAT. Amplitude of ICP rises, expressed as percentage of MAP measured during the corresponding ICP rise, and duration of ICP rises were determined in rats pretreated, 15 min prior to i.c.v. 7-OH-DPAT (10 µg), with i.v. oxytocin antagonist vehicle, oxytocin antagonist 0.25 or 1 µg kg⁻¹. Amplitude and duration of SVP increases, frequency of burst within a contractile response of BS, as well as duration of the BS contractile response, were determined. Bars and error bars represent the means ± s.e. mean of *n* number of rats. **P* < 0.05 versus control group; one-way ANOVA + Newman-Keuls' *post hoc* test. ANOVA, analysis of variance; BS, bulbospongiosus muscle; ICP, intracavernosal pressure; i.v., intravenous; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino)tetralin; MAP, mean arterial pressure.

Table 1 Effects of i.v. oxytocin antagonist on i.c.v. 7-OH-DPAT-induced sexual responses

	OT antagon, i.v.		
	0	0.25 µg kg ⁻¹	1 µg kg ⁻¹
Number of ejaculations	0.9 ± 0.2 (10)	0.6 ± 0.2 (10)	0.9 ± 0.2 (10)
Latency of first ejaculation(s)	887 ± 160 (7)	866 ± 76 (4)	852 ± 182 (7)
Number of SVP responses	2.4 ± 0.5 (10)	2.3 ± 0.9 (10)	2.6 ± 0.7 (10)
Latency of first SVP response(s)	897 ± 172 (7)	808 ± 195 (5)	764 ± 73 (7)
Number of BS responses	2.6 ± 0.4 (10)	2.6 ± 0.8 (10)	2.9 ± 0.4 (10)
Latency of first BS response(s)	906 ± 160 (7)	818 ± 162 (5)	770 ± 82 (7)

Abbreviations: BS, bulbospongiosus muscle; i.c.v., intracerebroventricular; i.v., intravenous; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; OT, oxytocin; SVP, seminal vesicle pressure.

The OT antagonist (OT antagon.) was injected via i.v. route 15 min prior to 7-OH-DPAT (10 µg, i.c.v.). SVP and BS EMG were recorded over 30 min following 7-OH-DPAT delivery. The number of ejaculations, and SVP and BS responses, was counted during the 30-min 7-OH-DPAT post-injection period. The latency of the first ejaculation, and SVP and BS responses after i.c.v. 7-OH-DPAT delivery, was also determined. The values are the means ± s.e. mean of data from *n* number of rats. Statistical analysis was performed by Kruskal-Wallis + Dunn's *post hoc* test for comparison of the number of sexual responses. One-way ANOVA + Newman-Keuls' *post hoc* test for comparison of the latency of sexual responses.

response was modified by i.t. delivery of oxytocin antagonist at L6 (Table 4). However, duration of 7-OH-DPAT-induced BS responses was significantly decreased in rats pretreated with oxytocin antagonist at L6 (i.t.) as compared with

the vehicle given at L6 (Student's *t*-test, *P* < 0.05). The burst frequency of BS contractions was unchanged by oxytocin antagonist given to L6 i.t. (Figure 4). ICP responses elicited by 7-OH-DPAT were not altered in rats pretreated

with i.t. oxytocin antagonist at the L6 level (Table 2; Figure 4).

When delivered at the T13 level, the oxytocin antagonist did not exert any effect on 7-OH-DPAT-induced sexual responses (Tables 2 and 4; Figure 4).

Table 2 Effects of oxytocin antagonist, administered through different routes, on ICP responses induced by 7-OH-DPAT

	Number of ICP responses	Latency of first ICP response(s)
<i>OT antagon., i.v.</i>		
0	2.7 ± 0.4 (10)	794 ± 156 (9)
0.25 µg kg ⁻¹	2.6 ± 0.8 (10)	838 ± 163 (6)
1 µg kg ⁻¹	2.9 ± 0.4 (10)	867 ± 82 (9)
<i>OT antagon., i.c.v.</i>		
0	3.6 ± 1.0 (10)	645 ± 135 (9)
0.001 µg	3.5 ± 0.7 (10)	488 ± 77 (9)
0.01 µg	1.9 ± 0.5 (10)	1146 ± 124 ^{b,d} (7)
0.1 µg	0.8 ± 0.5 ^{a,c} (10)	1354 ± 205 ^{b,d} (3)
<i>OT antagon., i.t.</i>		
T13		
0	3.2 ± 0.8 (10)	654 ± 125 (8)
0.1 µg	2.7 ± 0.5 (10)	793 ± 194 (9)
L6		
0	2.5 ± 0.5 (10)	933 ± 95 (9)
0.1 µg	2.3 ± 0.7 (10)	898 ± 138 (6)

Abbreviations: ICP, intracavernosal pressure; i.c.v., intracerebroventricular; i.t., intrathecal; i.v., intravenous; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; OT, oxytocin.

The OT antagonist (OT antagon.) was injected via i.v., i.c.v. or i.t. route 15 min prior to 7-OH-DPAT (10 µg, i.c.v.). ICP was recorded over 30 min following 7-OH-DPAT delivery. The number of ICP responses was counted during the 30-min 7-OH-DPAT post-injection period. The latency of the first ICP response after i.c.v. delivery of 7-OH-DPAT was also determined. The values are the means ± s.e. mean of data from *n* number of rats. Statistical analysis was performed by Kruskal–Wallis + Dunn's *post hoc* test for comparison of the number of ICP responses: ^a*P* < 0.05, different from corresponding control; ^c*P* < 0.05, different from OT antagon., 0.001 µg. One-way ANOVA + Newman–Keuls' *post hoc* test for comparison of latency of the first ICP response: ^b*P* < 0.01, different from corresponding control; ^d*P* < 0.01, different from OT antagon., 0.001 µg.

Discussion and conclusions

The present study demonstrates that brain oxytocin receptors are of primary importance in mediating the pro-ejaculatory and pro-erectile effects of the dopamine D₃ receptor-preferring agonist, 7-OH-DPAT, in anaesthetized rats. It was also found that spinal oxytocin receptors at L6 played a modulating role in the pro-ejaculatory activity of 7-OH-DPAT.

When sexual responses are elicited in the male by 7-OH-DPAT, a significant decrease was observed in the BS burst frequency in rats given the oxytocin antagonist via i.v. route (Figure 2). The other parameters that were measured, and especially occurrence of BS responses and ejaculation, were unchanged (Tables 1, 2; Figure 2). As the oxytocin antagonist used in the present study is a peptide, it is very likely that it did not cross the blood–brain barrier. Therefore, the effects of i.v. injection of this compound are due to its peripheral actions. There are no data available in the literature that may help to explain the peripheral mode of action of the oxytocin antagonist on BS contractile activity. Oxytocin receptors have been found in the epididymis (Filippi *et al.*, 2002) and in the testis (Nicholson *et al.*, 1984). It has been proposed that oxytocin when bound to its peripheral receptors promotes sperm transport during the emission phase of ejaculation by increasing the contraction of seminal tract smooth muscle cells (Filippi *et al.*, 2003). This peripheral action of oxytocin might explain the facilitation of ejaculation found in copulating rats after systemic delivery of oxytocin (Arletti *et al.*, 1985; Stoneham *et al.*, 1985). The present results do not support this view, since 7-OH DPAT-induced ejaculation was not affected by i.v. pretreatment with the oxytocin antagonist. Because of the high affinity of oxytocin receptors for the oxytocin antagonist used (EC₅₀ ~ 1 nM), we assume that the highest dose tested was sufficient to block most of the peripheral oxytocin receptors.

Oxytocinergic nerve terminals originating in the parvocellular part of the paraventricular nucleus of the hypothalamus (PVN) have been identified in the vicinity of preganglionic parasympathetic neurons in the L6–S1 spinal

Table 3 Effects of i.c.v. oxytocin antagonist on i.c.v. 7-OH-DPAT-induced sexual responses

	<i>OT antagon., i.c.v.</i>			
	0	0.001 µg	0.01 µg	0.1 µg
Number of ejaculations	0.6 ± 0.2 (10)	0.3 ± 0.2 (10)	0.1 ± 0.1 (10)	0.0 ± 0.0 ^a (10)
Latency of first ejaculation(s)	618 ± 215 (5)	432 ± 31 (4)	1136 (1)	— (0)
Number of SVP responses	3.4 ± 1.0 (10)	3.5 ± 0.7 (10)	1.5 ± 0.5 (10)	0.4 ± 0.5 ^{a,c} (10)
Latency of first SVP response(s)	644 ± 136 (9)	454 ± 53 (9)	1156 ± 124 ^{b,d} (7)	1355 ± 206 ^{b,d} (3)
Number of BS responses	4.2 ± 1.1 (10)	4.0 ± 0.7 (10)	2.0 ± 0.5 (10)	0.8 ± 0.5 ^{a,c} (10)
Latency of first BS response(s)	652 ± 106 (9)	459 ± 81 (9)	1166 ± 160 ^{b,d} (7)	1361 ± 182 ^{b,d} (3)

Abbreviations: BS, bulbospongiosus muscle; i.c.v., intracerebroventricular; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; OT, oxytocin; SVP, seminal vesicle pressure.

The OT antagonist (OT antagon.) was injected via i.c.v. route 15 min prior to 7-OH-DPAT (10 µg, i.c.v.). SVP and BS EMG were recorded over 30 min following 7-OH-DPAT delivery. The number of ejaculations, and SVP and BS responses, was counted during the 30-min 7-OH-DPAT post-injection period. The latency of the first ejaculation, and SVP and BS responses after i.c.v. delivery of 7-OH-DPAT, was also determined. The values are the means ± s.e. mean of data from *n* number of rats. Statistical analysis was performed by Kruskal–Wallis + Dunn's *post hoc* test for comparison of the number of sexual responses: ^a*P* < 0.05, different from corresponding control; ^c*P* < 0.05, different from OT antagon., 0.001 µg. One-way ANOVA + Newman–Keuls' *post hoc* test for comparison of the latency of sexual responses: ^b*P* < 0.01, different from corresponding control; ^d*P* < 0.01, different from OT antagon., 0.001 µg.

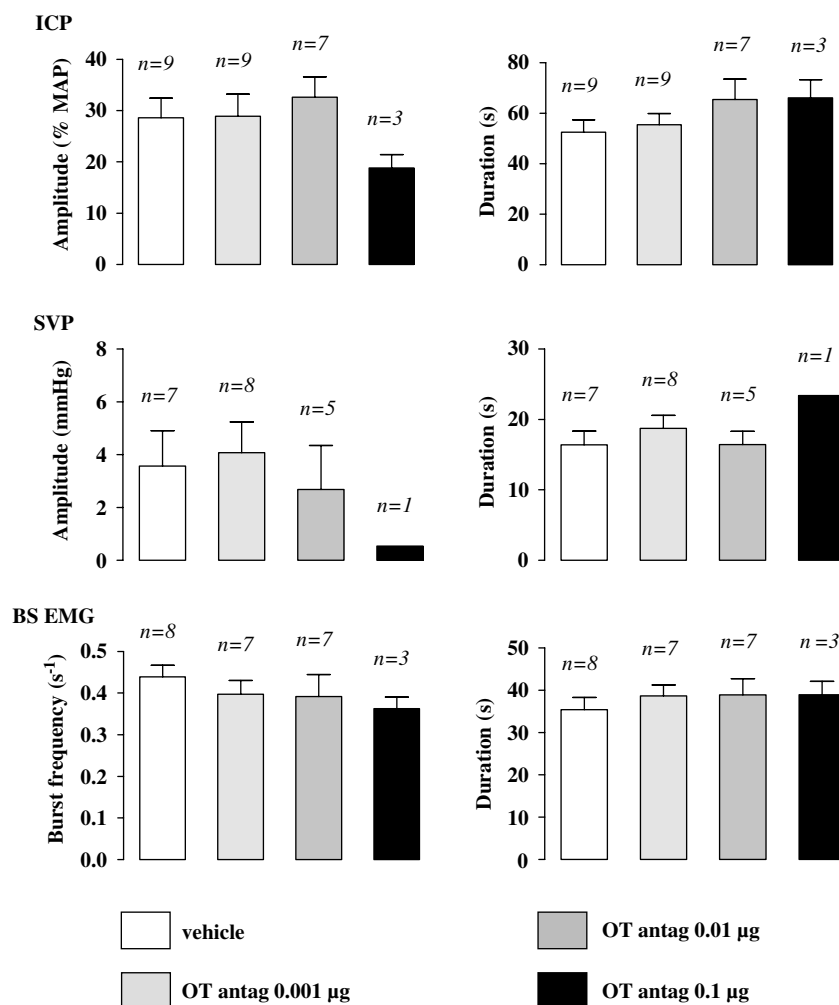


Figure 3 Effects of i.c.v. injection of oxytocin antagonist (OT antagon.) on the quantitative parameters of sexual responses induced by 7-OH-DPAT. Amplitude of ICP rises, expressed as percentage of MAP measured during the corresponding ICP rise, and duration of ICP rises were determined in rats pretreated, 15 min prior to i.c.v. 7-OH-DPAT (10 μg), with i.c.v. oxytocin antagonist vehicle, oxytocin antagonist 0.001, 0.01 or 0.1 μg . Amplitude and duration of SVP increases, frequency of burst within a contractile response of BS, as well as duration of the BS contractile response, were also determined. Bars and error bars represent the means \pm s.e.mean of n number of rats. BS, bulbospongiosus muscle; ICP, intracavernosal pressure; i.c.v., intracerebroventricular; MAP, mean arterial pressure; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin.

Table 4 Effects of i.t. oxytocin antagonist on i.c.v. 7-OH-DPAT-induced sexual responses

	OT antagon., i.t.			
	T13		L6	
	0	0.1 μg	0	0.1 μg
Number of ejaculations	0.9 \pm 0.2 (10)	0.9 \pm 0.3 (10)	0.9 \pm 0.2 (10)	0.3 \pm 0.1 ^a (10)
Latency of first ejaculation(s)	898 \pm 125 (7)	1080 \pm 163 (7)	1005 \pm 141 (6)	928 \pm 375 (3)
Number of SVP responses	2.6 \pm 1.0 (10)	3.0 \pm 0.7 (10)	2.4 \pm 0.7 (10)	1.2 \pm 0.6 (10)
Latency of first SVP response(s)	651 \pm 125 (8)	886 \pm 171 (8)	773 \pm 122 (8)	766 \pm 141 (5)
Number of BS responses	3.7 \pm 0.8 (10)	3.4 \pm 0.7 (10)	2.7 \pm 0.5 (10)	2.3 \pm 0.7 (10)
Latency of first BS response(s)	656 \pm 107 (8)	892 \pm 189 (8)	779 \pm 102 (8)	774 \pm 113 (5)

Abbreviations: BS, bulbospongiosus muscle; i.c.v., intracerebroventricular; i.t., intrathecal; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin; OT, oxytocin; SVP, seminal vesicle pressure.

The OT antagonist (OT antagon.) was injected via i.t. route 15 min prior to 7-OH-DPAT (10 μg , i.c.v.). SVP and BS EMG were recorded over 30 min following 7-OH-DPAT delivery. The number of ejaculations, and SVP and BS responses, was counted during the 30-min 7-OH-DPAT post-injection period. The latency of the first ejaculation, and SVP and BS responses after i.c.v. delivery of 7-OH-DPAT, was also determined. The values are the means \pm s.e.mean of data from n number of rats. Statistical analysis was performed by Man-Whitney's test for comparison of the number of sexual responses (same spinal level): ^a $P < 0.05$, different from corresponding control; Student's *t*-test for comparison of the latency of sexual responses.

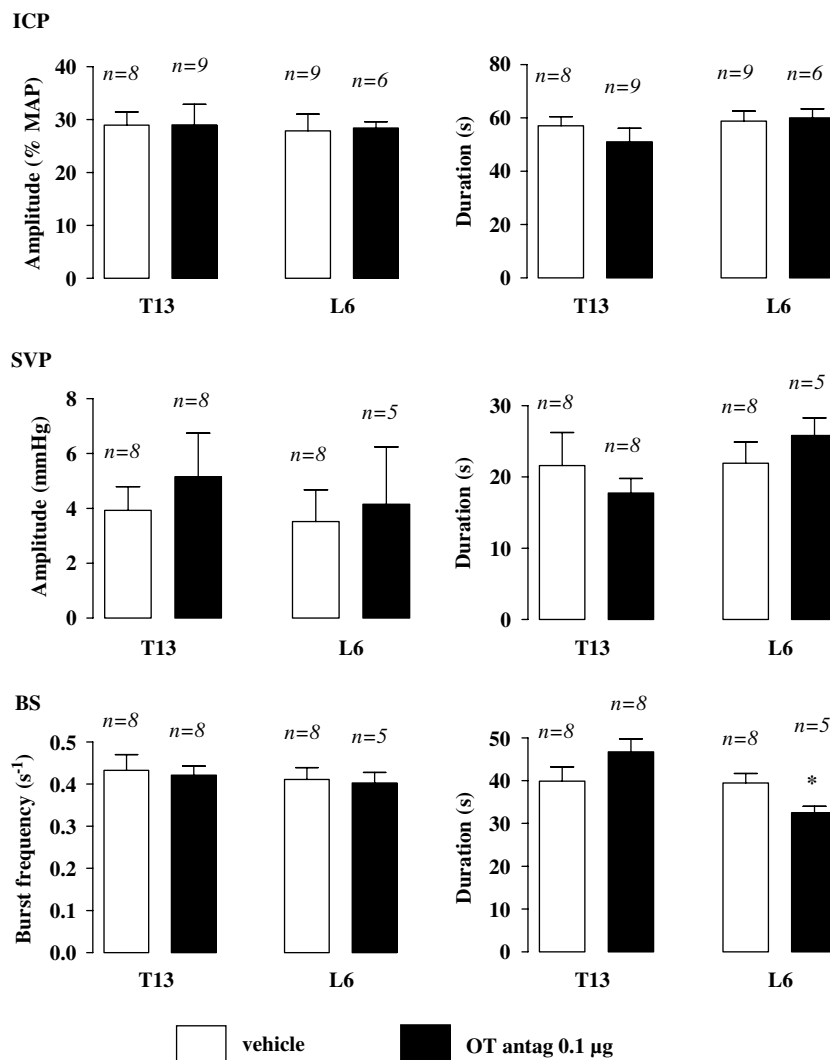


Figure 4 Effects of i.t. injection of oxytocin antagonist (OT antagon) on the quantitative parameters of sexual responses induced by 7-OH-DPAT. Amplitude of ICP rises, expressed as percentage of MAP measured during the corresponding ICP rise, and the duration of ICP rises were determined in rats pretreated via i.t. route, 15 min prior to i.c.v. 7-OH-DPAT (10 µg), with oxytocin antagonist vehicle or oxytocin 0.1 µg of the antagonist at the level of the thirteenth thoracic (T13) or the sixth lumbar (L6) segment of the spinal cord. Amplitude and duration of SVP increases, frequency of burst within a contractile response of BS, as well as duration of the BS contractile response, were determined. Bars and error bars represent the means ± s.e.mean of *n* rats. Statistical analysis was performed by one-way ANOVA + Newman-Keuls' *post hoc* test; **P* < 0.05 versus control group. ANOVA, analysis of variance; BS, bulbospongiosus muscle; i.c.v., intracerebroventricular; i.t., intrathecal; MAP, mean arterial pressure; 7-OH-DPAT, 7-hydroxy-2-(di-*N*-propylamino) tetralin.

segments (Tang *et al.*, 1998). Moreover, i.t. delivery of oxytocin at the L6 level, but not at the level of the thoracic sympathetic neurons (that is, T12–T13), induces ICP increase in anaesthetized rats, indicating that activation of oxytocin receptors at the L6 level exerts a pro-erectile effect (Giuliano *et al.*, 2001). These findings are partly corroborated by the present results showing that injection of the oxytocin antagonist at either the T13 or L6 spinal level did not impair 7-OH-DPAT-induced erection (Table 2). It is not clear why injection of the oxytocin antagonist at the L6 level was without any effect on 7-OH-DPAT-induced erection, although a difference in the pro-erectile mechanisms that were recruited is a possibility. It should be noted that ICP increases elicited by 7-OH-DPAT occurred after initiation of SVP increases and BS contractions. This indicates that the ejaculatory response preceded the erectile response under

the conditions of our study. This is in contradiction with the sequence of events taking place in a natural context and led us to assume that, in the 7-OH-DPAT model erections could well be reflexive responses. Reflexive erection is a spinal reflex induced by pelvic afferent inputs. Oxytocin has been reported to mediate non-contact erection (psychogenic erection) and erection elicited by centrally acting compounds (Melis *et al.*, 1989, 1997), but involvement of oxytocin in reflexive erection has not been documented.

Impairment of 7-OH-DPAT-induced ejaculation was observed in rats injected with the oxytocin antagonist via i.t. route at the L6 level (Table 4). This may be explained by a significant decrease in the mean duration of BS responses (Figure 4). The reason for this decrease is, however, unknown. No immunohistochemical studies have reported the existence of oxytocin receptors or fibres in Onuf's

nucleus, where the motoneurons controlling the BS are located. In addition, there is no clear evidence for involvement of oxytocin in mediation or modulation of somatomotor outputs. However, binding sites for oxytocin have been detected in neurons of the dorsal grey commissure in the L6–S1 spinal segments (Veronneau-Longueville *et al.*, 1999), which send projections to lumbosacral somatic motoneurons (Sasek *et al.*, 1984). From this, it can be suggested that, upon L6 delivery, the oxytocin antagonist, by acting on dorsal grey column neurons, modulated the activity of the somatic motoneurons controlling the BS, and this resulted in changes in BS contraction parameters.

Experimental evidence supports a role of brain oxytocin in the expression of sexual behaviour, including penile erection and ejaculation. Anatomical studies using immunohistochemistry have shown the presence of oxytocin and/or oxytocin receptors in several brain areas known to be involved in the control of erection and other aspects of male sexual behaviour, such as the PVN, the bed nucleus of the stria terminalis, the medulla oblongata and both lobes of the pituitary gland (Freund *et al.*, 1987; Elands *et al.*, 1988; Adan *et al.*, 1995). Oxytocin, given centrally in nanogram amounts to male rats, elicits erection (Argiolas *et al.*, 1985) and facilitates copulatory behaviour (Arletti *et al.*, 1985). Conversely, i.c.v. administration of oxytocin antagonists prevents oxytocin-induced erection (Melis *et al.*, 1989) and non-contact erection by presenting to a male rat a non-accessible oestrous female (Melis *et al.*, 1997). Furthermore, i.c.v. delivery of oxytocin antagonists impairs the copulatory behaviour of male rat by decreasing mount, intromission and ejaculation frequencies (Argiolas *et al.*, 1988a). A role for oxytocin receptors in mediating the pro-sexual activity of dopamine agonists has also been described. Indeed, erection and facilitation of male sexual behaviour induced by the non-selective dopamine agonist apomorphine are inhibited by blockade of brain oxytocin receptors (Argiolas *et al.*, 1988b; Melis *et al.*, 1989). The present reversal of 7-OH-DPAT-induced erection and ejaculation by i.c.v. delivery of the oxytocin antagonist (Tables 2 and 3) is in agreement with these previous observations. Interestingly, dopamine has been identified as one of the neurotransmitters capable of activating PVN oxytocin neurons, inducing penile erection and facilitating copulation (Argiolas and Melis, 2004; Succu *et al.*, 2007). As a whole, these data suggest a close relationship between dopamine and oxytocin in the central control of sexual responses. We have recently found that 7-OH-DPAT delivered into the medial preoptic area elicits ejaculation in anaesthetized rats (Kitrey *et al.*, 2007). Neuroanatomical studies have established the existence of projections from the medial preoptic area to the parvocellular part of the PVN, where oxytocin-synthesizing neurones are localized, which in turn send axons to spinal lumbosacral preganglionic neurons involved in the control of male sexual responses (Luiten *et al.*, 1985; Simerly and Swanson, 1988; Tang *et al.*, 1998). Therefore, it can be hypothesized that stimulation of dopamine D₃ receptors in the medial preoptic area, and to a lesser extent D₂ receptors, increases PVN oxytocin release, which activates spinal autonomic nuclei controlling peripheral events related to erection and ejaculation. Whether this mechanism can account for all the behavioural effects of

oxytocin and synthetic ligands of oxytocin receptors is yet to be ascertained, but it is likely that interactions between oxytocin and other neurochemical systems take place. Notably, interaction between 5-hydroxytryptaminergic and oxytocinergic neurotransmissions is important in a sexual context (de Jong *et al.*, 2007).

In conclusion, brain oxytocin receptors mediate ejaculation induced by 7-OH-DPAT, and L6 spinal oxytocin receptors have a modulating role in 7-OH-DPAT-induced ejaculation, whereas peripheral oxytocin receptors appear to be involved marginally. These findings provide further insights into the role of oxytocin in male sexual functions and might be helpful for future pharmacological research, in particular for treatment of ejaculatory dysfunctions, including premature ejaculation.

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Conflict of interest

The authors state no conflict of interest.

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