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Meriaux C[1], Jahanshahi A[2], Hohnen R[1], Temel Y[3], van Koeveringe G[1]
1. *Department of Urology, Maastricht university medical center, the Netherlands,* ***2.*** *Department of Translational Neuroscience, Maastricht University Medical Center, The Netherlands,* ***3.*** *Department of Neurosurgery, Maastricht University Medical Center, The Netherlands*

COMMUNICATION BETWEEN THE LOWER URINARY TRACT AND THE CENTRAL NERVOUS SYSTEM: FUNCTIONAL MAPPING OF THE PERIAQUEDUCTAL GRAY MATTER

Hypothesis / aims of study

Altered reflex and perceptual responses within the lower urinary tract – brain axis have emerged as a generally accepted model to explain pathologies such as overactive bladder syndrome complex. In the spinal – brainstem – spinal system involved in urine storage and micturition processes, the periaqueductal gray (PAG) plays a key role. In order to develop more specific options for the treatment of urinary dysfunctions, an enhanced understanding of the transmission and the processing by the PAG of urinary sensory information takes on critical importance. For this reason, the aim of this study is to determine which structures of the PAG are activated during the bladder electrical stimulation.

Study design, materials and methods

32 adult male Sprague Dawley rats (body weight 300-350 g at the time of surgery) were randomly assigned to one of the five groups as shown in Table 1.

Table 1: Summary of rat subjects used in the present study

Groups	Number of rats	Electrode Implantation	Stimulation
1 - Control	6	None	None
2 - Sham D-U	6	Dome - Base	None
3 - Sham U-U	6	Base - Base	None
4 - Stim D-U	7	Dome - Base	Electrical Stimulation
5 - Stim U-U	7	Base - Base	Electrical Stimulation

In groups 2, 3, 4 and 5, a bipolar stimulation electrode was implanted surgically in the bladder wall. The wires of the electrode were tunnelled in the back of the animal and connected to a plug anchored to the skull in order to connect easily the electrode to the stimulator for bladder electrical stimulation in awake animals. After a 2 weeks recovery period, electrical stimulation of the bladder in freely-moving rats (groups 4 and 5) was administrated with a constant current stimulator giving bipolar square wave pulses of 0.48 msec duration. A subtile contraction has been shown using the same stimulation parameters in a previous pilot study. Continuous stimulation for 1 hour was used with a frequency set at 20 Hz and the intensity adjusted at 10 mA to lead to bladder contraction without inducing voiding. The animals underwent perfusion-fixation with Somogyi solution and the brain tissues were dissected, snap frozen and cut into 30 µm coronal slices (Bregma -5.3 to -8.8 mm). Brain sections were processed for c-fos immunohistochemistry and photographed (4x and 10x magnification) using a camera mounted on a bright-field microscope. Cells exhibiting c-fos immunoreactivity were counted from 2 rostral and 2 caudal PAG images per rat. The mean number of cells was corrected for surface area and expressed as cells/mm^2. Histological data were analysed statistically with inter-group comparisons using *t*-test and are presented as mean ± S.E.M. values.

Results

During electrical stimulation of the bladder, rats behaved normally and no increased frequency in voiding was observed compared to sham and control animals. In this study, the topographical localization of c-fos expression in the PAG following electrical stimulation of the bladder has been investigated to detect stimulation-induced neural activity. Electrical stimulation of the urinary bladder increased the number of c-fos positive neurons in the PAG compared to sham and control groups (non-stimulated animals). C-fos immunoreactive cells were predominantly present in the caudal vlPAG. The qualitative assessment showed a strong density of c-fos positive cells localized within the ventrolateral aspects of the caudal PAG close to the aqueduct in stimulated compared to sham D-U animals (Figure 1).

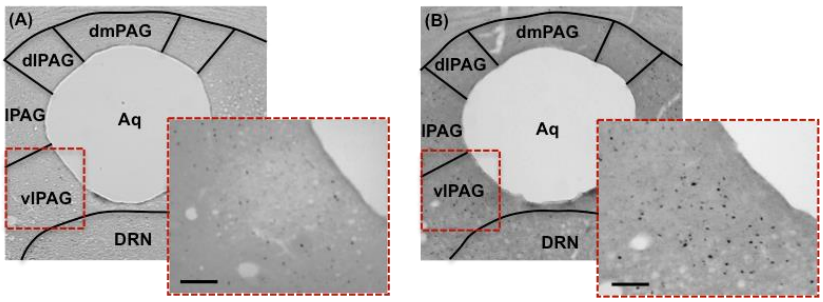

Figure 1: Effect of bladder stimulation on c-fos expression in the caudal vlPAG (Bregma -7.8 mm). Representative picture taken from a non-stimulated (sham) rat (A) and stimulated rat (B) with bipolar stimulation electrode implanted in the bladder wall at the dome and base levels. Aq: Aqueduct, DRN: dorsal raphe nucleus, dlPAG: dorsolateral PAG, dmPAG: dorsomedial PAG, lPAG: lateral PAG and vlPAG: ventrolateral PAG. Calibration bar = 100 µm.

No significant statistical difference in number of c-fos positive cells has been found between the control animals and the sham animals. For D-U electrode implantation, c-fos counts in the ventrolateral subdivision of the caudal PAG were significantly greater in stimulated compared to sham rats. It was also the case for the lateral and dorsomedial subdivisions of the caudal PAG (Figure 2). For U-U electrode implantation, the number of c-fos immunoreactive cells was not statistically different in the caudal PAG of stimulated and sham animals (Figure 2). In the rostral part of the PAG, the number of c-fos positive cells in the lPAG and DMPAG and lPAG, dlPAG and dmPAG for U-U and D-U stimulated rats respectively, was higher compared to the sham matched animals (Data not shown).

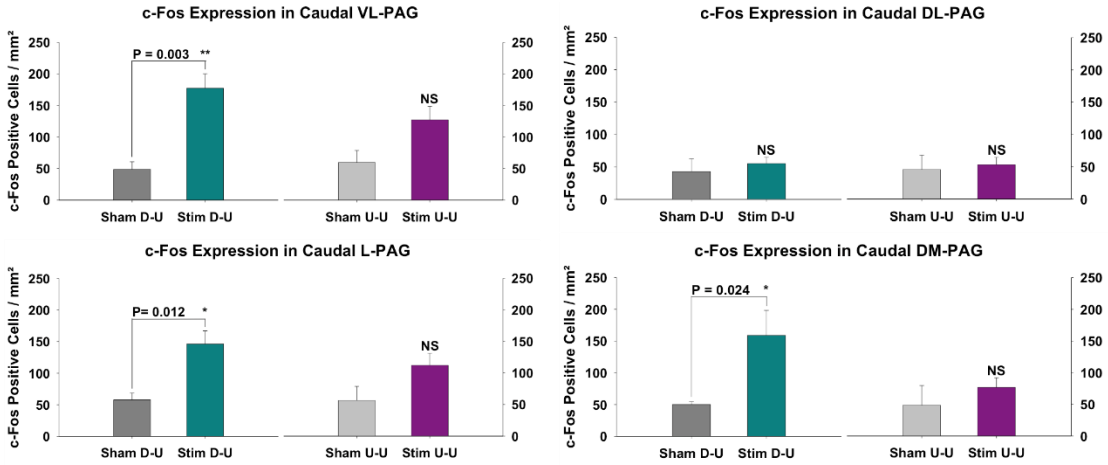

Figure 2: Number of c-fos positive neurons in different subdivisions of the caudal PAG.

Interpretation of results

In the caudal PAG of stimulated D-U rats, the number of c-fos positive cells in the ventrolateral subdivision was higher compared to the lateral, dorsolateral and dorsomedial columns. These results support a critical role of caudal vlPAG. The lack of effect in U-U implantation could be explained by the smaller stimulation field or by the electrical field being located closer to the bladder neck.

Concluding message

Bladder contractions induced by electrical stimulation influence neural activity in the PAG, especially in the medial area of the ventrolateral subdivision of caudal part.

To characterize and elucidate the function of the activated neurons in the propagation of signals generated by the bladder, additional investigations by immunohistochemistry and *in vivo* electrophysiology experiments are required.

Disclosures

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