Research article

Central effects of clozapine in regulating micturition in anesthetized rats

Pedro L Vera*[1,3,5], Alejandro J Miranda-Sousa[2,3], Raul C Ordorica[2,3,5] and Irving Nadelhaft[1,3,4,5]

Address: [1]Bay Pines VA Medical Center Research and Development Service Bay Pines, FL. 33744, USA, [2]James A. Haley VA Medical Center, Urology Section, Tampa, FL 33612, USA, [3]University of South Florida, Dept of Surgery, Division of Urology, Tampa, FL 33612, USA, [4]University of South Florida, Dept of Pharmacology, Tampa, FL. 33612, USA and [5]H. Lee Moffitt Cancer and Research, Institute Division of Urology, Interdisciplinary Oncology Group, Tampa, FL 33612, USA

E-mail: Pedro L Vera* - pvera@hsc.usf.edu; Alejandro J Miranda-Sousa - amiranda@hsc.usf.edu; Raul C Ordorica - rordoric@tampabay.rr.com; Irving Nadelhaft - inadelha@hsc.usf.edu

*Corresponding author

Abstract

Background: We previously showed that systemic administration of the atypical neuroleptic clozapine in the rat altered a number of urodynamic variables and inhibited the external urethral sphincter. Since clozapine acts at several receptor types both at the periphery and the central nervous system, the site of action remained uncertain. Therefore, the purpose of this study was to determine the effects of central administration of clozapine on the bladder and the external urethral sphincter during cystometry and to examine differences in spinal versus supraspinal administration. We extended our observations by delivering clozapine centrally in anesthetized rats instrumented with either an intrathecal (L6-S1 spinal segment) or an intracerebroventricular (lateral ventricle) catheter.

Results: Clozapine decreased micturition volume and increased residual volume possibly by acting at a supraspinal site. Expulsion time and amplitude of the high frequency oscillations were reduced by clozapine possibly by acting at a spinal site. Bladder capacity was increased after central clozapine but probably due to a peripheral effect. Clozapine acting at spinal and supraspinal sites increased pressure threshold. Contraction time and peak pressure were not affected by clozapine. The EMG from the external urethral sphincter was also reduced following clozapine centrally and suggests a spinal and a supraspinal site of action.

Conclusions: The results from the present study suggest that spinal and supraspinal central sites mediate clozapine's action in inhibiting expulsion parameters and the external urethral sphincter of the rat. Therefore, the reduction in the voiding efficiency observed after clozapine appears to be mediated by spinal and supraspinal sites.

Background

Clozapine is a widely used atypical neuroleptic with affin- ity for multiple receptors, including dopamine, serotonin, alpha adrenergic, muscarinic and histaminergic receptors

[1,2]. Although clozapine is effective in the treatment of schizophrenia refractory to traditional antipsychotic medication, it also has a number of significant side effects ranging from the potentially fatal but rare agranulocytosis to weight gain, constipation, seizures and urinary incontinence [3]. Urinary problems have been reported in the clinical literature with incontinence present in up to 44% of patients [4] and enuresis in 27% of patients [5]. Since clozapine has potent anti-muscarinic and anti-alpha adrenergic effects [2], it has been proposed that peripheral effects on the lower urinary tract might be responsible for the micturition disturbances [6]. Incontinence has also been reported following therapy with other atypical neuroleptics such as risperidone [7–9] and recently, olanzapine [10].

We have previously shown that intravenous administration of clozapine to anesthetized rats [11,12] altered several micturition parameters including a decreased amount of volume voided per micturition and a concomitant increase in the residual volume. In addition, clozapine also inhibits the external urethral sphincter. Other, newer, atypical neuroleptics (e.g. olanzapine and risperidone; [13]) were also found to have similar effects but with differing potency compared to clozapine. Since risperidone has little or no anti-muscarinic activity, a primary anti-muscarinic peripheral effect may be ruled out as the cause of micturition disturbances following risperidone administration, and suggest a possible central role for atypical neuroleptics in regulating micturition.

The purpose of the present study was to determine the effects of clozapine administered centrally at two different sites, spinal (L6-S1 spinal segments) vs. supraspinal (lateral ventricle), on micturition and the external urethral sphincter during cystometry in the anesthetized rat. By limiting the application site to a specific area of the central nervous system and comparing the results with our previous findings after systemic administration [12] it might be possible to determine whether the effects of clozapine are mediated at a central or peripheral level. Moreover, a comparison of the two central routes might indicate whether the central effect involves spinal or supraspinal structures. We report in this study that most of the effects of clozapine on micturition are mediated by its central effects. Furthermore, there are differences in spinal versus supraspinal effects.

Results
Effects of central administration of clozapine on urodynamic parameters during single cystometry (Table 1I)
Figures 1 and 2 show representative examples of the effects of clozapine on single cystometry in anesthetized rats after intrathecal (i.t.; L6-S1 spinal segment) and intracerebroventricular (i.c.v.; lateral ventricle) administration, respectively.

Bladder capacity was increased by clozapine given i.t. or i.c.v. only at the highest dose tested (Fig 1G; 2G). After 50 nmoles of clozapine i.t. the bladder capacity was 0.47 ± 0.09 ml, compared to 0.29 ± 0.027 ml after vehicle administration (Fig 3A). Similarly, after 50 nmoles of clozapine i.c.v the bladder capacity was 0.51 ± 0.11 ml, compared to 0.25 + 0.027 ml after vehicle administration (Fig 3A).

Table 1: 1. Effects of intrathecal (L6/S1 spinal level) administration increasing doses of clozapine on urodynamic and EMG parameters in anesthetized rats. Values are Mean ± S.E.M.

Dose (nmoles)	Urodynamic parameters								EUS EMG parameters			
	BC (ml)	MV (ml)	RV (%)	PT (mm Hg)	ct (sec)	PP (mm Hg)	ET (sec)	HFO (mm Hg)	Phase 1 (%)	Phase 2 (%)	Phase 3 (%)	Burst Amp. (%)
Control	0.29 (± 0.04)	0.18 (± 0.022)	34 (± 7.5)	3.4 (± 0.46)	16.9 (± 1.32)	13.1 (± 0.82)	2.4 (± 0.30)	1.5 (± 0.10)	100	100	100	100
0.5	0.30 (± 0.02)	0.17 (± 0.018)	43 (± 4.1)	3.3 (± 0.79)	17.0 (± 2.00)	13.3 (± 1.20)	1.9 (± 0.55)	1.3 (± 0.26)	98 (± 10.3)	89 (± 26.9)	116 (± 12.0)	84 (± 17.0)
5.0	0.32 (± 0.06)	0.11** (± 0.031)	69** (± 6.2)	3.3 (± 0.49)	22.1 (± 1.49)	12.3 (± 0.66)	0.7**,a (± 0.46)	0.4**,b (± .23)	91 (± 20.0)	37* (± 24.0)	94.7 (± 21.8)	32**,a (± 20.5)
50	0.47** (± 0.09)	0.08** (± 0.019)	79** (± 4.3)	6.0** (± 0.86)	21 (± 3.39)	13.6 (± 0.72)	0.9* (± 0.93)	0.5** (± 0.19)	93 (± 24)	40* (± 14.0)	72 (± 14.8)	66 (± 22.2)

Table 2: 2. Effects of intracerebroventricular (Lateral ventricle) admnistration of increasing doses of clozapine on urodynamic and EMG parameters in anesthetized rats. Values are Mean ± S.E.M.

	Urodynamic parameters								EUS EMG parameters			
Dose (nmoles)	BC (ml)	MV (ml)	RV (%)	PT (mm Hg)	CT (sec)	PP (mm Hg)	ET (sec)	HFO (mm Hg)	Phase 1 (%)	Phase 2 (%)	Phase 3 (%)	Burst Amp. (%)
Control	0.25 (± 0.027)	0.16 (± 0.018)	33 (± 7.4)	2.9 (± 0.29)	19.4 (± 1.5)	12.4 (± 0.46)	3.0 (± 0.28)	1.6 (± 0.11)	100	100	100	100
0.5	0.26 (± 0.026)	0.11*,b (± 0.02)	54* (± 8.5)	3.6 (± 0.30)	21.6 (± 3.0)	12.0 (± 0.90)	2.0 (± 0.33)	1.4 (± 0.19)	117 (± 9.4)	68* (± 13.9)	95 (± 4.7)	101 (± 7.0)
5.0	0.30 (± 0.022)	0.11* (± 0.019)	62** (± 3.8)	3.3 (± 0 44)	22.2 (± 25)	12.6 (± 0 73)	1.8* (± 0.35)	1.3 (± 0.07)	128 (+15 1)	54** (± 8.4)	101 (± 10.0)	94 (± 5.5)
50	0.51** (± 0.11)	0.084** (± 0.014)	80** (± 5.2)	7.2** (± 1.46)	18.7 (± 1.9)	13.6 (± 0.34)	1.3** (± 0.39)	0.6** (± 0.20)	62* (± 14.9)	26** (± 9.9)	50* (± 14.9)	65* (± 17.2)

* $p < 0.05$ compared to control; ** $p < 0.01$ compared to control; [a] $p < 0.05$ compared to same dose, different route; [b] $p < 0.01$ compared to same dose, different route

Micturition volume was significantly decreased after 5.0 and 50 nmoles of clozapine i.t. (Fig 3B) to 0.11 ± 0.031 and 0.08 ± 0.019 ml, respectively compared to 0.18 ± 0.022 ml after vehicle. In the case of intracerebroventricular administration, 0.5 nmoles of clozapine decreased the micturition volume to 0.11 ± 0.02 ml compared to 0.16 ± 0.018 ml after vehicle (Fig 3B). The effect of 0.5 nmoles of clozapine i.c.v. on micturition volume was significantly different from the effect of the same dose given i.t. Further reductions in micturition volume were observed after 50 nmoles of clozapine i.c.v.

Residual volume was significantly increased after 5.0 and 50 nmoles of clozapine i.t. to $69 \pm 6.2\%$ and $79 \pm 4.3\%$, compared to $34 \pm 7.5\%$ after vehicle administration (Fig 3C). Clozapine i.c.v. resulted in a significant increase in the residual volume at all doses, including 0.5 nmoles. After 0.5 nmoles i.c.v. the residual volume was $54 \pm 8.5\%$ compared to $33 \pm 7.4\%$ after vehicle. This residual volume was not significantly different from the one observed after the same dose i.t. Larger doses of clozapine i.c.v. resulted in further increases in the residual volume to 62 ± 3.8 and $80 \pm 5.2\%$ after 5.0 and 50 nmoles, respectively.

Clozapine i.t. or i.c.v caused an increase in the pressure threshold only at the highest doses tested (Fig 1G; 2G; 4A). After 50 nmoles i.t. the pressure threshold was 6.0 ± 0.86 compared to 3.4 ± 0.46 mm Hg after vehicle, whereas after i.c.v. administration the same dose increased the pressure threshold to 7.2 ± 1.46 mm Hg compared to 2.9 ± 0.29 mm Hg after vehicle.

Clozapine i.t. or i.c.v had no effect on either peak pressure or contraction time in the range of doses tested (Fig 4B,5A) in this study.

Expulsion time was significantly decreased by clozapine at 5.0 and 50 nmoles either i.t. or i.c.v (Fig 5B). After vehicle i.t. the expulsion time was 2.4 ± 0.3 sec, which decreased to 0.7 ± 0.46 and 0.9 ± 0.93 sec after 5 and 50 nmoles i.t. Similarly, after vehicle i.c.v the expulsion time was 3.0 ± 0.28 sec and 5.0 and 50 nmoles of clozapine i.c.v decreased it to 1.8 ± 0.35 and 1.3 ± 0.39 sec, respectively. The effect of 5.0 nmoles of clozapine i.t. on the expulsion time was significantly different from the effect observed after i.c.v administration of this dose (Fig 5B).

Clozapine also decreased the amplitude of (and in some animals even abolished) the high frequency oscillations (HFO) after i.t. administration (Fig 1; 5C). After 5.0 nmoles of clozapine the HFO were 0.4 ± 0.23 compared to 1.5 ± 0.1 mm Hg after administration of vehicle. In 4 of the 6 animals in this group, 5 nmoles of clozapine i.t. abolished the HFO. The reduction in amplitude observed at this dose after i.t. administration of significantly different from the effects observed after the same dose i.c.v. (Fig 5C). A greater dose of clozapine did not cause a larger effect although the amplitude of the HFO after 50 nmoles of clozapine i.t. was still significantly reduced (0.5 ± 0.19 mm Hg) when compared to vehicle administration. Clozapine i.c.v decreased the amplitude of the HFO only at 50 nmoles (0.6 ± 0.2 mm Hg compared to 1.6 ± 0.11 mm Hg after vehicle administration; Fig 2), and the HFO were abolished in only 1/6 of the animals in this group.
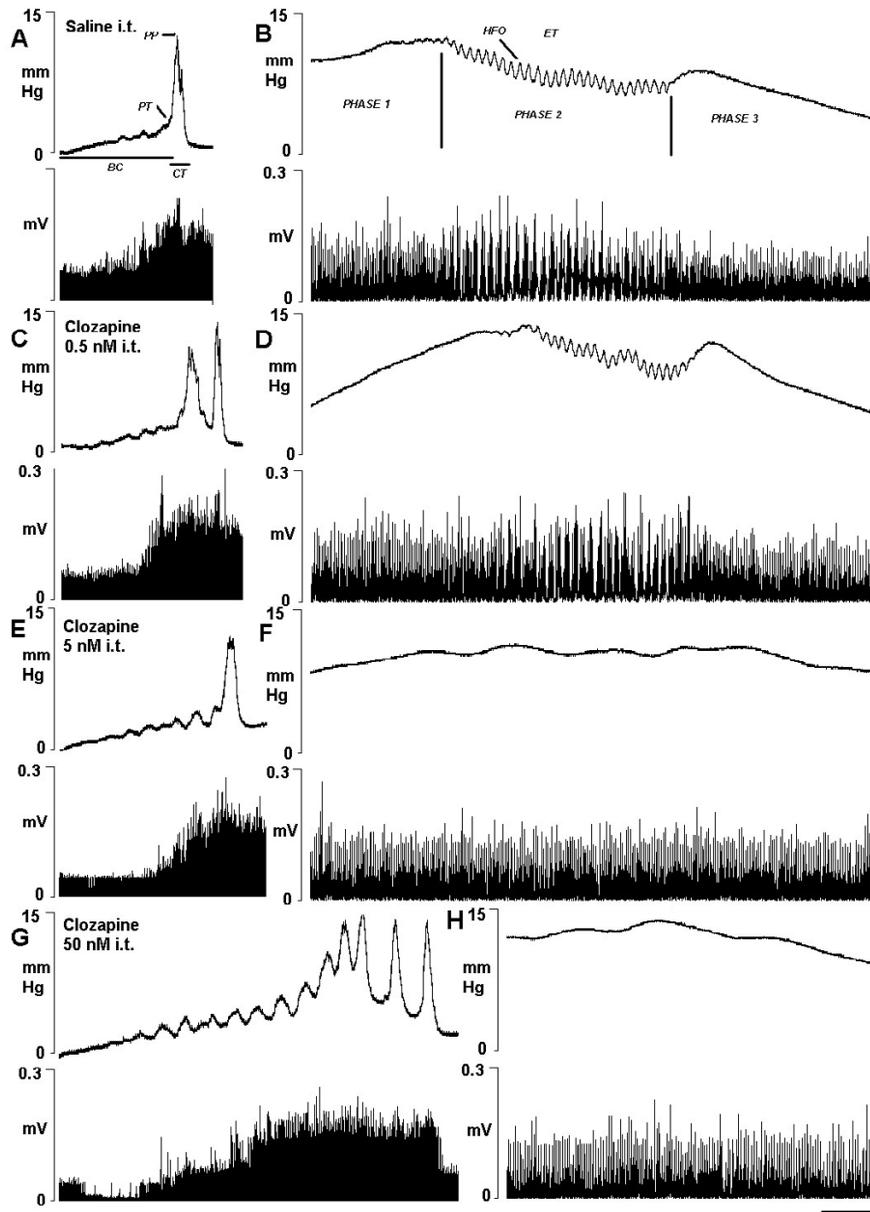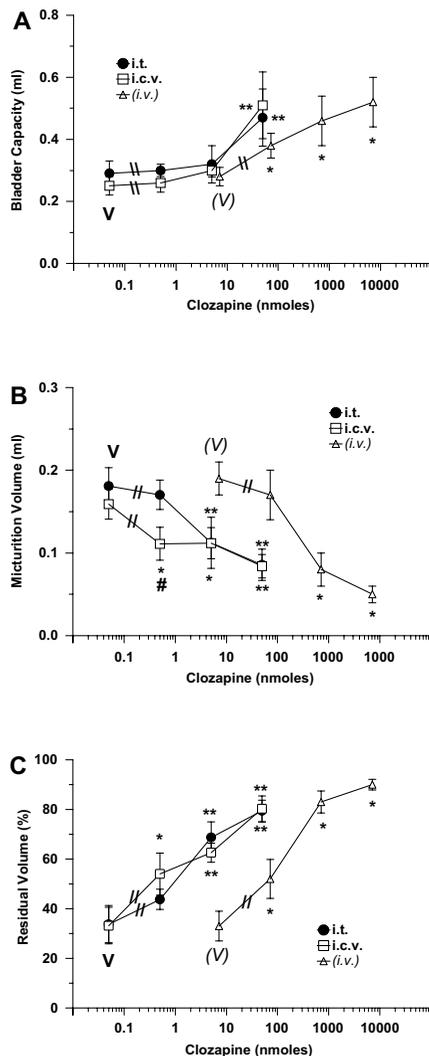
Figure 1
Representative traces of the effects of increasing dose of clozapine administered intrathecally (over the L6/S1 spinal segments) on the cystometrogram and external urethral sphincter of anesthetized rats (n = 6). Top panel shows bladder pressure during filling (0.11 ml/min) while bottom panel shows the integrated EMG recorded from the external urethral sphincter. Panels A and B also show some of the urodynamic parameters measured during cystometry: BC = amount of fluid infused into the bladder to elicit a contraction; PT = pressure at which contraction begins; PP = peak pressure during contraction; CT = contraction time; HFO = high frequency oscillations recorded during expulsion; ET = time between peak pressure and end of high frequency oscillations. The EMG activity was examined by dividing the contraction into 3 phases [19]: a contraction phase (Phase 1); an expulsion phase (Phase 2); a closing phase (Phase 3). A) Cystometrogram (CMG) following administration of vehicle (saline) intrathecally. (B) Expanded time scale shows the HFO and the concomitant bursting pattern in the EUS EMG. C & D) 0.5 nmoles of clozapine i.t. does not have an effect on the CMG or the EUS EMG. E & F) 5 nmoles of clozapine i.t. abolished the HFO (4/6 rats) and the bursting pattern in the EUS EMG. G & H) 50 nmoles of clozapine i.t. increased the bladder capacity and also abolished the HFO and the bursting pattern in the EUS EMG of some rats (2/6). Unstable, non-voiding contractions were occasionally seen following clozapine administration and preceding the final voiding contraction (1C; 1G). Calibration bar: A,C,E,G = 1 min; B,D,F,H = 1 sec.
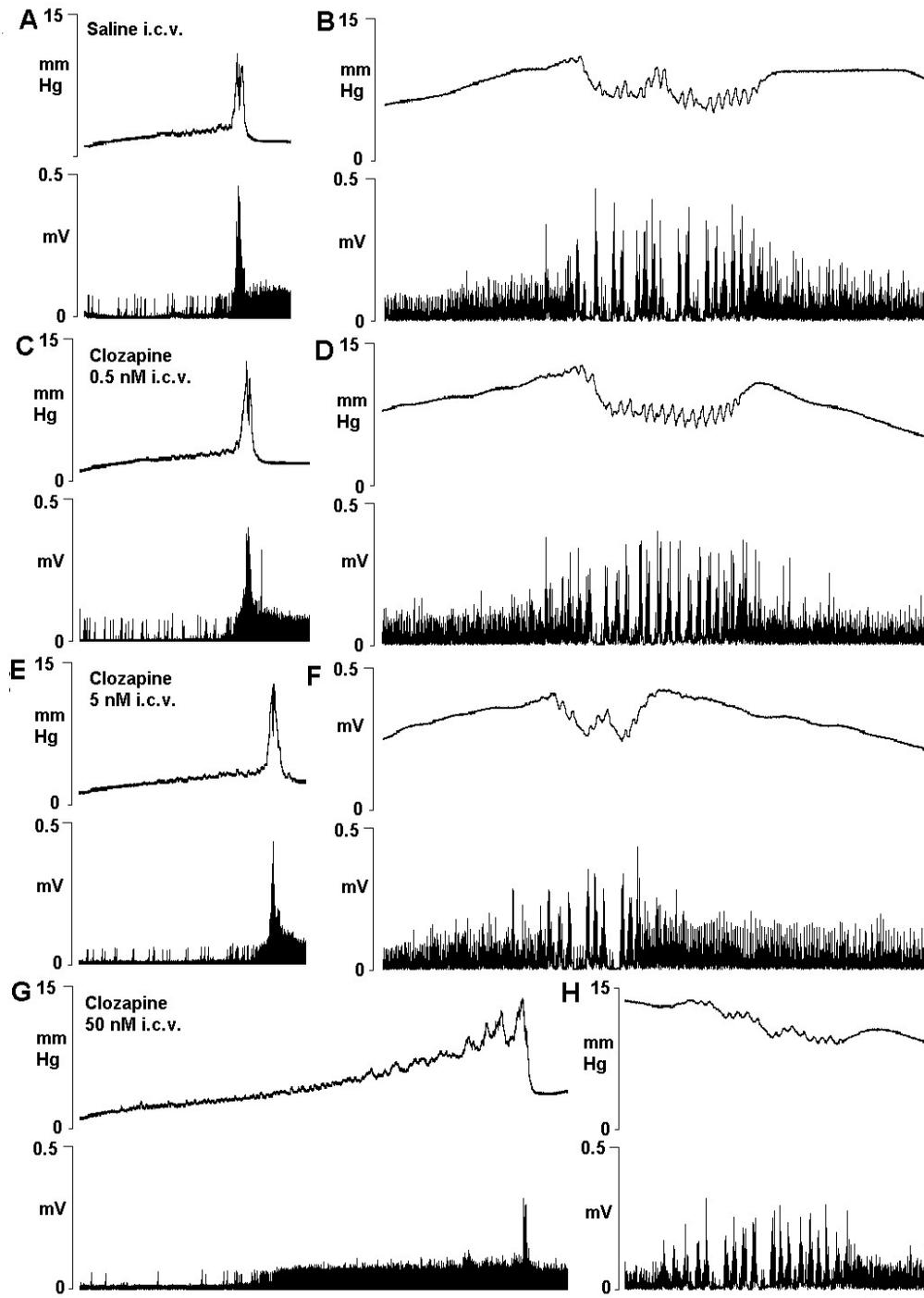
Figure 2
Representative traces of the effects of clozapine administered intracerebroventricularly (lateral ventricle) on the cystometrogram and activity of the external urethral sphincter of anesthetized rats. A & B) CMG after giving saline i.c.v. C & D) 0.5 nmoles of clozapine i.c.v. did not show appreciable effects on the CMG or the EUS EMG. E & F) CMG after 5 nmoles of clozapine i.c.v. Note a reduction in expulsion time and the amplitude of the HFO. G & H) 50 nmoles of clozapine i.c.v. increased the bladder capacity. However, the HFO are still present although reduced. In none of the rats were the HFO abolished by clozapine i.c.v contrasting the effects observed after spinal administration (Fig. 1). Non-voiding contractions are observed in this animal prior to the final voiding contraction (2G). Calibration bar: A, C,E,G = 1 min; B,D,F,H = 1 sec.

Figure 3
Effects of increasing doses of clozapine administered intrathecally (i.t.) or intracerebroventricuarly (i.c.v.) on bladder capacity, micturition volume and residual volume. For comparison purposes, the effects of intravenous admnistration of clozapine (data from [12]) are also shown. Doses are presented in a log scale, with vehicle marked as V for the results of the present experiments, and (V) for vehicle in the i.v. doses. A) Bladder capacity increased only after 50 nmoles of clozapine either i.t. or i.c.v.; B) Micturition volume decreased after clozapine i.t. or i.c.v. However, 0.5 nmoles of clozapine i.c.v. significantly reduced the micturition volume and this effect was also significantly different from that obtained after the same dose i.t. C) Residual volume was increased by clozapine i.t. or i.c.v. All doses of clozapine i.c.v. produced significant increases in the residual volume, whereas only 5 and 50 nmoles i.t. produced significant results. * = $p < 0.05$; ** = $p < 0.01$; # = $p < 0.05$ when compared to the effects of the same dose, different route.

Effects of central administration of clozapine on the activity of the external urethral sphincter during single cystometry (Table II)
Clozapine i.t. did not have an effect on the activity of the EUS during Phase 1 (the rising phase during a contraction), and only the highest dose of clozapine i.c.v (50 nmoles) was found to decrease the activity of the EUS (Fig 6A) to $62 \pm 14.9\%$ of that seen during administration of vehicle.

Phase 2 of the EUS EMG (occurring during the time of HFO) was decreased by clozapine either i.t or i.c.v. 5 and 50 nmoles of clozapine i.t. decreased the EMG to 37 ± 24 and $40 \pm 14\%$ of that observed during administration of vehicle, respectively (Fig 6B). In fact, in 4/6 animals, 5.0 nmoles of clozapine i.t. abolished the bursting pattern of the EMG observed during phase 2 (Fig 1F). Clozapine administered i.c.v at a dose of 0.5 nmoles decreased the EUS EMG to $68 \pm 13.9\%$ of the activity observed after vehicle administration (Fig 6B). Larger doses (5 and 50 nmoles) of clozapine i.c.v further reduced the EUS activity during this phase to $54 \pm 8.4\%$ and $26 \pm 9.9\%$. Clozapine i.c.v, however, was not observed to abolish the bursting pattern during this phase (Fig 2), except in 1/6 animals.

Phase 3 of the EUS EMG (recorded during the falling phase of a bladder contraction) was not affected by clozapine administered intrathecally (Fig 6C). However, the largest dose of clozapine given i.c.v (50 nmoles) decreased the activity to $50 \pm 14.9\%$ of the activity observed during vehicle administration.

Finally, the amplitude of the bursts observed during phase 2 in the EUS EMG were decreased to $32 \pm 20.5\%$ by 5.0 nmoles of clozapine i.t. (Fig 6D). A similar dose of clozapine i.c.v had no effect. However, 50 nmoles of clozapine i.c.v. decreased the amplitude of the bursts to $65 \pm 17.2\%$ of that observed during vehicle administration.

Discussion
In the present experiments, central application of clozapine (intrathecally over the L6/S1 spinal segments or intracerebroventricularly into the lateral ventricle) resulted in a number of changes in the urodynamic parameters of anesthetized rats. The major effect of clozapine was to decrease the voiding efficiency of the bladder by inhibiting expulsion parameters, such as micturition volume, residual volume and expulsion time. In addition, the activity of the EUS also decreased upon central application of clozapine. One problem in delivering substances centrally is the possibility that peripheral spread may confound the results. Since clozapine crosses the blood-brain barrier readily [14], the maximum dose selected in the present experiment was restricted to the first appearance of a decrease in blood pressure as an indication of possible
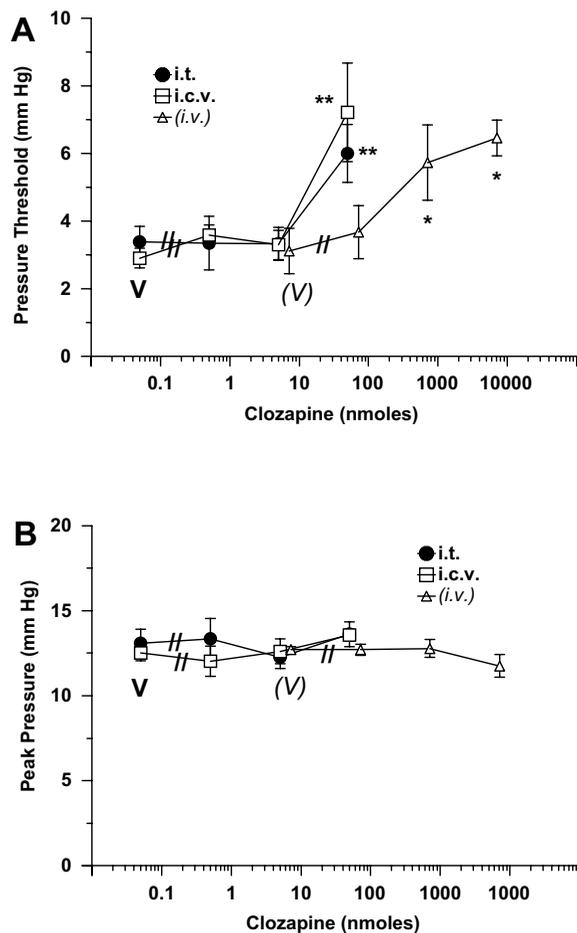
Figure 4
Effect of clozapine i.t. or i.c.v on pressure threshold and peak pressure. Data from intravenous administration [12] are shown for comparison. A) Pressure threshold increased only after the highest dose (50 nmoles) of clozapine either i.t. or i.c.v.; B) Clozapine i.t. or i.c.v. had no effect on the peak pressure. V= vehicle administration (saline) for i.t. or i.c.v. groups; (V) = vehicle administration for i.v. group. * = p < 0.05; ** = p < 0.01

peripheral alpha 1 antagonism due to peripheral leakage. We compared the effects obtained in the present study against those observed previously after intravenous administration [12] expecting that central effects would require significantly less application of clozapine than systemic administration. We considered a minimum difference of 10× magnitude in the central vs. peripheral dose that elicited a significant effect as an indication of possible central action. Our doses of 0.5, 5 and 50 nmoles of clozapine correspond to serum levels of 8, 80 and 800 ng/ml (assuming a blood volume of 8% of body weight)

and are in the range of therapeutic levels (260–387 ng/ml [5,15]).

A second problem in administering a substance at a particular central location is that of redistribution to other parts of the central nervous system. Since application of a substance at the lumbosacral spinal level may travel to the brain after some time and vice versa, we established two criteria to help determine a possible central site of action: 1) the first dose to elicit a significant effect by either i.t. or i.c.v administration; 2) significant differences at the same dose but different routes (i.t. vs i.c.v).

Figure 7 is a summary of our findings when interpreted in light of the criteria stated above for determining (a) central vs peripheral effects and (b) spinal vs supraspinal site of action. The effects are presented as percent change over the control (vehicle) dose, for each of the central routes in the present experiment and our previously reported findings for systemic administration [12]. Theses changes are plotted at the dose (mg/kg) that first yielded significant results. The route that we consider most likely to be the site of action for clozapine is italicized.

Bladder capacity was increased to a similar degree by an equivalent dose of clozapine, regardless of route (Fig 7A; 3A), making a determination of most likely site of action difficult. Given that the peripheral effects at low doses were similar to the effects after the highest central dose of clozapine (by either route) it appears likely that this effect is due to peripheral actions of clozapine. Bladder capacity was reported to increase following i.c.v. administration of muscarinic antagonist (atropine [16]) or i.t. alpha2 antagonists (yohimbine [17]; atipemazole [18]). Given clozapine's strong affinity for muscarinic and alpha2 receptors [2] it is possible that central effects are also contributing to the increase in bladder capacity.

Micturition volume, on the other hand, showed a reduction after smaller doses of clozapine i.c.v or i.t. when compared to the i.v. dose (Fig 7B;3B). Therefore, central effects of clozapine in controlling micturition volume are likely. Furthermore, when comparing the effects of i.t. vs. i.c.v administration, a 5 nmoles dose of clozapine i.c.v. elicited a reduction in micturition volume that was significantly greater from the effects observed after the same dose i.t. (Fig. 3B). Therefore, supraspinal effects of clozapine in regulating micturition volume are likely with spinal effects perhaps contributing. Clozapine i.t. also decreased the micturition volume, however the dose that first showed a significant effect was higher than the i.c.v. but smaller than the i.v. dose. Spinal antagonism of either alpha1 or alpha2 adrenergic receptors was reported to increase micturition volume [17] in anesthetized rats. However, intrathecal atipamezole (alpha2 antagonist)
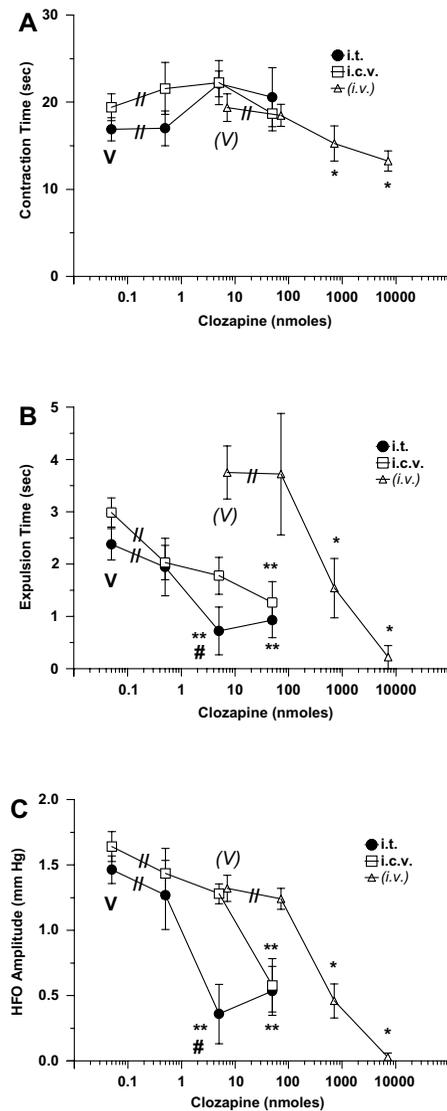
Figure 5
Effect of clozapine i.t. or i.c.v. on contraction time, expulsion time and amplitude of the high frequency oscillations (HFO). Data from intravenous administration of clozapine [12] are presented for comparison. A) Contraction time was not affected by clozapine i.t. or i.c.v.; B) Expulsion time was decreased by both clozapine i.t. or i.c.v. However, 5 nmoles of clozapine i.t. resulted in a greater reduction in expulsion time than the same dose given i.c.v. Only the highest dose (50 nmoles) resulted in a decrease in the expulsion time when given i.c.v. C) The amplitude of the HFO was significantly reduced by 5 and 50 nmoles ofclozapie i.t. but only by 50 nmoles of clozapine i.c.v. The effect of 5 nmoles of clozapine i.t. was significantly different from the same dose given i.c.v. V= vehicle administration (saline) for i.t. or i.c.v. groups; (*V*) = vehicle administration for i.v. group. * = p < 0.05; ** = p < 0.01; # = p < 0.05 when compared to the effects of the same dose, different route.

increased residual volume in awake rats [18]. Therefore, it is difficult to interpret clozapine's effects on micturition volume in terms of spinal alpha adrenoceptor antagonism and possibly the supraspinal effects predominate.

Clozapine given i.t. or i.c.v. also increased the residual volume (Fig 7C; 3C). Since the effects were observed at doses that were lower than those after i.v. administration, a peripheral effect of clozapine to increase residual volume appears unlikely. The smallest dose of clozapine given i.c.v. (0.5 nmoles) increased residual volume to 160% (Fig 7C), suggesting that supraspinal effects predominate with possible contributing effects from spinal sites. Ishiura et al. [16] reported a decrease in voiding efficiency (comparable to an increase in residual volume) following atropine i.c.v. or i.t. suggesting that muscarinic receptors at supraspinal and spinal sites are involved and may account for our effects after clozapine i.t. or i.c.v. A decrease in residual volume has been reported following spinal antagonism of alpha2 receptors with yohimbine [17] but atipamezole produced an increase in the residual volume [18], similar to our findings with clozapine i.t.

Clozapine increased pressure threshold after i.c.v. or i.t. administration only after 50 nmoles (Fig 7D; 4A). It should be noted that both central doses were at the range observed to result in cardiovascular changes and therefore the possibility of peripheral leakage of clozapine must be considered. Still the effective central doses are approximately 14× less than the first dose observed to produce significant results intravenously, and suggests a possible central site of action.

Peak pressure was not changed by clozapine after either route in the present study after central administration, consistent with our findings afer intravenous administration [12]. Ishiura et al. [16] reported a decrease in maximal voiding pressure (equivalent to our peak pressure) after atropine i.v., i.c.v or i.t. in awake rats undergoing continuous cystometry. Given that clozapine has a relatively high affinity for muscarinic receptors [1,2] it is surprising that we have not seen an effect on peak pressure. Clozapine, and also olanzapine [11,13] were able to decrease the contraction amplitude after electrical stimulation of the pelvic nerve. Since the contraction pressures observed during cystometry were smaller than those observed after electrical stimulation of the pelvic nerve (and against a closed urethra) it is possible that the antimuscarinic effects of clozapine on bladder contraction pressure are not detected because during cystometry maximal bladder pressures are not necessary for emptying.

Contraction time was not affected by clozapine i.c.v or i.t. (Table 1 and 2; Fig 5A) however it was clearly decreased after intravenous administration [12]. Therefore, we sus-
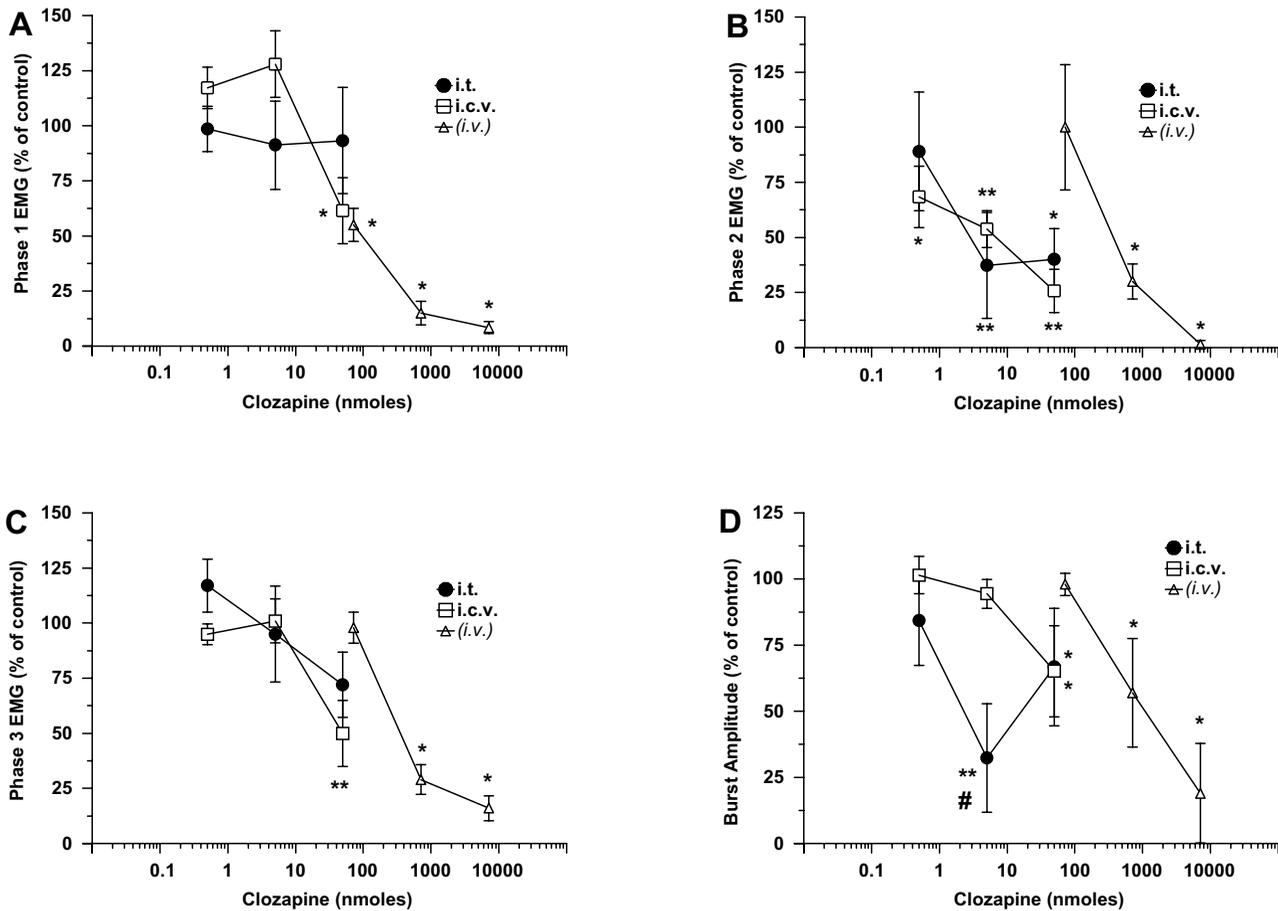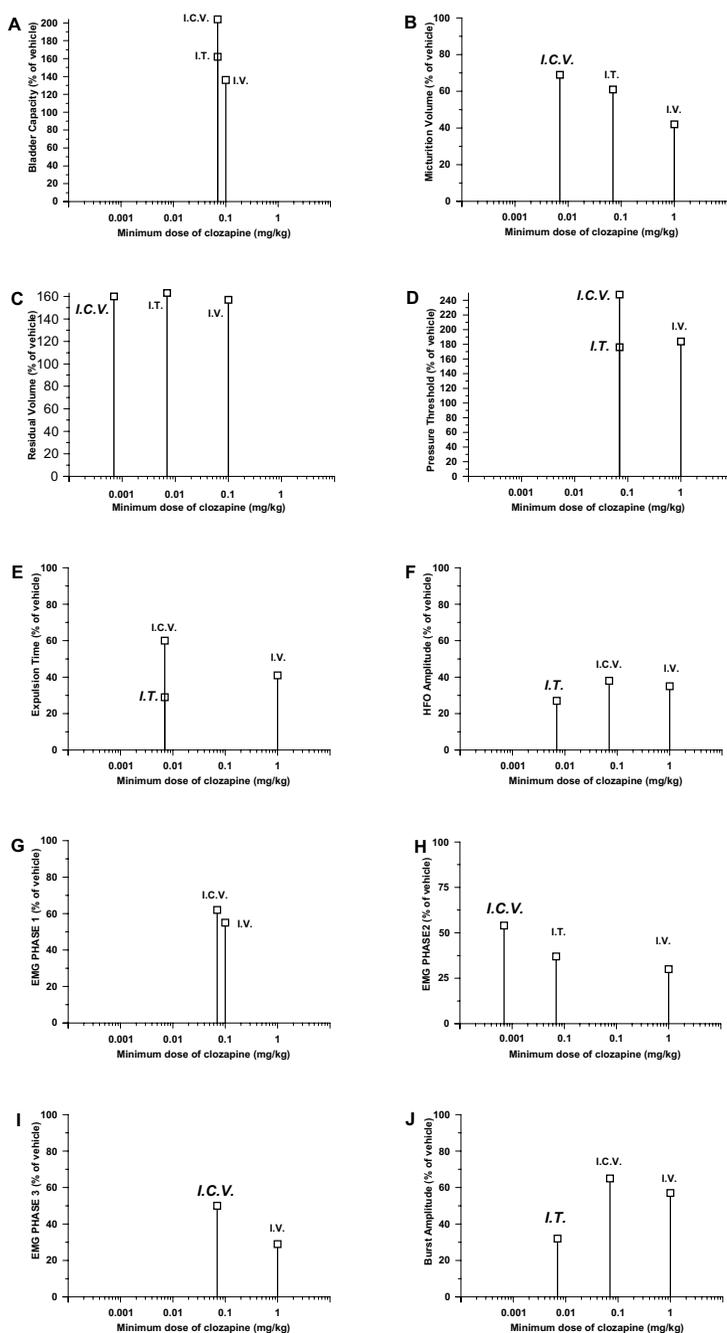
Figure 6
Effects of clozapine i.t. or i.c.v. on the EMG recorded from the EUS during cystometry in anesthetized rats. Data from i.v. administration of clozapine [12] are presented for comparison. A) Clozapine i.t. had no effect on the integrated EMG recorded during phase 1 of the contraction. Only the highest dose of clozapine (50 nmoles) i.c.v. decreased the EMG during this phase to 62%; B) Clozapine i.t. or i.c.v. decreased the integrated EMG during phase 2 of the contraction. All doses of clozapine i.c.v. significantly reduced the EMG whereas only 5 and 50 nmoles i.t. resulted in significant reductions. C) Only the highest dose of clozapine i.c.v (50 nmoles) decreased the EMG during this phase of the contraction. Clozapine i.t. had no effects. D) The amplitude of the individual bursts of EMG occurring during phase 2 of the contraction were reduced by clozapine i.t and i.c.v. However, 5 nmoles of clozapine i.t. produced a reduction of EMG that was significantly different from the effects of the same dose given i.c.v. Only the highest dose (50 nmoles) was effective in reducing the EMG when administered i.c.v. V= vehicle administration (saline) for i.t. or i.c.v. groups; (*V*) = vehicle administration for i.v. group. * = $p < 0.05$; ** = $p < 0.01$; # = $p < 0.05$ when compared to the effects of the same dose, different route.

pect that solely peripheral effects may explain the effects of clozapine on contraction time. Antimuscarinic agents have been show to decrease contraction time (e.g. atropine [19]) and since clozapine has high affinity for muscarinic receptors it is likely that peripheral anti-muscarinic effects of clozapine are responsible for the reduction in contraction time after systemic administration.

Expulsion time was reduced by clozapine after i.t. or i.c.v. administration at much smaller doses than were observed to produce similar results i.v. (Fig 7E; 5B). Therefore, cen-

tral effects of clozapine are likely responsible for the reduction in expulsion time. Since the intrathecal effects were greater than those observed after i.c.v. administration it is possible that a spinal action predominates with possible contribution from supraspinal sites.

The amplitude of the high frequency oscillations observed during the expulsion time in the rat micturition cycle [20,21] was reduced by clozapine i.t. at a dose of 5 nmoles (Fig 8F). This dose was 10× smaller than the first i.c.v. dose observed to have a significant effect and approxi-

Figure 7
Summary of the effects of clozapine given i.t., i.c.v. or i.v. (data from [12]) on urodynamic parameters and the activity of the EUS during cystometry in anesthetized rats. The first dose to yield a significant effect has been plotted for all 3 routes. Doses have been converted to mg/kg for ease of comparison. Peak pressure data are not shown since no significant differences were found with clozapine after any route. A) Bladder capacity is increased to a similar extent by comparable doses of clozapine, regardless of the route. Therefore, a peripheral effect may be predominating. In the case of micturition volume (B) and residual volume (C), both clearly show a central effect since the first dose to yield a significant effect is 142× smaller than the i.v. dose. Both of these parameters show a possible supraspinal site of action. D) Pressure threshold increased only after the highest dose of clozapine i.c.v or i.t., however, that dose was still 14× smaller than the first dose i.v., suggesting a possible central effect. E) Expulsion time shows a 142× difference between the central dose and the peripheral dose. In addition, intrathecal administration shows a larger effect than after i.c.v., suggesting a possible spinal site of action. F) The effect of clozapine on the amplitude of the HFO appears to be mediated by a spinal site. G) Phase 1 of the EMG was decreased after equivalent doses of clozapine i.v. or i.c.v. Intrathecal administration had no effect. H) Phase 2 of the EMG (where the bursting occurs) clearly showed a central effect since a much smaller dose (142×) was required to produce an effect. In addition, the reduction after i.c.v. was greater than after i.t. therefore suggesting a possible supraspinal site of action. I) Phase 3 of the EMG was not affected by clozapine intrathecally. Only the highest dose of clozapine given i.c.v decreased the EMG during this phase. That dose was 14× smaller than the first dose to produce a significant effect after i.v. administration suggesting a possible supraspinal site of action. J) The amplitude of the individual bursts during phase 2 of the EMG was decreased by central effects of clozapine at smaller doses than those observed i.v. Also, the intrathecal administration was much more effective in reducing the amplitude (abolished in 4/6) animals, suggesting a possible spinal site of action.

mately 142× smaller than the first i.v. dose to show a significant effect. Therefore, a spinal site mediating the effects of clozapine on the amplitude of high frequency oscillations appears likely. Previous results [12] suggest that D2 receptors modulate the amplitude of the HFO, since raclopride (selective D2 antagonist) decreased the amplitude of the HFO by 30%.

In addition to clozapine's effects on urodynamic parameters, the EMG recorded from the external urethral sphincter also showed changes after clozapine i.t. or i.c.v. The EMG during phase 1 of the contraction only showed a reduction after the largest dose of clozapine i.c.v.. This dose is equivalent to our lowest dose i.v. and in fact all doses i.v. previously showed a significant reduction [11]. Intrathecal administration had no effect on the EMG at phase 1.

The EMG during phase 2, corresponding to the period of high frequency oscillations was decreased by clozapine after i.t. or i.c.v administration at doses that were much lower than those necessary to produce an effect after i.v. Therefore, the effects of clozapine in reducing the EMG during this phase appear to be central in origin. Since the first significant effect was obtained after i.c.v. administration (Fig 7H) it is possible that supraspinal effects predominate with spinal sites contributing.

The EMG during phase 3 (closing phase) of the contraction was decreased only after the highest dose of clozapine (50 nmoles) i.c.v. (Fig 6C). This dose was still 14× smaller than the first significant dose i.v. and therefore a central effect of clozapine is probable.

Finally, the amplitude of the individual bursts of EMG recorded from the external urethral sphincter during phase 2 of the contraction also decreased after clozapine i.t. or i.c.v. (Fig 6D). Since the doses that yielded significant reductions were smaller than those observed after i.v. administration, a central effect is likely. Moreover, since 5 nmoles of clozapine i.t. produced a significant reduction when compared to the same dose i.c.v., a spinal effect of clozapine in mediating the reduction of the burst amplitude of the EMG is possible.

In the anesthetized rat, the external urethral sphincter contracts and relaxes during the expulsion phase in a manner that is complimentary to the high frequency oscillations observed in the bladder pressure record [19] whereas in humans the external sphincter not active during voiding. Pharmacological blockade of the external urethral sphincter in the rat resulted in decreased micturition volume and increased residual volume [20–23] suggesting that the activity of the external urethral sphincter contributes to efficient voiding in the rat. Therefore, central administration of clozapine, by reducing the activity of the EUS, contributes to the decrease in voiding efficiency by reducing micturition volume and increasing residual volume.

Alpha1 adrenergic antagonists have been shown to inhibit pudendal reflexes in anesthetized cats [24–26]. However, a systemic dose of prazosin (alpha1 antagonist) did not inhibit the EUS EMG activity during high frequency oscillations in the rat [27]. Thus, although alpha1 antagonism has been shown to modulate pudendal reflexes in the cat, their role in modulating the activity of the EUS during micturition in the rat appears unclear.

In summary, our results in the present experiments suggest that most of the effects of clozapine on urodynamic parameters can be ascribed to central effects. Expulsion parameters, such as micturition volume, residual volume, expulsion time, and amplitude of the high-frequency oscillations, appear to be mediated by the central action (spinal or supraspinal) of clozapine. The activity of the EUS also appears to decrease after central application of clozapine. Therefore, central effects of clozapine appear to decrease the voiding efficiency of the bladder in the rat. Contraction time clearly showed a peripheral effect only, whereas changes in bladder capacity were difficult to explain from central effects and probably reflect peripheral effects of clozapine.

Clozapine is metabolized mainly at the liver resulting in several metabolites [5,14]. One of the major metabolites, N-desmethylclozapine has been shown to have pharmacological activity both in vitro [28] and in vivo in rats [29]. In addition, N-desmethylclozapine is found in large concentrations in the serum of schizophrenic patients [5,15] and in rats [30]. The contribution of N-desmethylclozapine to clozapine's central effects has been questioned recently, since the levels of N-desmethylclozapine in the brain were much lower than those of clozapine [30] suggesting that N-desmethylclozapine does not cross the blood-brain barrier as readily as clozapine. Since we observed effects from central application of clozapine, we consider it unlikely that the effects of metabolites contributed significantly. However, whether any of the major clozapine metabolites also have a role in regulating micturition remains to be determined.

Conclusions
Atypical neuroleptics are useful in treating patients that are refractory to "traditional" antipsychotic medication and produce fewer extrapyramidal side effects. However, other side effects still occur with varying severity and frequency [31] and continue to pose a challenge to effective treatment. Urinary disturbances as a result of clozapine therapy have been well documented, and include inconti-

nence, enuresis and urgency [4,5,32]. Other atypical antipsychotics, such as risperidone [7] and olanzapine [10] have been reported to produce urinary incontinence.

We have previously shown [11–13] that systemic administration of clozapine, olanzapine and risperidone to anesthetized rats reduced voiding efficiency. Risperidone had smaller maximal effects than olanzapine and clozapine and had no direct (peripheral) inhibitory effects on the amplitude of bladder contractions. In the present study we show that clozapine acts at supraspinal and spinal sites to inhibit certain urodynamic parameters and the external urethral sphincter of the rat resulting in decreased voiding efficiency.

If these effects also occur in patients, they may contribute to the urinary disturbances reported following clozapine therapy. The exact receptor types (or combination of receptor types) responsible for clozapine's central effects on micturition were not investigated in the present study. However, isolating particular receptors that contribute to the effects of clozapine might be useful in designing neuroleptics that may avoid these side effects or in providing an adjunct therapy to relieve some of the side effects.

Materials and Methods
Surgical procedures
The experiments were conducted in compliance with the USDA Animal Welfare Act and amendments thereto and the revised Guide for the Care and use of Laboratory Animals DHEW (NIH) and were approved by the Animal Studies Subcommittee of the Bay Pines Veterans Administration Medical Center.

Surgical procedures have been described in detail elsewhere [12]. Rats (female Sprague-Dawley; n = 16; 230–270 g; Harlan; IN) were anesthetized with halothane and placed on a heating pad. A catheter (PE-50) was introduced into the jugular vein to administer urethane (1.1 g/kg) over a period of 20 minutes while decreasing the level of halothane to prevent respiratory depression. Rats were instrumented with either an intrathecal cannula placed over the L6/S1 spinal segment or a cannula into the right lateral ventricle.

An incision was made over the dorsal aspect of the neck and the overlying muscles were retracted to expose the atlanto-occipital membrane. An intrathecal catheter (PE 10) was introduced through a small slit in the atlanto-occipital membrane and positioned over the L6/S1 spinal cord segments [33]. Saline soaked gelfoam was placed around the catheter and the neck muscles and skin were sutured. Spinal segmental location of the catheter was verified post-mortem by performing a laminectomy.

Following a small craniectomy, a stainless-steel cannula (27 ga) was placed in the lateral ventricle at the following coordinates: AP - 1.0, ML = 1.2; V= 3.2 mm [34]. The cannula was held in place with skull screws and dental acrylic. At the end of the experiment, 5 μl of fast-green (1%) was infused through the cannula while observing the CSF though a small slit in the atlanto-occipital membrane. Almost immediate visualization of the fast-green in the fourth ventricle was taken to indicate appropriate placement of the cannula into the lateral ventricle.

After an abdominal incision, both ureters were tied distally and cut centrally and allowed to drain onto cotton gauzes that were directed outside the animal. A catheter (PE-90) was introduced into the bladder dome and tied in place with a purse string suture. A catheter (PE-50) was introduced into the right femoral artery for blood pressure recording. Stainless-steel wires (0.003 in.; A-M Systems; WA) insulated except at the tip were introduced into the external urethral sphincter for EMG recording (DAM 50; WPI; bandwidth= 3 to 3 kHz; gain: 1000–10,000).

Urodynamic studies
In pilot animals, the dose and duration of effects was determined by administering clozapine (saline, 0.5,5,50,100 nmoles) at 10 min intervals either intrathecally (n = 2) or intracerebroventricularly (n = 2) during continuous cystometry (infusion rate = 0.11 ml/min) while recording bladder pressure, external urethral sphincter EMG, and blood pressure. Fifty (50) nmoles of clozapine administered by either route resulted in a decrease in arterial pressure (mean decrease in MAP was 20 ± 2 and 15 ± 3 mm Hg for intrathecal and intracerebroventricular administration, respectively. The onset time ranged from 1–1.5 min from the start of the infusion). Since clozapine has alpha1 antagonist effects [2] it is possible that the blood pressure decreases were due to spread of clozapine into the periphery following central administration. Doses smaller than 50 nmoles did not elicit a drop in arterial pressure. In addition, 50 nmoles represents a dose close to the smallest intravenous dose used previously (0.1 mg/kg [12]). Therefore, during single cystometry, the dose range was limited to 0.5 to 50 nmoles in order to reduce possible peripheral spread of clozapine.

Single cystometry studies were conducted as follows. The bladder was emptied and allowed to equilibrate to air pressure for 5 minutes before beginning each cystometrogram. Room temperature saline was infused into the bladder (0.11 ml/min) while recording bladder pressure and the infusion was stopped when a contraction occurred. Volume expelled was determined by placing cotton gauze at the urinary meatus and weighing before and after micturition. External urethral sphincter EMG (EUS-EMG) was

recorded throughout the cystometrogram and for some time after the filling had stopped. Increasing doses of clozapine (Sigma, vehicle, 0.5, 5, 50 nmoles in a volume of 5 µl; followed by a 7 µl saline wash) were administered through the intrathecal catheter (n = 6; Mean weight = 245 gm) or through the intracerebroventricular cannula (n = 6; Mean weight = 245 gm) at approximately 10-minute intervals. Clozapine was dissolved in a minimal amount of 0.1 N hydrochloric acid, and brought up to volume with saline (final pH = 6). Cystometrograms were started approximately 3 minutes after each drug administration. At the end of the experiment the rat was euthanized with an overdose of urethane (3.0 mg/kg; i.v.).

Data analysis
Bladder pressure, EUS-EMG and blood pressure during the single cystometrograms were displayed in an electronic chart recorder (RC Electronics; Goleta, CA) and analyzed off-line (Dataview, W.J. Heitler, U. St Andrews, Scotland). The following parameters were examined from the cystometrogram as described in detail earlier [12]: bladder capacity (amount of fluid infused to elicit a contraction); micturition volume (amount of fluid expelled); residual volume ([bladder capacity-micturition volume]/[bladder capacity] ×100); pressure threshold (pressure at which contraction begins); peak pressure (maximal pressure during contraction); contraction time; expulsion time (time between peak pressure and end of high frequency oscillations); amplitude of high frequency oscillations. The EMG activity was examined by dividing the bladder contraction into three phases in a modification of the technique of Chien et al. [19] a contraction phase (phase 1); an expulsion phase (phase 2) and a closing phase (phase 3).

The raw EMG was rectified, integrated (0.5 second bin) and the area under curve of the EMG corresponding to each phase of the bladder contraction was measured (Sigma Scan/Image; Jandel Scientiflcs, San Rafael, Ca). Drug effects for the EMG were calculated as percent of control.

Values are presented as Mean + S.E.M. Repeated measures ANOVA (GB Stat; Dynamic Microsystems; MD) were performed on all parameters and when statistical significance (p<0.05) was obtained, comparisons between control and different drug dosages and between different routes (i.t. vs. i.c.v.) were made using Fisher's protected t-test [35] .

List of Abbreviations
CMG = cystometrogram

EMG = electromyogram

EUS = external urethral sphincter

HFO = high frequency oscillations

i.c.v. = intracerebroventricular

i.t. = intrathecal

i.v. = intravenous

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