The Time-Frequency Analysis of the Pudendo-to-Pudendal Nerve and Pelvic-to-Pudendal Nerve Reflexes in Anesthetized Intact Rats

Hui-Yi Chang Chi-Wei Peng Jia-Jin J. Chen Chen-Li Cheng[*,1]
William C deGroat[2]

Institute of Biomedical Engineering, National Cheng Kung University, Tainan, 701, Taiwan R.O.C.
[1]*Division of Urology, Department of Surgery, Tai-Chung Veterans General Hospital, Tai-Chung, 407, Taiwan R.O.C.*
[2]*Department of Pharmacology, University of Pittsburgh School of Medicine, Pennsylvania, USA*

Received 8 February 2004; Accepted 16 March 2004

Abstract

Two main functions of lower urinary tract, storage and periodic elimination of urine, are regulated by a complex neural control system located in the brain and spinal cord which coordinates the activity of the reservoir (urinary bladder) and the outlet (bladder neck, urethra and urethral sphincter). These organs are regulated by three sets of peripheral nerves: sacral parasympathetic (pelvic nerves), thoracolumber sympathetic nerves (hypogastric nerves) which innervate the bladder trigone and prostate, and sacral somatic nerves (pudendal nerves) which innervate external urethral sphincter (EUS). The relationship between the bladder and EUS is controlled by reflex pathways in the lumbosacral spinal cord that are activated by primary afferent input from the bladder or the urethra. This study was conducted to examine the reflexes that mediate bladder and sphincter coordination. We compared the properties of the pelvic nerve afferent to pudendal nerve reflex (pelvic-to-pudendal nerve reflex) and the pudendal nerve afferent to pudendal nerve reflex (pudendo-to-pudendal nerve reflex).For data analysis, we utilized the time-frequency analysis in Matlab to verify the components of pelvic-to-pudendal nerve reflex. The result of pelvic-to-pudendal nerve reflex indicates that there are two frequency bands: low-frequency nerve action potential low frequency (below 50 Hz) and high frequency nerve signal (50-100 Hz). Moreover, our result showed that the analytic method could extract the components in the reflex signals in lower urinary system.

***Keywords*:** Bladder; Pelvic nerve; Pudendal nerve; Time-frequency analysis

Introduction

The lower urinary tract has two main functions: storage and periodic elimination of urine. These functions are regulated by a complex neural control system located in the brain and spinal cord which coordinates the activity of the two components of the lower urinary tract: the reservoir (urinary bladder) and the outlet (bladder neck, urethra and urethral sphincter). Normally these structures exhibit reciprocal activity. During urine storage the reservoir is quiescent and the intravesical pressure remains in low pressure whereas activity in the outlet gradually increases during bladder filling to maintain continence. These organs are regulated by three sets of peripheral nerves: sacral parasympathetic (pelvic nerves) and thoracolumber sympathetic nerves which innervate the bladder and proximal urethra, and sacral somatic nerves which innervate the external urethral sphincter (EUS) [8].

The relationship between the bladder and EUS is controlled by reflex pathways in the lumbosacral spinal cord that are activated by primary afferent input from the bladder or the urethra. Bladder afferent axons pass through the pelvic nerves and urethral afferent axons pass through the pelvic floor muscle and pudendal nerves [2,3]. The pudendal nerve is considered a major component of the innervation of the pelvic structures, carrying efferent nerve fibers to the urethral sphincters as well as afferent nerve fibers from the sensory ending. Furthermore, its complexity is increased due to its relationship with sacral plexus, composed of fibers from lumbosacral trunk.

This study was conducted to examine the reflexes that mediate bladder and sphincter coordination. We compared the properties of the pelvic nerve afferent to EUS reflex (pelvic-to-EUS reflex) and pudendal nerve reflex (pelvic-to -pudendal reflex) and the pudendal nerve afferent to pudendal

*Corresponding author: Chen-Li Chen
Tel: +886-23741215 ; Fax: +886-4-23593160
E-mail: cheng20011@yahoo.com.tw

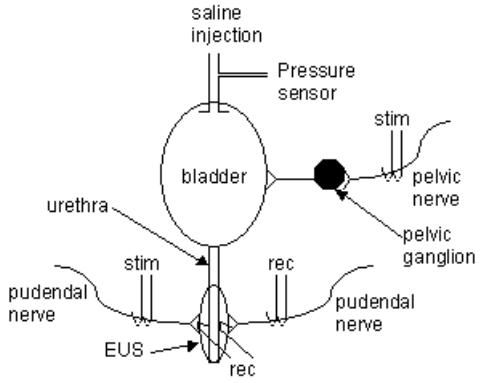


Figure 1. Overview of the experimental setup. stim: stimulation electrode, rec: recording electrode.

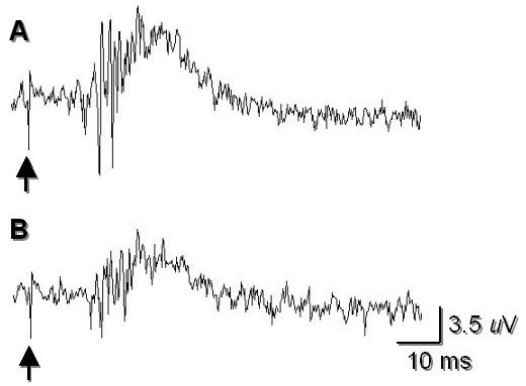


Figure 2. The pudendo-to-pudendal reflex was reduced by distension of the urinary bladder. A: the reflex was elicited by a single shock to the contra-lateral pudendal nerve (arrow) when the bladder was empty. B: the amplitude and area of reflex was reduced when the bladder was distended by 0.2 ml of saline.

nerve reflex (pudendo-to-pudendal reflex). We have examined the effect of bladder distension on these reflexes and the role of glutamic acid as a transmission in these reflexes by studying the effects of drugs that both N-methyl-D-asparate (NMDA) and alpha-Amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) glutamatergic receptors. These drugs have been studied previously on bladder and EUS functions [9-11]. Furthermore, signal processing techniques were utilized to extract the properties of the pelvic-to-pudendal reflex and pelvic-to-EUS reflex in terms of reflex latencies and frequency response after eliminating the interference from muscle activities.

Methods

Animal preparation

Female and male Sprague-Dawley rats (N=5, weight between 250-350 g) were anesthetized with urethane (1.2 mg/kg sc) and supplemental doses were given when necessary. The jugular vein was catheterized by polyethylene tube (PE-50) for administration of drugs. The urinary bladder was exposed via a midline abdominal incision and the half of pubic symphysis was removed to expose the middle urethra, the bilaterally pudendal nerves, pelvic nerve on one side and EUS. Polyethylene tubing (PE-50) was inserted through the apex of bladder dome into the bladder lumen and connected to a pressure transducer and the infusion system filled with physiological saline. The pudendal nerves were through incision of public symphysis and exposed it. Then the nerves were carefully dissected bilaterally. The left pelvic nerve of the animal was dissected carefully from the underlying tissue around bladder neck.

Cystometrograms (CMGs) were displayed on an oscilloscope and plotted on a rectilinear paper recorder. During the measurements, the abdominal cavity was filled with warm paraffin oil. Neural activity was amplified using an AC preamplifier (P15 Grass Instruments), displayed on an oscilloscope and averaged on a digital computer.

Experimental Design

To record the EUS electromyogram (EMG), two fine insulated silver wire electrodes (0.05 mm diameter) with exposed tips were inserted into the lateral sides of midurethra, where the muscle fibers of the EUS were identified. Bipolar silver-wire electrodes were positioned on the left pelvic nerve and bilaterally on the pudendal nerves (Figure 1). Reflexes were evoked by different frequencies of stimulation between 0.1 Hz to 5 Hz.

NMDA and AMPA glutamatergic receptors have an important role in the normal reflex pathways controlling the bladder and EUS. This study utilized the drugs, including MK-801 (dizocilpine, Merck, Sharp & Dohme Res. Labs., West Point, PA) and LY215490 ((3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[2-(1*H*-tetrazol-5-yl-)ethyl]-decahydroisoquinoline-3-carboxylic acid, Lilly Res. Labs.), which were both dissolved in normal saline for i.v. administration. Drug doses were calculated for the base of each compound.

Data analysis

The signals of pelvic-to-pudendal reflex and pelvic-to-EUS reflex were sampled at 2 kHz and amplified 20,000-fold and badnpass filtered (high-frequency cuff-off at 2,000 Hz, Low-frequency cuff-off at 0.1 Hz) to remove the high frequency interference and low frequency artifact. The sampling rate is 2000 Hz. The acquired signals after cutting off primarily stimulus artifacts and removing a linear trend, were processed by using the Wingner-Ville distribution for time-frequency analysis in Maltlab [1,5]. Winger-Ville distribution of time-frequency analysis shows the best possible resolution in time and frequency with no significant cross-term distortion. We are interested in applying the iterative sharpening of time and frequency resolution to provide a new modeling paradigm for observing the relationship between micturition reflex pathways. Furthermore, the theory of time-frequency representations is the concept of one- or two-dimensional Fourier transforms of the time-frequency plane. These plots offer alternative ways to quantify the changes in frequency and to observe the latency of reflex.

Results

The pudendo-to-pudendal nerve reflex

Figure2 shows the pudendo-to-pudendal reflex by a single

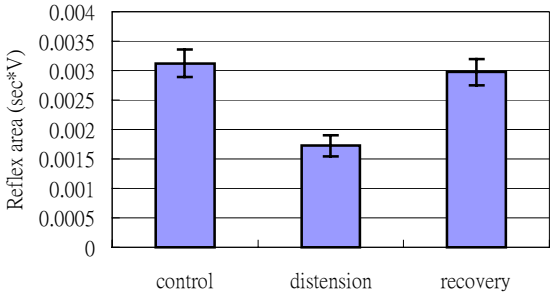


Figure 3. Effect of bladder distension on the pudendo-to-pudendal reflex. The bladder was distended with 0.2 ml of saline. (n=5, Mean ± S.E.M)

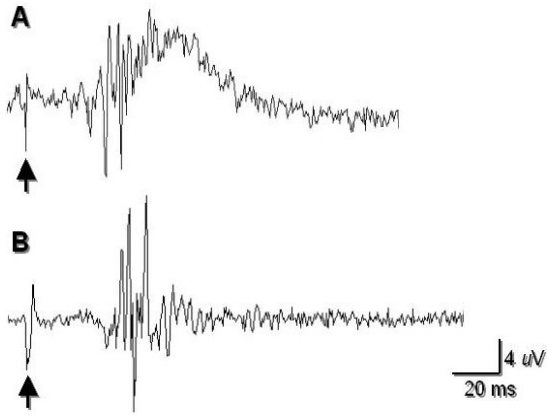


Figure 4. Comparison of the pudendo-to-pudendal and pelvic-to-pudendal reflexes when the bladder was empty. A: The pudendo-to-pudendal reflex elicited by a single shock to the contra-lateral pudendal nerve (arrow). B: The pelvic-to-pudendal reflex elicited by a single shock to the ipsilateral pelvic nerve (arrow). Responses represent the average of 20 individual reflexes recorded on a computer.

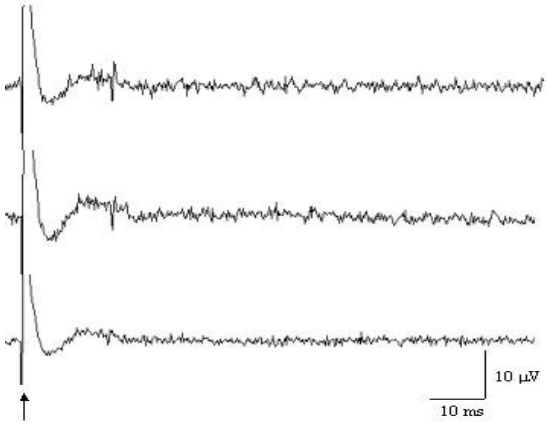


Figure 5. Upper panel shows the pelvic-to-pudendal nerve reflex elicited by single shock (12 V, 1 Hz, 0.05 ms pulse duration) when the bladder was distended. Middle panel shows that LY215490 (2.5 mg/kg) has a minor effect on reducing the reflex. However, lower panel shows that. MK801 (0.3 mg/kg) reduced the amplitude and reflex area following administration of LY215490.

pulse of electrical stimulation when the bladder was empty or was distended with 0.2 ml of saline. When the bladder was empty, electrical stimulation of one pudendal nerve with a single shock elicited a reflex to the contra-lateral pudendal nerve (Figure 2A) at latencies range from 18 to 20 msec. The reflex latency and amplitude was similar at different frequencies of stimulation between 0.5 ~ 5 Hz. The stimulus threshold was 0.3~0.8 volts at 0.5 Hz with 0.05 ms pulse duration. The amplitude and reflex area of pudendal-to- pudendal reflex were markedly suppressed (Figure 2B). The average reduction in the amplitude and reflex area of five tests was summarized in Figure 3. Around 37.6% of reduction in reflex area was observed in bladder distension condition.

The pelvic-to-pudendal reflex

Compared to pudendo-to-pudendal reflex (Figure 4A), the pelvic-to-pudendal reflex was elicited by electrical stimulation (3 volts, 0.5 Hz, 0.05 ms pulse duration) to ipsilateral pelvic nerve. Reflex responses were recorded from pudendal nerve axons (Figure 4B) at latencies range between 32-38 ms, mean 36.96 ± 0.05 ms, and at stimulus threshold between 1~2 volts at 1 Hz with 0.1 ms pulse duration.

Drug effects on reflex pathways

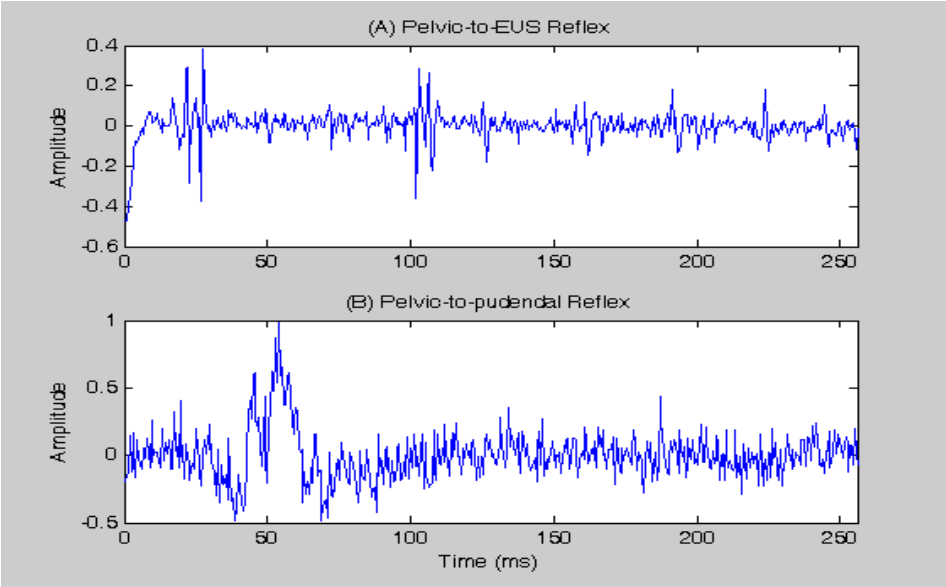
The previous study indicated that MK801 was significantly suppressed the amplitude of bladder contraction in normal rats [12-13]. Figure 6 shows the inhibitory effect of MK801 (0.3 ~ 5 mg/kg), an NMDA glutamate receptor antagonist, on the pelvic-to-EUS reflex. Long latency discharges were abolished by MK801; whereas the amplitude of short latency responses were suppressed 50% but not completely blocked. The pudendo-to-pudendal reflex was also reduced 20% by MK801 when the bladder was full or empty within 10 to 20 min after i.v. administration.

In normal rats, LY215490 in small i.v. dose (1-3 mg/kg) suppressed bladder contraction amplitude by 29% and EMG by 41%[11]. However, LY215490 (1~3 mg/kg, i.v.) had a minor additional effect following administration of MK801 in the present study. We observed the change in each experiment last to an hour after i.v. administration (Figure 5). Similar effects were obtained when the drugs were injected in reverse order. There is no significant decreased on the pelvic-to-pudendal reflex by injecting MK801, LY215490 or both of them.

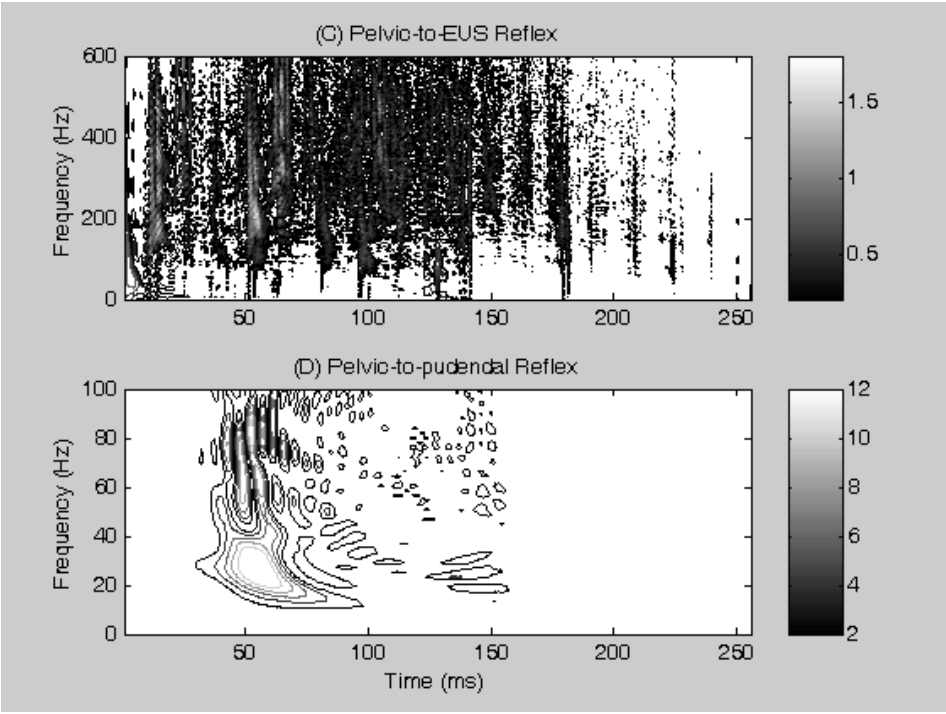
Time-frequency analysis

Figure 6(a) shows the pelvic-to-EUS reflex that was elicited by 3 volts at 0.5 Hz with 0.05 ms pulse duration and recorded from EUS. At the same time, the pelvic-to-pudendal reflex was also elicited by the same electrical pulse and recorded from the ipsilateral pudendal nerve. In time domain, these two reflexes have different latencies. The latency of the pelvic-to-EUS reflex is around 25 ms, and the pelvic-to-pudendal reflex is around 45-60 ms.

By utilizing the time-frequency analysis, the plot indicated two separated frequency ranges of the pelvic-to-EUS reflex, one is between 200 and 600 Hz and the other is below 100 Hz. Some higher activities were obviously observed at the late latencies in the time-frequency plot as well as easily



(a)



(b)

Figure 6. (a) The raw signals of pelvic-to-EUS reflex and pelvic-to-pudendal reflex (lower trace); (b) the results of time-frequency analysis for pelvic-to-EUS reflex and pelvic-to-pudendal reflex.

recognized the later responses of the pelvic-to-EUS reflex. The time-frequency analysis was helpful to make sure the late responses of the pelvic-to-EUS reflex because it is hard to differentiate in time domain. Figure 6(b) show the time-frequency plot for the pelvic-to-pudendal reflex. Several narrow bands (below 100 Hz) appeared at the time axisbetween 50~70 ms. The frequency components could be further classified into two frequency bands, one is low frequency below 50 Hz and the other is high frequency between 50~100 Hz.

Discussion and Conclusion

In the present study, we have examined the physiological properties of the pudendo-to-pudendal nerve and pelvic-to-pudendal nerve reflexes. The pudendo-to-pudendal nerve was inhibited by distending bladder. The drug effects of the pudendo-to-pudendal nerve and pelvic-to- pudendal nerve reflexes are different. NMDA antagonist (MK801) was markedly depressed the pudendo-to-pudendal nerve reflex, but AMPA antagonist (LY215490) did not. However, the pelvic-to-pudendal nerve reflex was not influenced by either MK801 or LY215490. Furthermore, it was very useful to utilize the

time-frequency analysis on to study early and late responses in the pelvic-to-EUS reflex as well as the variety of response frequencies in the pelvic-to-pudendal nerve reflex.

McKenna and Nadelhaft indicated that the pudendo-pudendal reflex is a polysynaptic spinal reflex, and the initial component is not abolished by spinal transaction [6-7]. This is consistent with our finding that short latency pudendo-to-pudendal reflexes, which are suppressed by bladder distension, may play a role in continence mechanisms.

Compare with the potentiation in pelvic-to-pudendal activity by repetitive pelvic afferent nerve stimulation [4], the action potential in the pelvic-to-pudendal reflex evoked by single-shock pelvic stimulation in this study is significant on EUS EMG activity when the bladder was distended. NMDA or AMPA antagonists have no significant effect on this reflex mechanism. It may imply another systematic manners such as the impulse indirectly goes through pelvic afferent neurons and is evoked by another peripheral pathways.

LY215490 in small i.v. dose (1-3 mg/kg) suppressed bladder contraction amplitude by 29% and EMG by 41%; whereas a large dose (10 mg/kg) completely eliminated bladder and EUS EMG activity in intact rats[11]. However, in our study, LY215490, in a small dose (1~3 mg/kg), has minor inhibition on the pudendo-to-pudendal reflex evoked by artificial electrical stimulation. The i.v. or i.t. administraction of MK801 and LY215490 depressed reflex bladder and EUS activity in urethane-anesthetized rats [9-11]. In contrast with AMPA antagonist receptor, NMDA glutamatergic mechanisms are important in the pudendo-to-pudendal reflex. MK801 (0.3 ~5 mg/kg) siginificantly suppressed the pudendal-to-pudendal reflex within 10 to 20 minutes by i.v. administrated.

The time-frequency analysis for pelvic-to-EUS and pelvic-to-pudendal reflex shows that it is more clear to observe the various reflex components in time-frequency domain compared to that with amplitude information in time domain. The time-frequency approach could provide certain information to distinguish the timing of reflex latencies including the onset and off time that might be lost in time domain. The late responses of the pelvic-to-EUS reflex can be easily identified in time-frequency domain. Signals with two major frequency bands were found in the pelvic-to-pudendal nerve reflex. The signals of these tow frequency bands might be related to different conduction velocities of varied nerve fibers. Due to the property of nerve conduction, firing of varied sized of nerve fibers such as A-delta nerve fibers and c-fibers could be mixed which can not be differentiated from the time-domain analysis. It is known that A-delta nerve fiber is faster than c-fiber which were both existed in LUT.

In summary, there is an interesting finding that the pudendo-to-pudendal nerve was inhibited when bladder was distended. Furthermore, NMDA antagonist (MK801) was markedly depressed the pudendo-to-pudendal reflex, but AMPA antagonist (LY215490) did not. We have seen varied frequency band in different time lags can be observed in the nerve reflex signals. Further study is needed to verify whether the time-frequency approached can be used for differentiating the firing of varied size of nerve fibers which has b=not been possible in time-domain analysis.

Acknowledgement

This work is supported by National Science Council (NSC-91-2213-E-075A-002) and ITRI, Taiwan to CL Cheng and JJ Chen, and the Li Foundation, USA to HY Chang.

Reference

[1] L. Cohen, “Time-frequency distributions-A Review”, *Proc. of IEEE*, 77: 941-81,1989.

[2] W.C. de Groat and W.D. Steers, “Neuroanatomy and neuro-physiology of penile erection. In: *Contemporary Management of Impotence and Infertility*”, Williams and Wilkins, Baltimore, 1988.

[3] W.C. de Groat, “Central nervous system control of micturition. In: O'Donnell P. Urinary incontinence”, *Mosby, St Louis*, 1997.

[4] T.B. Lin, “Dynamic pelvic-pudendal reflex plasticity mediated by glutamate in anesthetized rats”, *Neuropharmacology,* 44(2): 163-70, 2003.

[5] N. Martin, J. Mars, J. Martin, and C. Chorier, “A Capon's time-octave representation application in room acoustics”, *Proc. of IEEE Transactions on Signal*, 43(8): 1842-1854, 1995.

[6] K.E. McKenna and I. Nadelhaft, “The organization of the pudendal nerve in the male and female rat”, *Journal of Comparative Neurology,* 248(4): 532-549, 1986.

[7] K.E. McKenna and I. Nadelhaft, “The pudendo-pudendal reflex in male and female rats”, *Journal of the Autonomic Nervous System,* 27(1): 67-77, 1989.

[8] P. Pacheco, M.Martinez-Gomez Whipple B. Beyer C. Komisaruk BR. Somato-motor components of the pelvic and pudendal nerves of the female rat. *Brain Research,* 490(1): 85-94, 1989.

[9] M. Yoshiyama, J.R. Roppolo, J. Rihml, B. Blastos, and W.C. de Groat, “The effects of MK-801, an NMDA receptor antagonist, on the micturition reflex in the rat”, *Neuroscience Letters*, 126(2): 141-144, 1991.

[10] M. Yoshiyama, J.R. Roppolo, and W.C. de Groat, “Effects of MK-801 on the micturition reflex in the rat--possible sites of action”, *Journal of Pharmacology & Experimental Therapeutics*, 265(2): 844-850, 1993.

[11] M. Yoshiyama, J.R. Roppolo, and W.C. de Groat, “Effects of LY215490, a competitive alpha-amino-3-hydroxy-5- methyl-lisoxazole-4-propionic acid (AMPA) receptor antagonist, on the micturition reflex in the rat”, *Journal of Pharmacology & Experimental Therapeutics,* 280(2): 894-904, 1997.

[12] M. Yoshimura, “Bladder afferent pathway and spinal cord injury: possible mechanisms inducing hyperreflexia of the urinary bladder”, *Progress in Neurobiology,* 57(6): 583-606, 1999.

[13] M. Yoshiyama, K.A. Erickson, S.L. Erdman,and W.C. de Groat, “Effects of N-methyl-D-aspartate (dizocilpine) and alpha-amino-3-hydroxy-4- isoxazolepropionate (LY215490) receptor antagonists on the voiding reflex induced by perineal stimulation in the neonatal rat”, *Neuroscience,* 90(4): 1415-20, 1999.