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Role of the anterior cingulate cortex in the control of micturition reflex in a rat model of Parkinson's disease

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ABSTRACT

Purpose: The present study examined dynamic changes in neural activity of the anterior cingulate cortex (ACC) and the midbrain periaqueductal gray (PAG) during the micturition reflex in a Parkinson's disease (PD) model as well as the effects of direct stimulation of the ACC on the micturition reflex.

Materials and Methods: Electrodes were inserted into the ACC or the PAG, and the effects of intravenous (i.v.) administration of ZM24138 (ZM: adenosine A2A receptor antagonist) on the pelvic nerve evoked field potentials were examined.   The effect of electrical stimulation of the ACC was also examined.

Results: PD rats showed bladder overactivity evidenced by a significant decrease in intercontraction intervals (ICI) compared with sham rats.   I.v. administration of ZM increased ICI in both groups with the inhibitory effects being greater in PD; and dose-dependently increased the amplitude of evoked potentials in the ACC of PD rats but not in sham rats. I.v. administration of ZM reduced the evoked potential amplitude in the PAG of both groups with the inhibitory effects being greater in PD vs. sham rats.   Electrical stimulation of the ACC significantly increased ICI.

Conclusions: These results suggest that ACC neurons have an inhibitory role in bladder control because neural activity in the ACC was significantly increased along with suppression of bladder overactivity after ZM administration in the PD model, and the stimulation of the ACC inhibited the

micturition reflex.   Understanding the roles of the ACC in the modulation of micturition could

provide further insights into the pathophysiology of OAB.

INTRODUCTION

Micturition is controlled by a complex-interaction of circuitry at different levels of the central nervous system. The midbrain periaqueductal gray (PAG) integrates bladder sensory information with input from forebrain centers and then sends signals to the pontine micturition center (PMC), which sends efferent information back to the spinal cord to control storage and voiding of urine [1]. Recent functional brain imaging studies have identified multiple regions of the forebrain that exhibit altered activity during bladder filling and micturition; and that the responses in certain regions are different in patients with overactive bladder (OAB) and in normal volunteers [2]. Brain imaging studies indicate that the anterior cingulate cortex (ACC) which increases activity when patients experience urgency may have an important role in monitoring bladder volume and also in modulating urinary continence mechanisms including contraction of the pelvic floor and sphincter muscles.

In the present study we examined the role of the ACC and PAG in the bladder dysfunction in a rodent model of Parkinson's disease (PD). In patients with PD, the prevalence of lower urinary tract symptoms (LUTS) identified by validated questionnaires is around 30% [3, 4]. Previous urodynamic studies of PD patients with LUTS have revealed that the most frequent abnormality is detrusor overactivity, resulting in nocturia, urinary urgency and frequency and urge incontinence [5,6]. However, few studies have addressed the dynamic changes in neuronal activity of the ACC, which as mentioned above may be a key region for controlling urine storage and voiding functions. In addition,

our group previously investigated the mechanisms underlying **bladder overactivity** in a rat PD

model, and discovered that **enhanced activity of the adenosine A2A receptor system in the brain**

contribute at least in part to bladder overactivity associated with PD because

intracerebroventricular administration of an adenosine A2A receptor antagonist increased

intercontraction intervals during cystometry in both PD and control rats with the inhibitory

effects being greater in PD vs. control rats [7].   However, it has not been investigated how

adenosine A2A receptors are involved in modulation of ACC or PAG activity in the normal or PD

condition.

The present study therefore examined changes in neural activity in the ACC as well as in the PAG

during the micturition reflex induced by pelvic nerve (PLN) afferent stimulation in the PD rat model

and the influence of an adenosine A2A receptor antagonist on that activity. Moreover, because there

are only a few reports of the effect of direct activation of the ACC on **bladder function, we also**

examined how electrical stimulation of the ACC affects the micturition reflex in rats.

MATERIALS AND METHODS

Animals

Female adult Sprague-Dawley rats weighing 210 to 308 gm were used with experimental protocols

approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Experiments

were performed in normal (n=6), 6-hydroxydopamine hydrochloride (6-OHDA)-lesioned (n=18), and sham operated rats (n=18).

Surgical procedures for experiments

6-OHDA injection

6- OHDA (8 μg) (Tocris Bioscience,Ellisville, MO, USA) was unilaterally injected into the left substantia nigra pars compacta (2.2 mm lateral, 5.3 mm posterior to bregma, 8.0 mm ventral to dura) under anesthesia with pentobarbital (60 mg/kg, intraperitoneally; Ovation, Deerfield, IL, USA) as previously described [8].   Rats receiving vehicle injection (0.1% ascorbic acid / 0.9% saline) into the substantia nigra pars compacta were used as sham-operated controls.

Electrode implantation and Cystometry

Two weeks later, PD and sham animals under urethane anesthesia (1.1g/kg subcutaneous injection; Sigma Chemical, St. Louis, MO, USA), a tungsten electrode was inserted stereotaxically into the ACC (3.2 mm lateral, 0.6 mm posterior to bregma, 1.8 mm ventral to dura) or the PAG (0.8 mm lateral, 7.8 mm posterior to bregma, 6.8 mm ventral to dura) according to the atlas of Paxinos & Watson (Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic, San Diego) (Fig.1).   Then, field potentials were recorded in the ACC or the PAG in separate groups of animals during electrical stimulation (1-15V, 100-200Hz and 30ms duration) of the pelvic nerve

(PLN) located proximal to the major pelvic ganglia.   During PLN stimulation, the bladder was kept in the emptied condition.   The signals were amplified, filtered, and displayed on an oscilloscope. ACC and PAG field potentials were digitized and averaged from >8 individual responses using a PowerLab system (AD Instruments, Colorado Springs, CO, USA).   Then, the area under the curve of electrical field potentials was calculated by Scope software (AD Instruments).

Electrical stimulation of anterior cingulate cortex

  In normal untreated rats anesthetized with urethane a tungsten electrode was inserted stereotaxically into the ACC (3.2 mm lateral, 0.6 mm posterior to bregma, 1.8 mm ventral to dura).   The bladder was exposed through a midline abdominal incision, and a PE-50 (Clay-Adams, Parsippany, NJ, USA) cannula was inserted into the bladder, and continuous cystometrograms with an infusion speed at 0.04ml/min were recorded.   After obtaining baseline micturition reflex parameters, electrical stimulation (1-10V, 100Hz and 30ms duration) was applied to the ACC for 30 min through a tungsten electrode.   Bladder activity was evaluated by measuring intercontraction intervals (ICI) , maximal voiding pressure (MVP), **pressure thresholds for inducing micturition (PT) and non-voiding contractions.**

Drug administration

Intravenous injections (i.v.) were made through a cannula (PE-10) inserted into the jugular vein. The effects of ZM241385 (adenosine A2A receptor antagonist, 0.01-10 mg/kg: ZM) were examined by single dose i.v. administration and compared with the effects of DMSO (vehicle) administration. Doses of drugs used in this study were determined according to previous studies [7]. Cumulative dose response curves were obtained in each rat by administering increasing doses of the drug at 15-20 min intervals.

Histological analyses

  At the end of the experiment, rat brains were subjected to an immunohistochemical analysis using antibodies for tyrosine hydroxylase as previously described [8]. Brain sections (40 μm) were incubated with normal goat serum followed by rabbit anti-TH (Abcam Inc., Cambridge, MA, USA), and then biotinylated goat anti-rabbit IgG secondary antiserum (Vector laboratories, Inc. Burlingame, CA, USA) was applied. Incubation was carried out with peroxidase-conjugated streptavidin (Vector laboratories, Inc. Burlingame, CA, USA). During incubations, sections were rinsed and reacted with 3,3'-diaminobenzidine (DAB) as a chromogen. After mounting sections on gelatin-chromalum-coated slides, sections were dehydrated and coverslipped. The sites for stimulation/recording with the tungsten electrodes were also confirmed in every animal's brain at the end of the experiments. Only data from rats in which the location of electrode tips was found in the ACC or the PAG region were used for the data analysis.

Statistical analysis

All data values are expressed as mean±standard error of mean, and analyzed using the statistical software package Prism (Graphpad Software, San Diego, CA, USA). Student's paired t-test was used for comparison of the data before and after drug administration. Repeated measures ANOVA followed by Tukey's multiple comparison test was used to compare changes in electrical field potentials as well as in ICI and MVP before and after electrical stimulation of the ACC. P values <0.05 were considered to be statistically significant.

RESULTS

Histological study

Immunohistochemical analyses showed a marked reduction of catecholamine-positive cells in the substantia nigra on the lesioned side, but not on the intact side, in 6-OHDA lesioned rats (**Fig. 2**), as similarly observed in our previous study [8].

Cystometric parameters in sham operated and Parkinson's disease model rats (Fig.3 A, B)

As shown in Fig. 3A and 3B, 6-OHDA lesioned rats exhibited a significant decrease in ICI compared with sham operated controls (6-OHDA: 7.3 ± 1.0 min, n = 6, Sham: 13.5 ± 1.3 min, n = 6; P < 0.05), indicating that the micturition reflex was facilitated by dopamine depletion.   **However, there were no significant changes in MVP or PT in 6-OHDA lesioned (34.8 ± 3.1 and 7.8 ± 0.7**

cmH$_2$O, n = 6) vs. sham rats (33.9 ± 4.4 and 6.8 ± 1.2 cmH$_2$O, n=6).   Moreover, the number

and amplitudes of non-voiding contractions were not significantly different among groups

(data not shown).

Evoked potentials in the ACC and the PAG (Fig.4)

PLN stimulation induced field potentials in the ACC and the PAG with the latency to their peaks of

42-55 ms and 37-48 ms, respectively.   I.v. administration of ZM increased the amplitude of evoked

potentials in a dose-dependent manner in the ACC of PD rats, with statistically significant increases

at 1 and 10 mg/kg (p<0.05).   These effects were seen within few minutes after ZM administration

and lasted over 30 minutes.   No significant changes in ACC evoked potentials were found in sham

rats.   In contrast, i.v. administration of ZM significantly reduced the amplitude of evoked potentials

in the PAG of both sham and PD rats. The dose that induced statistically significant increases of

ACC evoked potentials in PD rats (0.1 mg/kg) was lower than the dose (1 mg/kg) that produced

inhibition (Fig.4) in sham rats.   ZM treatment did not alter the latency of evoked potentials in either

sham or PD rats.

Electrical stimulation (Fig.5 A, B)

  In normal untreated rats electrical stimulation of the ACC significantly increased ICI without

changing MVP compared with the pre-stimulation values (Fig.5A, B).   When stimulation was

stopped, ICI immediately returned to control values. However, electrical stimulation of the ACC did

not change other parameters significantly.   Average baseline pressure, maximum voiding pressure

and threshold pressure were 5.5 ± 1.3, 33.4 ± 2.8, and 8.7 ± 1.2 cmH$_2$O before stimulation, 5.5 ± 1.2,

32.2 ± 2.6, and 9.6 ± 1.4 cmH$_2$O during stimulation, 5.0 ± 1.1, 31.0 ± 2.1, and 8.3 ± 1.0 cmH$_2$O after

stopping stimulation, respectively.   We also confirmed the reproducibility of ICI increases during

ACC stimulation by showing that repeated electrical stimulation of the ACC with an interval of 30

min produced similar inhibitory effects on ICI (data not shown).

DISCUSSION

The results of this study indicate that: (1) a rat model of PD produced by 6-OHDA-induced

dopamine depletion exhibits bladder overactivity, (2) ZM, an adenosine A2A receptor antagonist,

enhanced nerve activity in the ACC as evidenced by increased field potential responses to PNL

stimulation in PD rats, with the similar dose range that significantly reduced bladder overactivity in

PD rats, as shown in our previous study [7], (3) PLN-evoked neural activity in the PAG was reduced

following ZM treatment, and (4) electrical stimulation of the ACC suppressed the micturition reflex.

Therefore, based on the findings in our current and previous studies, we propose that activation of

the ACC exerts inhibitory effects on the micturition reflex, which might be insufficient when bladder

overactivity is induced by PD-like dopamine depletion in the brain. The effect of ZM to enhance

PLN evoked neural activity in the ACC and reduce activity in the PAG suggests that adenosine A2A

receptors modulate the sensory limb of the supraspinal micturition reflex pathway and that blocking

these receptors can suppress bladder overactivity in PD.

The ACC is a part of the limbic system that has extensive connections with many areas, such as

hypothalamus, brainstem (autonomic nervous system) [9], hippocampal region (memory), and

prefrontal regions (cognition and task performance) [10].   Also, it is known that the ACC has direct or

indirect connections with the PAG (**Fig. 6**) [10].   Early studies using functional brain imaging

revealed that, the ACC is involved in monitoring bladder filling [11], withholding urine, and starting

micturition [12,13].   However, subsequent studies suggested more complex functions such as a role in

integrating afferent information and internal motivational states [12]. Functional brain imaging

studies have shown that there are increased responses in the ACC during withholding urine in normal

volunteers [14] and that the ACC is activated when patients have the sensation of urgency and when

OAB patients tighten pelvic floor and sphincter muscles and relax the bladder, in order to maintain

continence [2]. Moreover, a previous study by Tai et al. using functional MRI in rats showed

activation in the posterior region of the cingulate cortex during urine storage which shifted to

activation in a more anterior region of the cingulate cortex during the micturition reflex [15]. In

accordance with these findings, the present study demonstrated that neural activity in the ACC

significantly increased along with suppression of bladder overactivity after ZM administration in the

PD model, suggesting that ACC neurons have an inhibitory role in the control of bladder activity.

This assumption is further justified by the findings of this study that direct stimulation of the ACC

inhibits the micturition reflex.

In this study, we used an animal model of PD to study bladder dysfunction. PD is the second most

prevalent neurodegenerative disorder with a progressive degenerative condition that is primarily

induced by dopamine depletion in the striatum, leading to motor symptoms as well as non-motor

symptoms including urinary urgency, frequency and incontinence [3]. **The incidence of lower**

urinary tract dysfunction is estimated to be as high as 30% in patients with PD [3] [16]. The

etiology of this dysfunction is largely unknown, although it is often associated with significantly

decreasing quality of life [16,17]. Previous functional brain imaging studies have shown some

overlaps as well as differences in brain activation sites during bladder filling between healthy

volunteers and patients with PD who had detrusor overactivity [18]. Moreover, PD patients have

decreasing ability to process sensory inputs as the disease progresses [19], whereas other studies have

demonstrated that PD patients require activation of larger cortical areas to manage tasks compared to

normal controls [20].   These studies indicate that integrated cortical dysfunctions may play a role in

the development of bladder dysfunction in PD.

Adenosine acts through different subtypes of G protein-coupled (A1, A2A, A2B and A3

receptors). A1 and A2A receptors are the main targets for the physiological effects of

adenosine in the brain.   A2A receptors have a unique cellular and regional distribution in the

basal ganglia, being particularly concentrated in areas richly innervated by dopamine such as

the caudate-putamen and the globus pallidus whereas A1 receptors are widely distributed in

the brain [21]. Increasing evidence suggests that compounds selectively targeting adenosine A2A

receptors can be used for a promising non-dopaminergic therapy for PD [22,23].   Istradefylline, an

adenosine A2A receptor antagonist, has been approved and launched in Japan for PD patients who

are experiencing the wearing-off phenomenon, a motor complication of PD.   We previously

performed a study using a rat model of PD that examined the effect of an adenosine A2A receptor

antagonist on the micturition reflex **and found that enhanced adenosine A2A receptor activity in**

the brain is involved in the emergence of bladder overactivity in PD rats, suggesting that

inhibition of A2A receptors could be effective for the treatment of bladder dysfunction in PD patients [7].

.   The current study further demonstrated that i.v. administration of ZM reduced the amplitude of evoked potentials in the PAG of both PD and sham rats with the inhibitory effects being greater in PD rats.   The latency of PAG activation after PNL stimulation in the current study (**Figure 4A**) is consistent with that (33-47 ms) reported in previous studies by Noto et al [24,25]. Thus, in contrast to the inhibitory function of the ACC, PAG is likely to have a facilitatory effect on the micturition reflex, as suggested by human brain imaging and animal studies [26,27].

This study shows the importance of the ACC and the PAG in processing of bladder sensory input during the micturition reflex.   While there are complex interconnections among many brain regions, each of these two regions is thought to be involved in various functions related to the micturition reflex.   A hypothetical scheme of the possible roles of cortical (ACC) and the brainstem structures (PAG) in the bladder control of normal and PD conditions is shown in **Figure 6**.   The ACC, a brain region involved in executive function including decision making, may control key brain regions involved in the micturition reflex (i.e., PAG and PMC) via direct projections to the PAG [28] (**Fig. 6A**). Based on human functional brain imaging studies and current results, the ACC has the potential for exerting an inhibitory influence on micturition, which is possibly enhanced by the nigro-striatal dopaminergic pathway (**Fig. 6A**), but reduced after damage to this pathway in the PD model (**Fig. 6B**). Enhancement of activity in the ACC in the PD model by doses of ZM that reciprocally inhibited

activity in the PAG and also reduced PD-induced bladder overactivity is consistent with putative

micturition inhibitory function of the ACC (**Fig. 6C**).   Understanding the processes by which the

ACC contributes to regulation of the micturition reflex may lead to the development of new

therapeutic approaches for OAB treatments, including pharmacotherapies such as adenosine A2A

receptor modulation.

CONCLUSIONS

The ACC has an inhibitory role in micturition control. The enhancement of PLN afferent evoked

responses in the ACC by the adenosine A2A receptor inhibition that also reduced bladder

overactivity in a rat model of PD raises the possibility that the drug acts by enhancing ACC

inhibitory control of the voiding.   Understanding the roles of CNS pathways including those in the

ACC in the modulation of micturition could provide further insights into the pathophysiology of

OAB. In addition synaptic transmission in the ACC is also a potential-target for drug therapy

designed to treat OAB symptoms.

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LEGENDS

Figure 1

The location of electrodes for recording field potentials in the anterior cingulate cortex (ACC) and periaqueductal gray (PAG) and for electrical stimulation of the ACC

Figure 2

Photomicrographs showing tyrosine hydroxylase immunostaining of the mid brain section where the substantia nigra is located in a 6-OHDA lesioned rat. The arrow indicates tyrosine hydroxylase-positive cells on the intact side (left) of the substantia nigra compared with the lesioned side (right), in which the number of tyrosine hydroxylase-positive cells was substantially decreased. Scale bar = 1 mm.

Figure 3

A: Representative cystometrograms of the micturition reflex in sham operated (Sham) and 6-OHDA lesioned rats (Parkinson's disease model). Physiological saline was infused into the bladder at a rate of 0.04 ml/min. Decreases in intercontraction intervals were observed in the 6-OHDA lesioned rat compared with the sham operated rat. **B:** Averaged intercontraction intervals. Longitudinal axis: intercontraction interval (min). Note that the intercontraction interval in Parkinson's disease model

rats (7.3 min, n=6) was significantly smaller than in sham operated rats (13.5 min, n=6). **P<0.01 vs

sham operated rats.

Figure 4

A: Representative electrical field potentials in the ACC and PAG evoked by electrical stimulation of

the pelvic nerve in Parkinson's disease model rats. **B:** Evoked potentials in the ACC and the PAG. I.v.

administration of ZM increased the amplitude of evoked potentials in a dose-dependent manner in

the ACC of Parkinson's disease model rats. However, no significant changes in ACC evoked

potentials were found in sham rats. I.v. administration of ZM reduced the amplitude of evoked

potentials in the PAG of both Parkinson's disease model and sham rats with significant inhibitory

effects at a lower dose in PD rats (0.1 mg/kg) vs. sham rats (1mg/kg). *P < 0.05, **P < 0.01.

Figure 5

A: Effects of electrical stimulation of the anterior cingulate cortex (ACC) on intercontraction

intervals (ICI) in urethane anesthetized rats. **B:** Averaged intercontraction intervals. Longitudinal

axis: ratio to control intercontraction interval (%). Note that the intercontraction interval during

stimulation (150.0 %, n=6) was significantly increased compared with the pre-stimulation control. *:

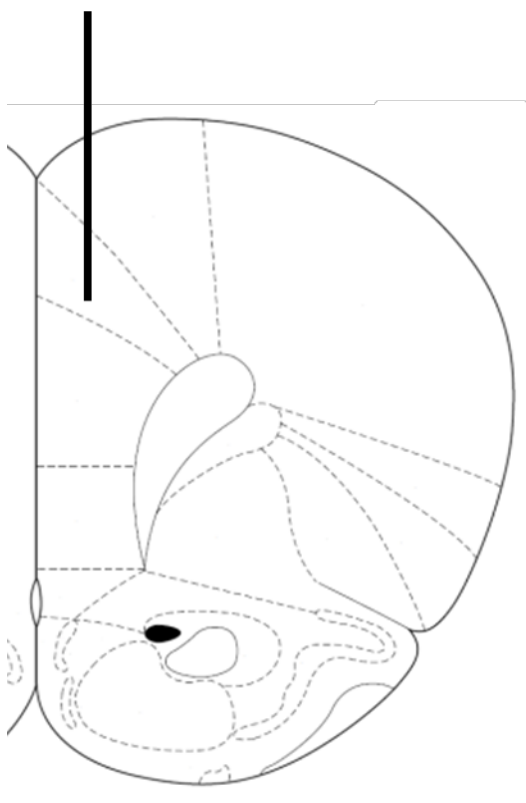p<0.05 vs. control group.

Figure 6

Hypothetical diagrams showing a working model of bladder dysfunction in Parkinson's disease. The micturition reflex is controlled by the spino-bulbo-spinal pathway passing through the periaqueductal gray (PAG) in the midbrain and the pontine micturition center (PMC) in the brainstem. This neural circuit is under the control of higher centers including the anterior cingulate gyrus (ACC) and other cortex regions.

A: Under normal conditions, tonic inhibition (-) from the ACC suppresses the micturition reflex and prevents the bladder overactive condition. **B:** In Parkinson's disease, dopaminergic neurons in the substantia nigra pars compacta (SN) are lost, and excitation (+) from the striatum to ACC are reduced, resulting in the loss of inhibitory function of ACC, which leads to facilitation of the micturition reflex via activation of the spino-bulbo-spinal pathway passing through PAG and PMC. **C:** In Parkinson's disease with ZM (adenosine A2A receptor antagonist) administration, neural activity of the ACC is enhanced to inhibit the micturition reflex and prevent the bladder overactive condition.
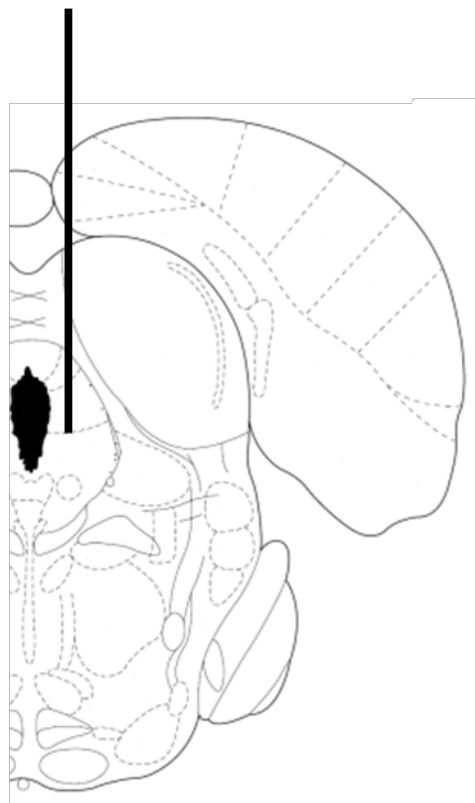
Fig. 1

ACC

PAG

AP   +3.2
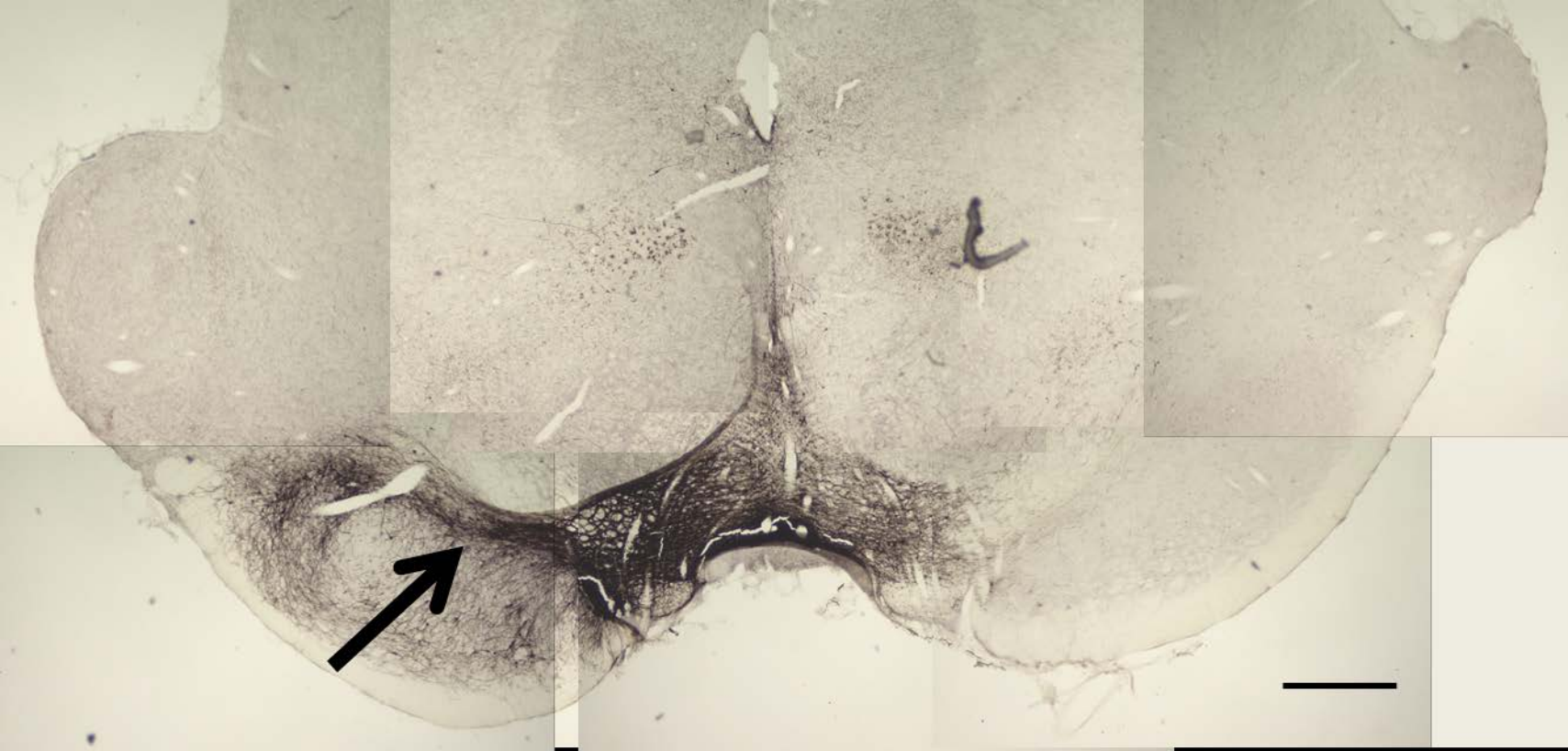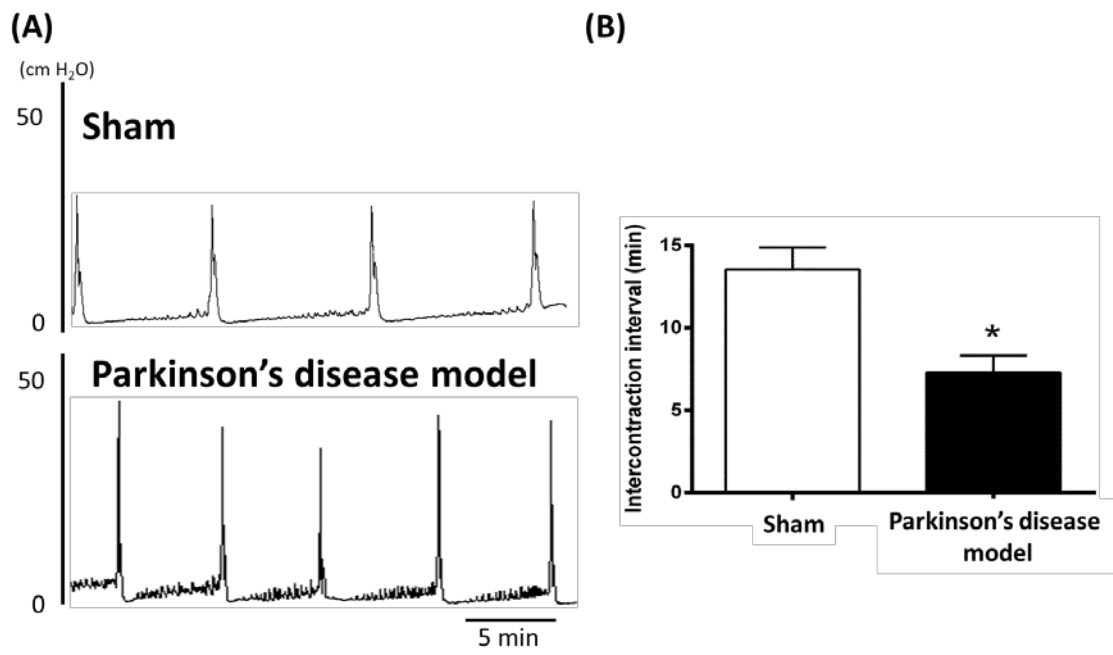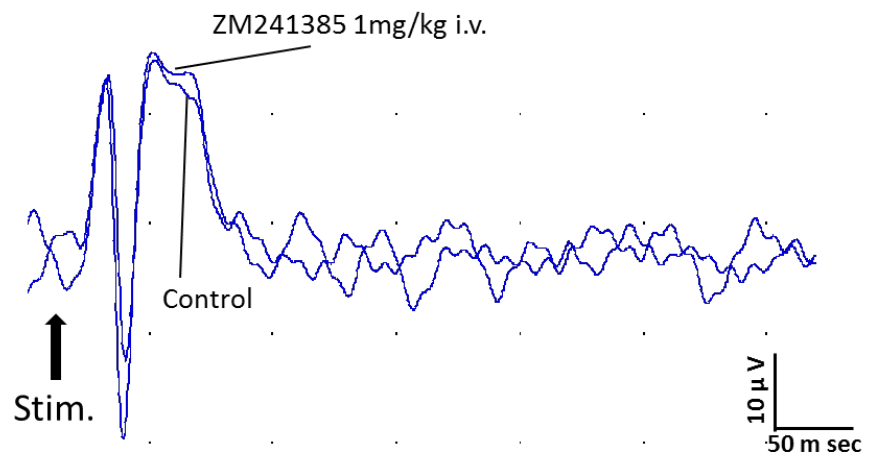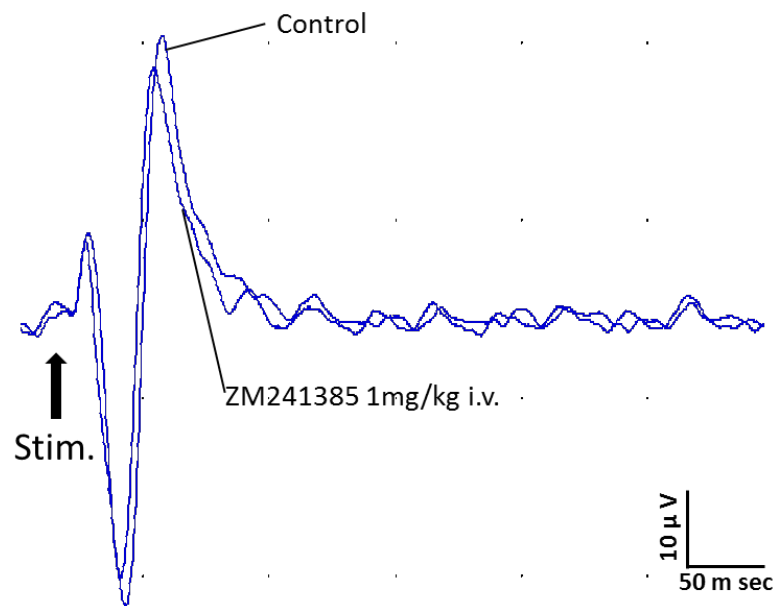LR   +0.6
DV -1.8

AP   -7.8
LR   +0.8
DV -6.8

Fig. 3

(A)

(B)

Fig.4

(A)

ACC

ZM241385 1mg/kg i.v.

Control

Stim.

10 μ V

50 m sec

PAG

Control

Stim.

ZM241385 1mg/kg i.v.

10 μ V

50 m sec

(B)

Sham

Parkinson's disease model

ACC

PAG

Fig. 5

A

10cmH₂O
5 min

stimulation

B

Ratio to baseline ICI (%)

*

Control   Stimulation   Stop stimulation

A

Normal

Cortex
Other regions

ACC

Striatum

(+)

SN

(-)

PAG

(+)

PMC

Spinal Cord

Stimulation

Bladder

B

Parkinson's disease

Cortex
Other regions

ACC

Striatum

(+)

SN

(-)

PAG

(+)

PMC

Spinal Cord

Stimulation

Bladder
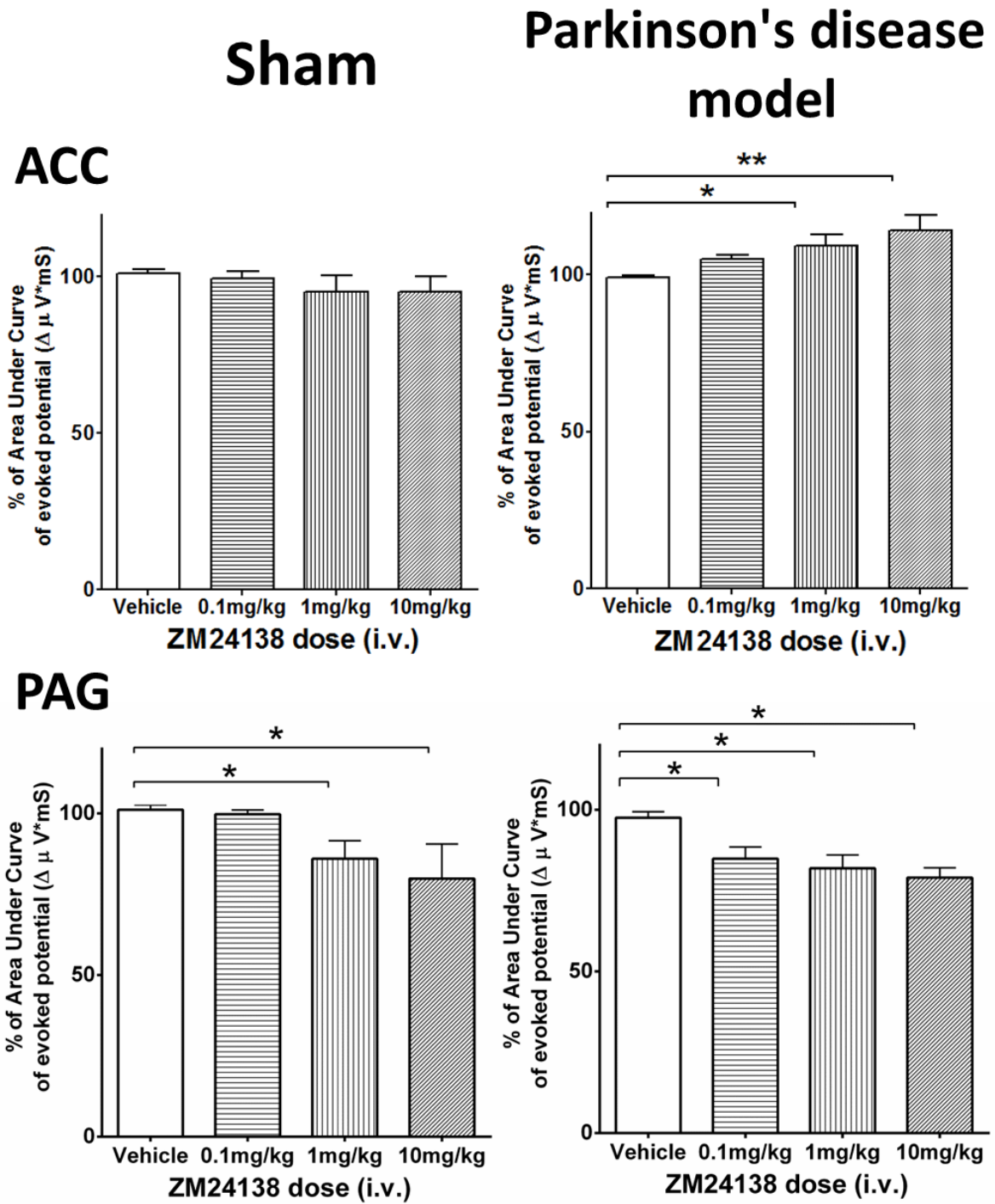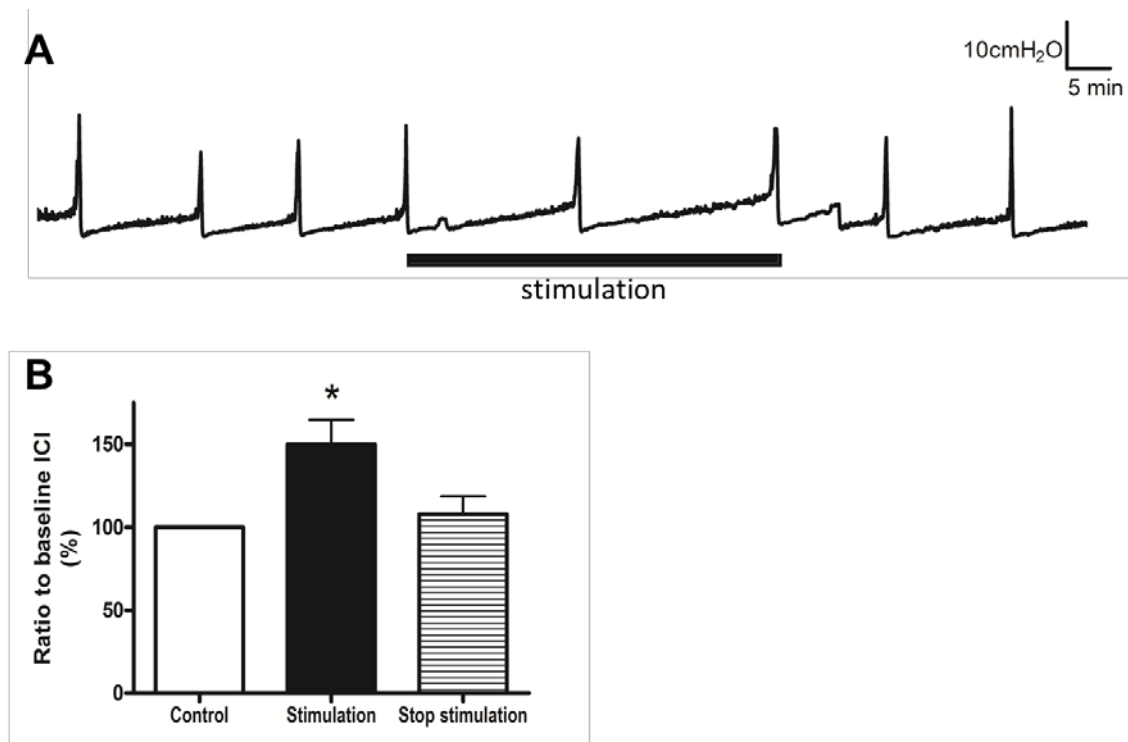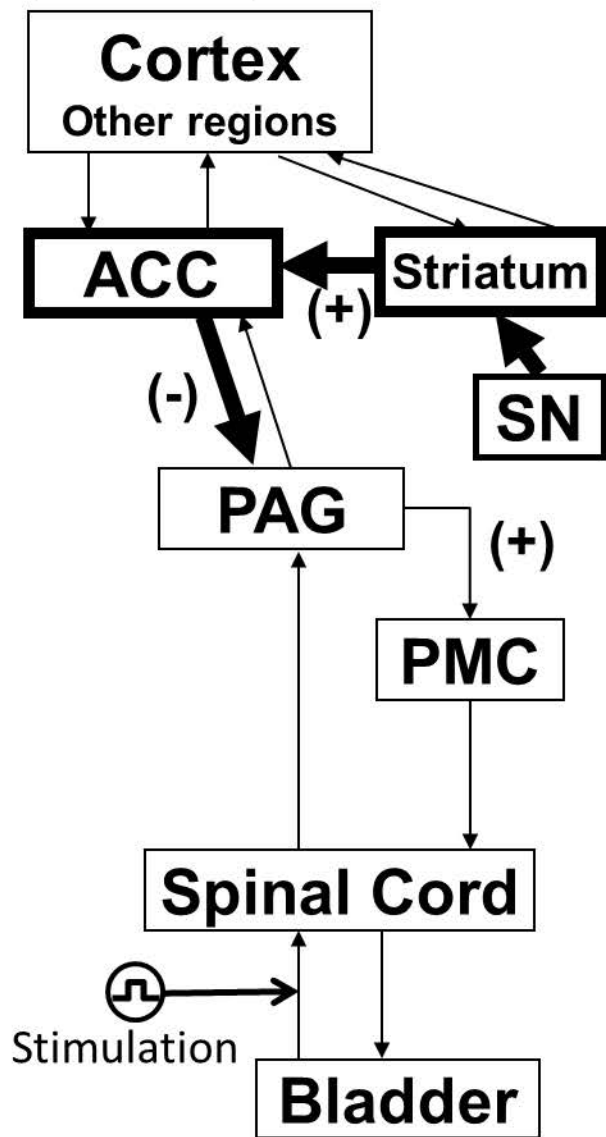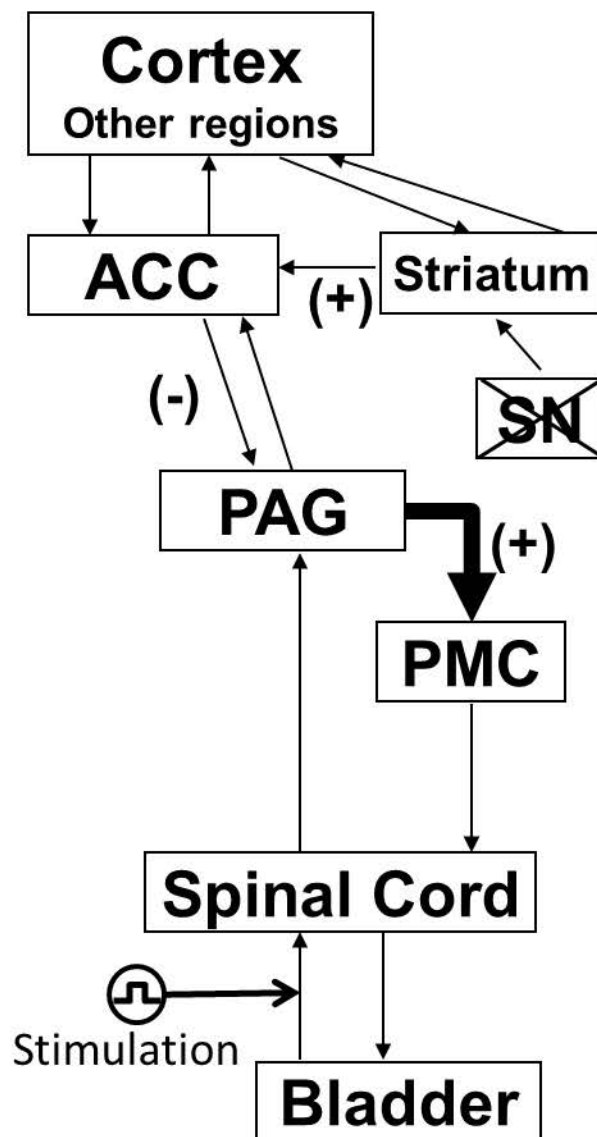
C

**Parkinson's disease
+ ZM administration**

Cortex
Other regions

ACC

Striatum

(+)

SN

ZM

(-)

PAG

(+)

PMC

Spinal Cord

Stimulation

Bladder