

Nicotine-activated descending facilitation on spinal NMDA-dependent reflex potentiation from pontine tegmentum in rats

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Submitted 15 November 2007; accepted in final form 18 February 2008

Pan S-F, Peng H-Y, Chen C-C, Chen M-J, Lee S-D, Cheng C-L, Shyu J-C, Liao J-M, Chen G-D, Lin T-B. Nicotine-activated descending facilitation on spinal NMDA-dependent reflex potentiation from pontine tegmentum in rats. *Am J Physiol Renal Physiol* 294: F1195–F1204, 2008. First published February 20, 2008; doi:10.1152/ajprenal.00539.2007.—This study was conducted to investigate the possible neurotransmitter that activates the descending pathways coming from the dorsolateral pontine tegmentum (DPT) to modulate spinal pelvic-urethra reflex potentiation. External urethra sphincter electromyogram (EUSE) activity in response to test stimulation (TS, 1/30 Hz) and repetitive stimulation (RS, 1 Hz) on the pelvic afferent nerve of 63 anesthetized rats were recorded with or without microinjection of nicotinic cholinergic receptor (nAChR) agonists, ACh and nicotine, to the DPT. TS evoked a baseline reflex activity with a single action potential (1.00 ± 0.00 spikes/stimulation, $n = 40$), whereas RS produced a long-lasting reflex potentiation (16.14 ± 0.96 spikes/stimulation, $n = 40$) that was abolished by D-2-amino-5-phosphonovaleric acid (1.60 ± 0.89 spikes/stimulation, $n = 40$) and was attenuated by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (7.10 ± 0.84 spikes/stimulation, $n = 40$). ACh and nicotine microinjections to DPT both produced facilitation on the RS-induced reflex potentiation (23.57 ± 2.23 and 28.29 ± 2.36 spikes/stimulation, $P < 0.01$, $n = 10$ and 20 , respectively). Pretreatment of selective nicotinic receptor antagonist, chlorisondamine, reversed the facilitation on RS-induced reflex potentiation caused by nicotine (19.41 ± 1.21 spikes/stimulation, $P < 0.01$, $n = 10$). Intrathecal WAY-100635 and spinal transection at the T₁ level both abolished the facilitation on reflex potentiation resulting from the DPT nicotine injection (12.86 ± 3.13 and 15.57 ± 1.72 spikes/stimulation, $P < 0.01$, $n = 10$ each). Our findings suggest that activation of nAChR at DPT may modulate N-methyl-D-aspartic acid-dependent reflex potentiation via descending serotonergic neurotransmission. This descending modulation may have physiological/pathological relevance in the neural controls of urethral closure.

acetylcholine; serotonin; WAY-100635; intrathecal; rats; N-methyl-D-aspartic acid; spinal reflex potentiation

THE IMPORTANCE OF UNDERSTANDING the descending innervations coming from the brain stem to the spinal circuitry involved in the micturition function has been emphasized because it may offer the strategy for developing pharmacological therapy in

micturition disorders (38). Researchers applied electric shocks to the pontine reticular formation to relate this site with micturition function and revealed that the pontine reticular formation plays an important role in descending control on the functions of the lower urinary tract (30, 31, 54, 55, 67). The cholinergic system in brain areas of the central nervous system regulates physiological functions, including micturition (18, 21, 40). Administration of cholinergic agonists to the pontine tegmentum affected urodynamic parameters in reflexive micturition cycles (31, 67). In addition, pharmacological blockage of the nicotinic cholinergic receptors (nAChR) in the pontine tegmentum using chlorisondamine, a nicotinic receptor antagonist, abolished the modulation exhibited by cholinergic agonists (40, 56), suggesting that activation of nAChR in the pontine tegmentum is involved in the descending modulation on the spinal circuitry mediating micturition functions (19, 52).

Activation of nicotinic receptor in the brain area enhances the release of neurotransmitter, including norepinephrine, dopamine, glutamate, acetylcholine, and serotonin (5-hydroxytryptamine; 5-HT) in the terminal of descending fiber at the spinal cord level (16, 36, 50, 57, 60, 75). 5-HT has been shown to play an important role in the descending control of reflexive micturition functions (9, 10, 17, 21, 39, 69). 5-HT receptors are widely distributed in the lumbosacral spinal cord, which is the origin of parasympathetic outflow regulating the lower urinary tract (24, 53, 54, 73). Among 5-HT-receptor subtypes, the 5-HT_{1A} receptor had been well investigated due to the early availability of a selective 5-HT_{1A} receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635; see Refs. 22, 23, 34, 69). Previous experiments have demonstrated that the activation of descending 5-HT_{1A} systems modulates the spinal integrated voiding cycles in rats and suggested the importance of spinal 5-HT_{1A} receptors in the descending control of the micturition function (39).

Activity-dependent reflex plasticity, for example, long-term potentiation (5, 6, 52) and windup phenomenon (46, 65, 78), has been widely studied in the central nervous system for their presumed relevance to memory (51) and hyperalgesia (58), respectively. Forms of reflex plasticity can be elicited by

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applying electric shocks (15) and by reagent injections/perfusions to specific sites (33). On the other hand, an established/establishing reflex plasticity may be modified by genetic (64), pharmacological (32, 58), surgical (37, 61, 68), or behavioral manipulations (3, 59). However, few studies explored the possibility of modulating activity-dependent reflex plasticity by activating a specific nucleus, which sends neural projection to the site, where the reflex plasticity occurs. Such a study may mimic the physiological modulation on reflex plasticity and offer gateways to elucidate the physiological/pharmacological relevancies in it (2).

Using *in vivo* animal preparations, we demonstrated that low-frequency repetitive stimulations (RS) on the pelvic afferent nerve elicited a form of activity-dependent reflex plasticity, spinal reflex potentiation (SRP) in the pelvic-urethra reflex activities (12, 13, 41, 42, 43, 44, 45). Such *in vivo* preparations used in these studies maintained the whole neural network within the the central nervous system intact, thus offering an animal model to explore the possibility that activity-dependent reflex plasticity can be regulated by a specific nucleus that projects nerve fibers to the site where plasticity occurs. We recently explored the descending modulations on the reflex potentiation coming from the pontine reticular formation and reported that electric stimulation at the dorsolateral pontine tegmentum might activate the descending fiber to the spinal cord to facilitate SRP induction (11). In the present study, we further explored the possible neurotransmitter to activate this descending system by microinjecting nAChR agonists to the dorsolateral pontine tegmentum. We also identified whether spinal 5-HT_{1A} receptor mediates such a descending innervations at the spinal cord level by intrathecal injection of 5-HT_{1A} receptor antagonist.

MATERIALS AND METHODS

General preparations. Sixty-three adult female Wistar rats weighing 250–350 g were anesthetized with urethane (1.2 g/kg ip). The National Science Council in Taiwan approved the animal care and experimental protocol. The trachea was intubated to keep the airway patent. A PE-50 catheter (Portex; Hythe, Kent, UK) was placed in the left femoral vein for administration of anesthetics when needed. Body temperature was kept at 36.5–37.0°C by infrared light and was monitored using a rectal thermometer. The corneal reflexes of the rats and responses to a noxious stimulation to the paw were monitored throughout the experiment. If responses were present, a supplementary dose (0.4 g/kg) of urethane was given through the venous catheter. After all the experiments were completed, the animals were killed via an intravenous injection of potassium chloride saturation solution.

Intrathecal catheter. The occipital crest of the skull was exposed, and the atlantooccipital membrane was incised at the midline with the tip of a 24-gauge needle. A PE-10 catheter was inserted through the slit and passed caudally to the L₆ level of the spinal cord, the level presumed to mediate the micturition reflex. Test reagents were injected 1 min before the TS/RS onset. The volume of fluid within the cannula was kept constant at 10 μ l in all experiments. When we tested reagents intrathecally, single 10- μ l volumes of drug solutions were administered followed by a 10- μ l flush of artificial cerebrospinal fluid. At the end of the experiments, the location of the injection site was marked by an injection of Alcian blue (10 μ l, 2%), followed by a laminectomy to verify the location of the cannula tip. The volume of drug injected in the spinal cord in this experiment may spread from 0.5 to 1.5 mm from the site of injection, as previously described (12).

The experimental animals, in which the cannula tip deviated by >0.5 mm from the target structure, were excluded from statistical analysis.

Pontine stimulation and microinjection. The procedure to identify the dorsolateral pontine tegmentum was done as follows. The rat was placed in a stereotaxic apparatus. A small craniotomy was performed, and a fine stainless steel needle electrode that can be used for both stimulation and injection was inserted in the dorsolateral pontine tegmentum for electric stimulation and for testing agent injections. A transurethral bladder cannula was inserted in the urinary bladder from the urethra opening. A ligation around the urethra opening was done to immobilize the transurethral cannula and avoid fluid leakage from the bladder through the urethra. For recording intravesicular pressure (IVP), we connected the transurethral bladder cannula to a pressure transducer (P23 ID; Gould-Statham, El Segundo, CA), and the IVP was continuously recorded on a computer system (MP30; Biopac, Santa Barbara, CA) through a preamplifier (7P1; Grass, Cleveland, OH). Saline was injected through the transurethra bladder cannula to determine the threshold volume for inducing rhythmic isovolumic contractions. After the threshold volume was obtained, small amounts of saline were withdrawn from the bladder until bladder contractions disappeared. The bladder volume was kept isovolumically below the threshold for inducing spontaneous bladder contractions during brain stem stimulation. The needle electrodes were then introduced stereotaxically in the lateral part of the dorsolateral pontine tegmentum (anterior-posterior, –8.4 to –9.4 mm; lateral, 1.0 to 1.5 mm; height, –6.5 to –7.0 mm from the bregma) in 0.25- or 0.5-mm steps using a micromanipulator (34). Electrical stimulation in the dorsolateral pontine tegmentum consisted of sequences of stimulation (1–15 V, 0.05 ms in pulse duration at 300 Hz, 5 ms train duration). The optimal sites for inducing an isovolumic contraction in the urinary bladder, with the largest amplitude (usually >15 mmH₂O), were determined in each experiment. After localization of the stimulation point at the pos varolli, the ligation around the urethra opening was cut, and the transurethral bladder cannula was withdrawn. The lower body of the rat was rotated to a supine position, and a midline abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally and cut proximally to the ligation sites. The proximal ends of the ureters drained freely into the abdominal cavity. Another wide-bore intravesical bladder cannula was tied in the apex of the bladder dome. The urinary bladder was drained free to rule out unnecessary afferent inputs. Abdominal skin flaps were tied to a metal frame to form a skin pool, and the abdominal cavity was filled with warm mineral oil. After all the experimental procedures were finished, the stimulation site was electrically injured by passing a direct current through, and then the site was reconstructed with reference to the lesion mark. Experimental animals of which the stimulation site deviated by >1 mm from the target structure were excluded from statistical analysis.

Nerve dissection and stimulation. The left pelvic nerve was dissected carefully from the surrounding tissue and was transected distally, whereas the right pelvic nerve was left intact. The central stump of the left pelvic nerve was placed on a pair of stainless steel wire electrodes and was stimulated by an electric current of square wave pulses with pulse durations of 0.1 ms applied by a stimulator (S88; Grass) through a stimulus isolation unit (SIU5B; Grass) and a constant current unit (CCU1A; Grass).

Reflex activities recording. Epoxy-coated copper wire (50 μ m; Giken, Tokyo, Japan) electromyogram electrodes were placed in the periurethra area intra-abdominally. This procedure was performed using a 30-gauge needle with a hooked electromyogram electrode positioned at the tip (1.0–1.5 mm). The needle was inserted in the sphincter ~1–2 mm lateral to the urethra and then withdrawn, leaving the electromyogram wire embedded in the sphincter. The electromyogram activities were amplified 20,000-fold, filtered (high frequency cut-off at 3,000 Hz and low at 30 Hz) by a preamplifier (P511AC; Grass), and then continuously displayed on an oscilloscope (TDS

3014, Telectronics, Wilsonville, OR) and the recording system with a sampling rate of 20,000 Hz (MP30; Biopac).

Spinal cord transection. In some experiments, the vertebrae were exposed along the cervicothoracic level and followed by a 4-cm laminectomy. After a pair of microscissors had carefully opened the dura, the spinal cord was transected at the T₁ level using a microfor-

Experimental arrangement and protocol. The schematic arrangement of reflex activity recordings, in response to the afferent nerve stimulation and pons varolli stimulation/injection, is shown in Fig. 1A. After the urinary bladder drained free via the intravesical bladder cannula, the pelvic afferent nerve was stimulated using the parameters as follows: the test stimulation (TS) was single shocks at fixed suprathreshold strengths with a frequency of 1/30 Hz and lasting for 10 min. The RS was single shocks with a frequency of 1 Hz also lasting for 10 min with an intensity identical to the TS. Train pontine

stimulation was train pulses synchronized with afferent fiber RS (1 Hz) with 300 Hz in pulse frequency and 5-ms train duration.

Spinal cord transection. In some experiments, after facilitation on RS-induced SRP had been established by pontine nicotine microinjection and the animal has been connected to a ventilator, the spinal cord was transected at the T₁ level to verify the pathway involved in facilitation on SRP.

Application of drugs. Drugs were administered by intrathecal injections, including 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline [NBQX, a glutamatergic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist, 100 μ M, 10 μ l; Sigma], D-2-amino-5-phosphonovaleric acid [APV, a glutamatergic N-methyl-D-aspartic acid (NMDA) receptor antagonist, 100 μ M, 10 μ l; Sigma], L-glutamate (a nonselective glutamatergic receptor agonist, 100 μ M, 10 μ l; Sigma), NMDA (a glutamatergic NMDA receptor agonist, 100 μ M, 10 μ l; Sigma), WAY-100635 (a selective

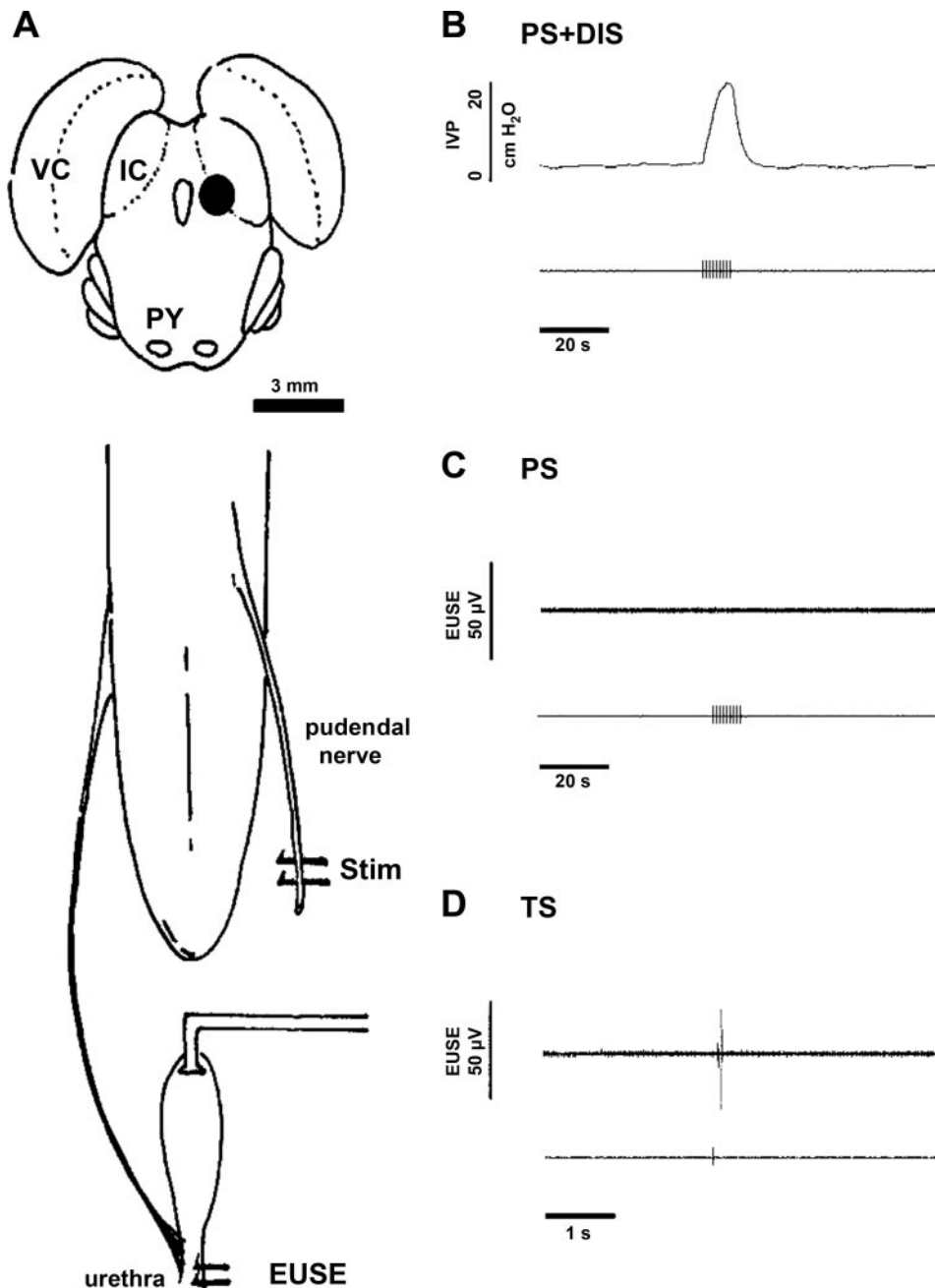


Fig. 1. Effects of pelvic afferent nerve stimulation and dorsolateral pontine tegmentum train stimulations. *A*: schematic arrangement of the external urethra sphincter electromyogram (EUSE) recording in response to pelvic afferent nerve and pontine stimulations or injections. VC, visual cortex; IC, inferior colliculi; PY, pyramidal tract. The black circle indicates the range of the locations of the stimulation/injection electrode tips. *B*: short train pulse pontine stimulation (PS) produced an increase in intravesicular pressure (IVP) in a isovolumically filled urinary bladder [PS + distension (DIS)]. *C*: after the urinary bladder was drained free, a short train pulse pontine stimulation (PS) evoked no action potential in the electromyogram activity. *D*: after the urinary bladder was drained free, a single action potential in the reflex activity was induced by pelvic afferent nerve test stimulation (TS).

serotonergic 5-HT_{1A} receptor antagonist, 100 μ M, 10 μ l; Sigma), ACh (a nonselective nAChR agonist, 100 μ M, 10 μ l; Sigma), (\pm)nicotine (a selective nAChR agonist, 100 μ M, 10 μ l; Sigma), and chlorisondamine (a selective nAChR antagonist, 100 μ M, 10 μ l; Sigma). All drugs were dissolved in artificial cerebrospinal fluid (in mM: 118 NaCl, 3 KCl, 25 NaHCO₃, 12 NaH₂PO₄, 1 MgCl₂, 1.5 CaCl₂, and 10 glucose; pH = 7.4) or dimethyl sulfoxide (DMSO) and applied in final DMSO concentration <1%. The solution of identical volume to tested agents was dispensed to serve as the vehicle. This selected dose of drug was used because previous studies showed that glutamate and NMDA could induce pelvic-pudendal reflex plasticity in urethane-anesthetized rats at this dose (44, 45). Besides, previous studies showed that 5-HT_{1A} receptor antagonist may modulate micturition reflex at the spinal cord level (20, 25, 26). Therefore, we used the above tested agents and adopted this effective dose. The dose-response curve was not obtained in our study.

Data analysis. All data in the text and Figs. 1–4 are mean values \pm SE. Statistical analysis of the reflex excitability between groups over the stimulation period was performed by means of ANOVA followed by a post hoc test. In all cases, a difference of $P < 0.05$ was considered to be statistically significant.

RESULTS

Complete data were obtained from 63 rats. Under some conditions, animals were excluded from analysis, including three rats did not complete the experimental procedures; in two rats the stimulation/injection electrode deviated by >1 mm, and in two rats the cannula tip for intrathecal injections deviated by >0.5 mm from the target structure. Therefore, there were 56 animals for statistical analysis. TS, RS, glutamatergic agonists/antagonists, and synchronized pontine stimulation were tested in all animals. There were 10 rats for spinal transection after pontine stimulation, 10 rats for pontine ACh injections, and 30 for pontine nicotine injections. In rats receiving pontine nicotine injections, 10 rats received pretreatment of chlorisondamine, 10 rats were further tested by intrathecal serotonergic antagonist injections, and the other 10 rats received spinal cord transection. In addition, there are an extra three rats each in glutamate agonist/antagonist and nicotine injection for vehicle control.

Pontine electrical stimulation. As shown in Fig. 1B, after saline of subthreshold volume has been instilled in the urinary bladder via the transurethral bladder cannula, pontine train stimulation produced a contraction wave in the isovolumically filled urinary bladder, indicating that the location of the electrodes was optimum. Next, the transurethra cannula was withdrawn. A midline abdominal incision exposed the urinary bladder and urethra, and an intravesical bladder cannula was inserted in the urinary bladder from the bladder dome so that the urinary bladder could drain freely. Train stimulation at the same site with identical parameters, after the bladder has drained freely, evoked no action potential in the external urethra sphincter electromyogram (Fig. 1C).

Baseline reflex activities. The reflex activities evoked by the pelvic afferent nerve TS (1/30 Hz) after the urinary bladder has drained freely, obtained from one of the 40 rats, are shown in Fig. 1D. A baseline reflex activity with a single action potential was elicited by the TS. The mean latency for the TS to evoke the activity was 57.42 ± 0.31 ms ($n = 40$).

RS-induced reflex potentiation. As shown in Fig. 2A, a longer-lasting reflex potentiation was induced by the afferent nerve RS (1 Hz) with the same intensity as the TS. The evoked

activity increased gradually following the onset of the RS, reached a plateau at ~ 2 min, and maintained this level until the cessation of stimulation. Summarized data in Fig. 2B show that the mean spike numbers evoked by the RS counted 10 min following stimulation onset increased significantly compared with the baseline reflex activities induced by the TS ($P < 0.01$, $n = 40$).

Glutamatergic agonists and antagonists. As shown in Fig. 2A, intrathecal administration of glutamate and NMDA both induced longer-lasting potentiation in the TS-induced reflex activities that is similar to that induced by the RS. On the other hand, intrathecal NBQX attenuated and intrathecal APV blocked the RS-induced reflex potentiation. Vehicle injections caused no effect on the test/RS-elicited reflex activity; therefore, the data were pooled to the TS/RS alone. The summarized data in Fig. 2B show that intrathecal glutamate and NMDA significantly increased the spike number evoked by the TS when compared with the TS ($P < 0.01$, $n = 40$). Meanwhile, intrathecal NBQX and APV significantly decreased the spike number evoked by the RS when compared with RS alone ($P < 0.01$, $n = 40$).

Synchronized pontine electrical stimulation. As shown in Fig. 3A, repetitive train pontine stimulation (1 Hz) evoked no action potential in the pelvic-urethra reflex activities. However, synchronized train pontine stimulation with the repetitive afferent nerve stimulation exaggerated the reflex potentiation compared with that elicited by afferent nerve RS alone. A spinal cord transection at the T₁ level abolished the facilitation caused by synchronized pontine stimulation but did not affect the reflex potentiation caused by pelvic afferent nerve RS. The summarized data in Fig. 3B show that the spike number elicited by the repetitive afferent fiber stimulation with the synchronized pontine stimulation was significantly higher than that induced by the repetitive afferent fiber stimulation alone ($P < 0.01$, $n = 40$). In addition, the facilitation on the RS-induced reflex potentiation caused by synchronized pontine stimulation was abolished by the spinal transection ($P < 0.01$, $n = 10$).

Effects of nAChR agonists. As shown in Fig. 4A, microinjection of ACh, a nonselective nAChR agonist, to the dorso-lateral pontine tegmentum facilitated the RS-induced reflex potentiation that is similar to the synchronized pontine electrical stimulation. On the other hand, a selective nAChR agonist, nicotine, also produced facilitation on the RS-induced reflex potentiation when it was microinjected in the identical site with the same concentration. Pretreatment of nAChR antagonist chlorisondamine before nicotine injection to the dorsolateral pontine tegmentum showed no effect on the reflex potentiation caused by RS but blocked the descending facilitation caused by nicotine injection. Figure 4B showed that both ACh and nicotine significantly increased the spike number evoked by the RS when compared with the RS alone ($P < 0.01$, $n = 10$ and 20, respectively), and chlorisondamine reversed the increase in spike number caused by nicotine injection ($P < 0.01$, $n = 10$).

Effects of 5-HT antagonist. Intrathecal pretreatment of WAY-100635, a 5-HT_{1A} receptor antagonist, did not affect the baseline reflex activity evoked by the TS (data not shown); however, as shown in Fig. 4A, it abolished the facilitation elicited by the pontine nicotine injection. Vehicle injections caused no effect on the RS-induced reflex potentiation; therefore, the data were pooled to the RS alone. The summarized data in Fig. 4B shows that WAY-100635 significantly de-

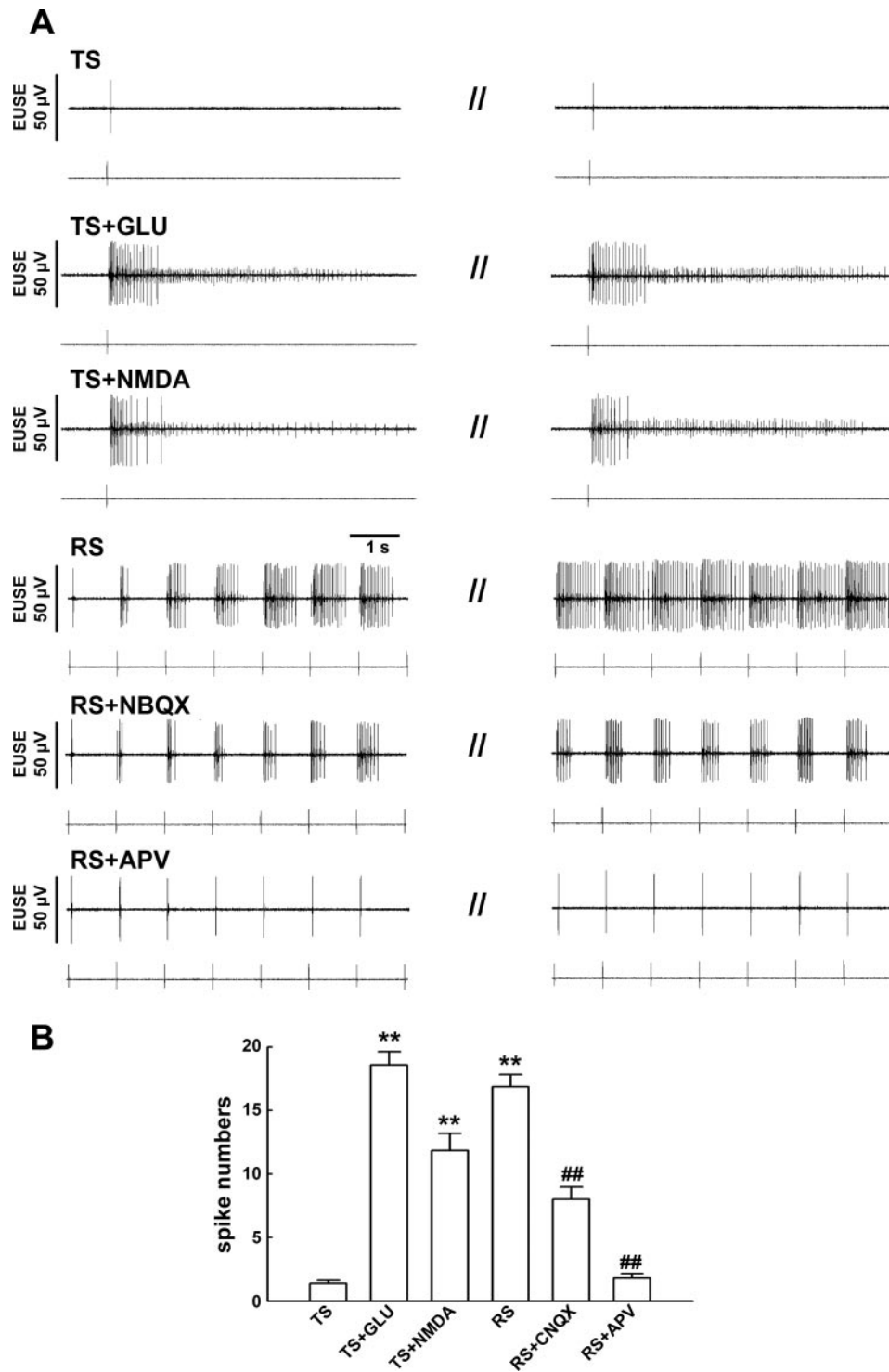


Fig. 2. The baseline reflex activity and repetitive stimulation (RS)-induced reflex potentiation. *A*: pelvic afferent nerve TS (1/30 Hz) evoked single action potentials in the EUSE. Glutamate (TS + Glu) and *N*-methyl-D-aspartic acid (NMDA) (TS + NMDA) both induce reflex potentiation in the TS-elicited reflex activities. RS (1 Hz) induced a longer-lasting reflex potentiation that was attenuated by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX) (RS + NBQX) and abolished by D-2-amino-5-phosphonovaleric acid (APV) (RS + APV). The tracings before and after the break symbol show the reflex activities at the onset of stimulation and at 10 min following the stimulation onset, respectively. *B*: mean spike numbers (mean \pm SE, $n = 40$) in reflex activity counted 10 min following stimulation onset induced by the pelvic afferent nerve stimulation with or without tested agents. $P < 0.01$, significantly different from that of the TS (***) and RS (##).

creased the spike number caused by RS in association with pontine nicotine microinjection when compared with RS with pontine nicotine injection ($P < 0.01$, $n = 10$).

Effect of spinal cord transection. As shown in Fig. 4A, a spinal cord transection at the T₁ level abolished the facilitation exhibited by nicotine injection. The summarized data in Fig. 4B show that spinalization at the T₁ level significantly decreased the spike number evoked by RS in association with

pontine nicotine microinjection when compared with RS in association with pontine nicotine microinjection ($P < 0.01$, $n = 10$).

DISCUSSION

Under physiological conditions, urine distension activates stretch receptors on the bladder wall and therefore generates action potentials, which propagate along the pelvic afferent nerve in the spinal dorsal horn. These impulses ascend via

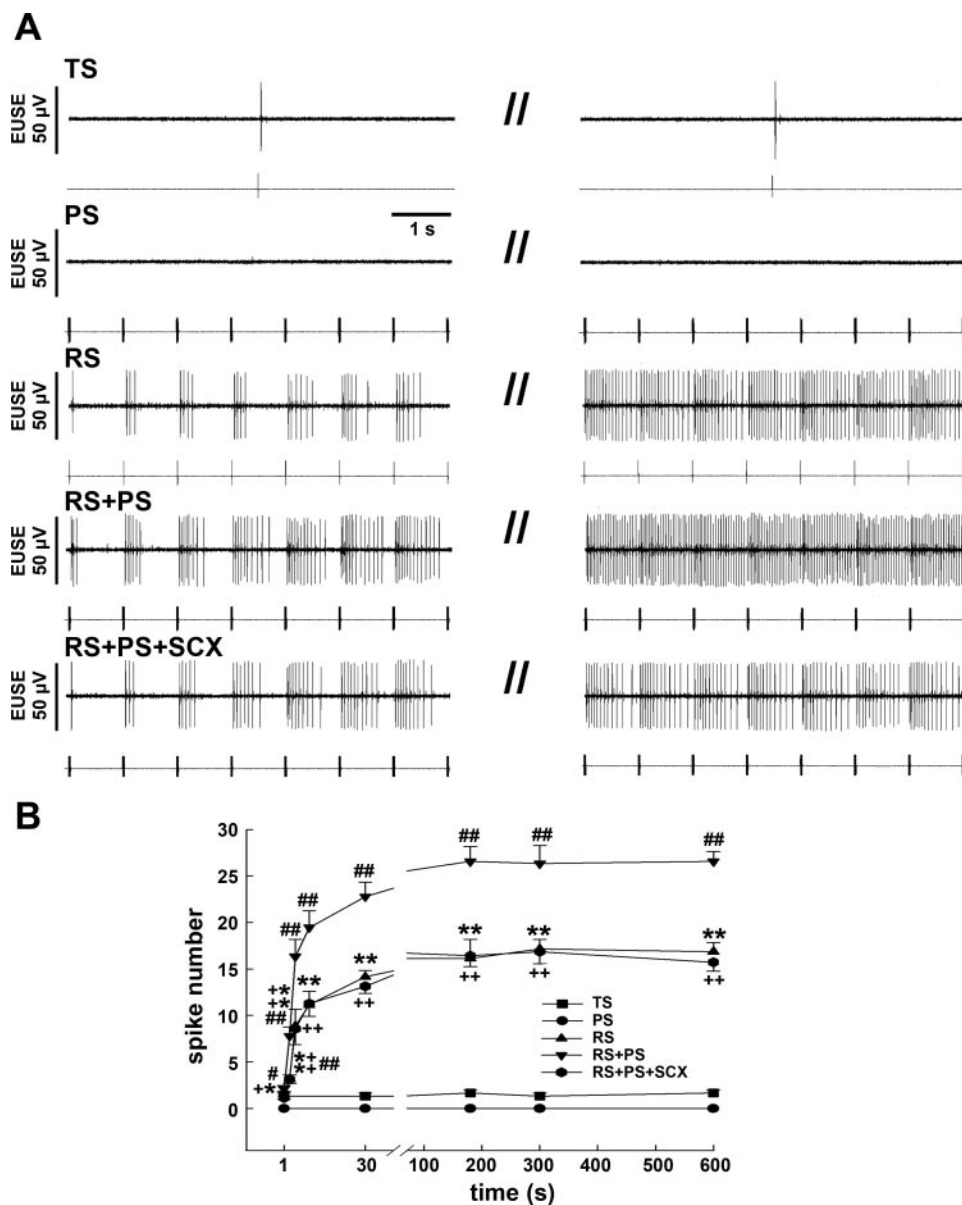


Fig. 3. Synchronized train pontine stimulation facilitated the RS-induced reflex potentiation. **A**: when compared with a baseline reflex activity with single action potentials in the EUSE elicited by the pelvic nerve afferent nerve TS, a long-lasting reflex potentiation was induced by the RS. Although the repetitive train pontine stimulation (PS) evoked no action potential, synchronized train pontine stimulation with pelvic afferent nerve RS facilitated the RS-induced reflex potentiation (PS + RS). A spinal cord transection at the T₁ level abolished the facilitation on the RS-induced reflex potentiation caused by synchronized train pontine stimulation (RS + PS + SCX). The tracings before and after the break symbol show the reflex activities at the onset of stimulation and at 10 min following the stimulation onset. **B**: mean spike nos. (mean \pm SE, *n* = 10) induced by the pelvic afferent nerve stimulation with or without synchronized pontine stimulation. *P* < 0.01, significantly different from activities induced by TS (**), RS (##), and RS in association with pontine stimulation (++)

interneurons to activate the pontine micturition center. If micturition is signaled by the center as appropriate, descending axons coming from this brain center activate sacral parasympathetic preganglionic neurons that traverse the pelvic efferent nerve and subsequently cause contraction in the bladder detrusor and relaxation in the urethra sphincter to induce voiding. On the other hand, a more rapid, somatic storage reflex, the guarding reflex, is initiated in response to a sudden increase in bladder pressure, such as during a cough, laugh, or sneeze. In the guarding reflex, afferent impulses caused by an abrupt increase in bladder pressure conduct through the pelvic afferent nerve to the sacral spinal cord, and activate the striated urethral muscle via interneurons to sphincter motor neurons in the Onuf nucleus, thereby “guarding” against urine leakage during a sudden unexpected increase in bladder pressure.

In the present study, train pulses stimulation at the dorsolateral pontine tegmentum produces detrusor contraction in the isovolumic filled bladder. Moreover, synchronized pontine

stimulation to the pelvic afferent stimulation exhibited an augmentation in the RS-induced SRP. However, the relevance of the facilitation on SRP caused by the dorsolateral pontine tegmentum descending projections has not yet been elucidated. We suggested that the descending projections coming from the dorsolateral pontine tegmentum might facilitate the guarding reflex activity, which is characterized by a urethra contraction caused by a sudden bladder pressure increase, and therefore promotes the reflexive urethra closure to avoid urine leakage during an abrupt bladder pressure increase. This result is quite correlated with urodynamic investigations that suggested the pontine micturition center may regulate the spinal cord circuitry controlling micturition functions via descending pathways in normal (31, 40) and stroke (80) rats. On the other hand, in this study, pharmacological activation of dorsolateral pontine tegmentum by microinjection of nAChR agonists exhibited descending facilitation on the RS-induced SRP. This result implied that the descending cholinergic pathway coming from the

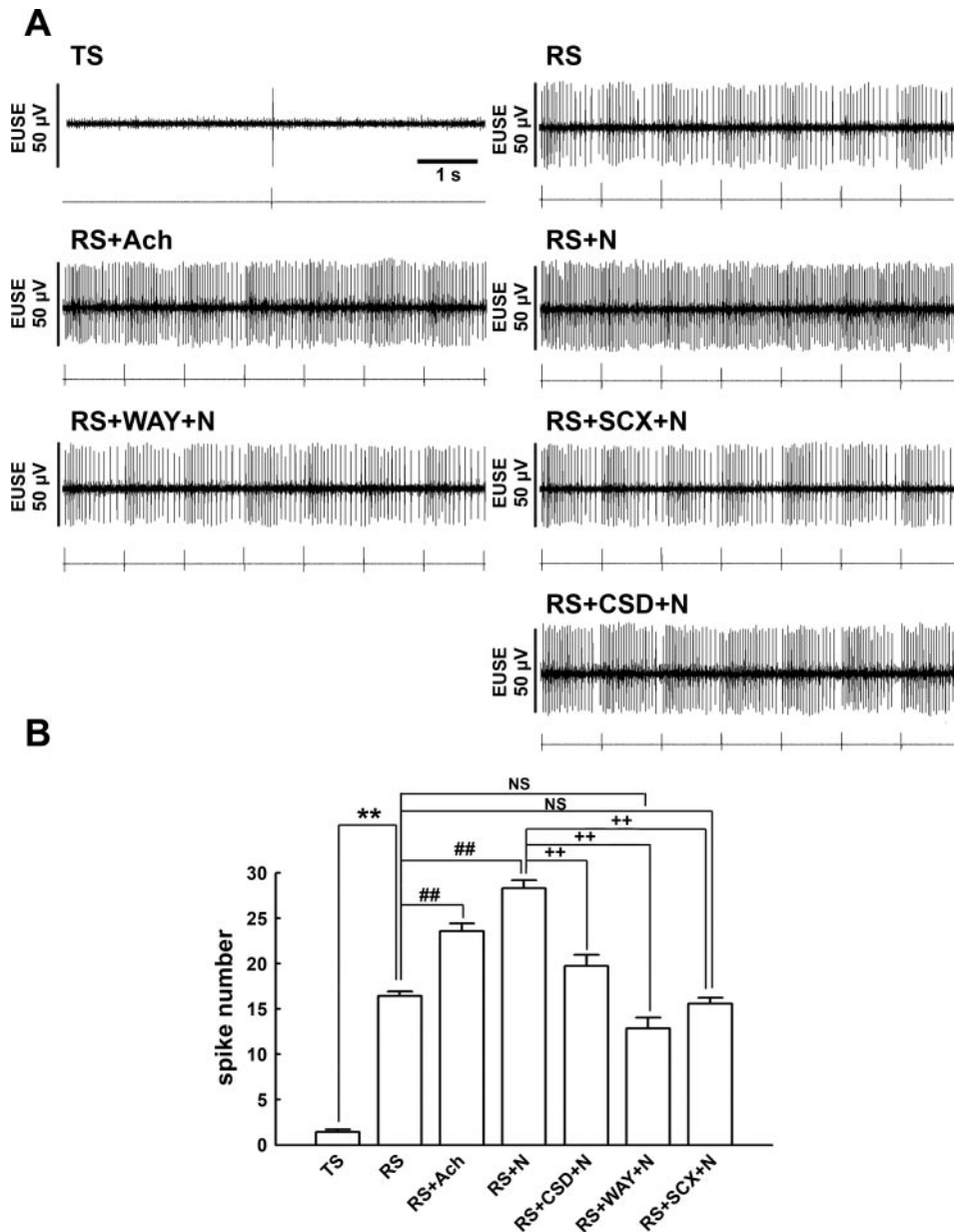


Fig. 4. Microinjection of cholinergic nicotinic receptor agonists to the dorsolateral pontine tegmentum facilitated the RS-induced reflex potentiation. *A*: when compared with a baseline reflex activity with a single action potential in the EUSE elicited by the pelvic nerve afferent nerve TS, a long-lasting reflex potentiation was induced by the repetitive afferent nerve stimulation (RS). Microinjection of ACh to the dorsolateral pontine tegmentum facilitated the RS-induced reflex potentiation (RS + ACh). Nicotine injection at the identical site with the same concentration also produced facilitation on the RS-induced reflex potentiation (RS + N) that was reversed by pretreatment of chlorisondamine (PS + CSD + N). Intrathecal WAY-100635 pretreatment at the lumbosacral level (RS + WAY + N) and a spinal cord transection at the high thoracic level (RS + SCX + N) both abolished the facilitation caused by nicotine injection in the dorsolateral pontine tegmentum. The tracings before and after the break symbol show the reflex activities at the onset of stimulation and 10 min following the stimulation onset. *B*: mean spike nos. (mean \pm SE, $n = 10$) counted 10 min following stimulation onset induced by the pelvic afferent stimulation with or without microinjection of tested agents. $P < 0.01$, significantly different from activities induced by TS (**), RS (##), and RS with pontine nicotine injection (++) .

brain area plays a role in regulation of guarding of urine from leakage, and nAChR seems to be a potential candidate for developing strategies in treatment of stress urine incontinence caused by urethra insufficiency resulting from neurological origins. This proposal is in agreement with pharmacological investigations in which intracerebroventricular nicotine injection increased the intercontraction interval in a continuously infused cystometry (29). However, there is a concern in this study about the possibility that, although the bladder was maintained empty during the stimulation period, pontine stimulation may induce undetectable bladder contraction, which increased the afferent impulse through the intact nerve trunk contralaterally to the stimulation, and result in potentiation in reflex activity. This possibility should be seriously considered in future investigations.

Serotonergic 5-HT_{1A} receptors have been identified at the lumbosacral spinal cord, where preganglionic neurons inner-

vating the lower urinary tract are located (54, 73). Two functionally distinct 5-HT_{1A} receptor populations, somatodendritic autoreceptors and postsynaptic receptors, mediate serotonergic neurotransmission within the spinal cord. Several 5-HT_{1A} receptor partial agonists have been identified that act as antagonists at postsynaptic receptors and agonists at presynaptic receptors (23). However, this combination of effects of 5-HT_{1A} receptor partial agonists causes complex and conflicting pharmacological results. Forster et al. (23) and Fornal et al. (22) found WAY-100635 to be a potent, selective, and silent 5-HT_{1A} receptor antagonist; therefore, this compound was widely used in pharmacological investigations on serotonergic neurotransmission. In the present study, we used WAY-100635 to evaluate the role of 5-HT_{1A} receptors in the regulation of micturition function at the spinal cord level. Our results showed that the descending facilitation on the SRP caused by pontine electric stimulation and nAChR agonists was abolished by the

pharmacological blockage using WAY-100635 at the spinal cord level, indicating that spinal 5-HT_{1A} neurotransmission at the lumbosacral spinal cord mediates the descending projections from the dorsolateral pontine tegmentum to regulate reflexive micturition function. This result is consistent with previous reports that the 5-HT_{1A} receptor antagonist abolished descending facilitation caused by electric stimulation at the dorsolateral pontine tegmentum. Moreover, such an abolition was reversed by the pretreatment of 5-HT_{1A} receptor agonist, indicating an excitatory role of spinal 5-HT_{1A} receptors in the descending control of the lumbosacral spinal reflex activity (7, 8, 25, 26, 40, 39, 70, 71). However, the mechanism by which 5-HT affects the glutamatergic NMDA-dependent SRP presented in this study is still waiting for elucidation. *In vitro* electrophysiological studies recording the dorsal horn field potential (47) and whole cell patch-clamp recording (62) have demonstrated that 5-HT may facilitate C fiber-evoked excitatory postsynaptic potential in a subpopulation of lamina III-VI spinal neurons. However, the detailed intracellular cascade involved and the physiological relevance needs further investigations.

Except being essential for urethral closure, pathological overactivation of the neural substrate involved in the pelvic-urethra reflex activity caused by injury or inflammation may produce urethra hyperreflexia or detrusor-sphincter dys-synergia (28), which are seen in patients with lower urinary tract syndrome (74, 79). Therefore, whether the blockage of descending facilitation on the SRP and urethra activity caused by WAY-100635 also implied that spinal serotonergic 5-HT_{1A} receptors can be substantial candidates to relieve the neurogenic hyperactive or dys-synergic urethra in patients with lower urinary tract syndrome (20, 72) is an interesting topic to be investigated further.

A repetitive activation of synaptic connections leads to activity-dependent reflex plasticity in brain areas (33, 48, 63). Forms of modulation on activity-dependent reflex plasticity, including long-term potentiation (1, 4) and central sensitization (76, 77), are still being examined because they may offer a gateway to clarify the physiological relevance and the possibility of pharmacological manipulation in activity-dependent neural responses (3, 61). It is widely accepted that glutamatergic NMDA neurotransmission underlies activity-dependent reflex plasticity in the central nervous system (66). Investigations on the neural control of the lower urinary tract, including the urinary bladder (81, 82, 84) and urethra (10, 83), have revealed the role of glutamatergic NMDA-dependent neurotransmission in the spinal-mediated micturition functions. In this study, we pharmacologically blocked spinal glutamatergic neurotransmission by intrathecal NBQX and APV injections and demonstrated that a glutamatergic NMDA-dependent neurotransmission was involved in the RS-induced SRP. These findings suggest that RS-induced SRP may share a similar glutamatergic NMDA-dependent neurotransmission to the well-investigated long-term potentiation (14, 27, 35, 49). However, there are some obvious differences between long-term potentiation and the SRP present in this study. First, LTP is induced by high-frequency tetanic afferent input that is usually beyond 100 Hz, whereas a low-frequency stimulation paradigm (of 1 Hz) was used in this study. In addition, LTP lasts for hours after tetanization, whereas SRP decays shortly after RS cessation. In our unpublished data, the evoked activity usually recovered to the baseline level within 1 min, even if the afferent fiber was

continuously stimulated with the TS following the RS offset. Furthermore, LTP applies to an increment in a single synaptic efficacy that is typically measured by a synaptic potential rather than action potentials, which were recorded in this study to reflect a reflex activity, and it is not a monosynaptic event. Therefore, we suggest that SPR, which was facilitated by descending serotonergic innervations in the present study, is possibly not an "LTP-like" synaptic transmission.

Recently, using *in vivo* animal preparations with their central nervous system intact, our laboratory has demonstrated that applying train pulses to the dorsolateral pontine tegmentum might activate serotonergic descending pathways to modulate the SRP, a form of activity-dependent reflex plasticity. This result implied that an established/establishing reflex plasticity could be modulated by a specific nucleus, which sends projections to the site where the plasticity occurs, and offered a novel model to investigate the neural regulation of plasticity induction. In this study, we further extended our work by activating the dorsolateral pontine tegmentum using nAChR agonists, and our data showed that microinjection of ACh and nicotine to the dorsolateral pontine tegmentum both facilitated the RS-induced SRP. Moreover, after nAChR agonist has established facilitation on SRP, a high thoracic spinal cord transection abolished the facilitation caused by reagent injection. These data showed that descending regulation on the induction of activity-dependent reflex plasticity at the spinal cord level can be elicited by activating a specific nucleus using a widely utilized neurotransmitter within the central nervous system, *i.e.*, implies that modulation of the induction of neural plasticity from another neural nucleus cannot only be elicited by using nonphysiological electric shock on a neuron but can also be done by activating the receptors on such a nucleus using physiological neurotransmitters. This *in vivo* animal preparation offers a model for investigating the regulation on the induction of plasticity using physiological neurotransmitter and for developing pharmacological therapeutic strategies on modulation of plasticity.

In summary, our findings in this study demonstrated that single-pulse pelvic afferent nerve stimulation evoked a baseline pelvic-urethra reflex activity, whereas RSs elicited long-lasting SRP, which can be facilitated by synchronized pontine stimulation. Pharmacological activation of nAChR at the dorsolateral pontine tegmentum by ACh and nicotine microinjections produces facilitation on the RS-induced SRP in a manner similar to the synchronized pontine stimulation, indicating involvement of nAChR in the activation of the descending projections coming from the dorsolateral pontine tegmentum to modulate the induction of activity-dependent neural plasticity at the spinal cord level. In addition, pharmacological blockage of serotonergic 5-HT_{1A} receptor at the spinal cord level and spinal cord transection both abolished the facilitation caused by nicotine injection at the dorsolateral pontine tegmentum, implying that spinal serotonergic 5-HT_{1A} receptor could be a potential candidate for developing pharmacological strategies in treatment of patients with neurological urethra hyperexcitability or dys-synergia.

GRANTS

This research was supported by the National Science Council in Taiwan Grants NSC-96-2314-B-040-012-MY2 to G. D. Chen and 96-2320-B-040-012 to T. B. Lin.

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