# Modulation of Neuropathic Pain by Neuropeptides at the Level of the Medulla in Rats

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# Modulation of Neuropathic Pain by Neuropeptides at the Level of the Medulla in Rats

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This certifies that the Master's Thesis of
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저 자 씀

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**ABSTRACT**

## Modulation of neuropathic pain by neuropeptides at the level of the medulla in rats

Neuropathic pain resulting from peripheral nerve injury is characterized by the development of abnormal pain states. It is accompanied by a number of neuroplastic alterations at the level of the spinal cord including upregulation or downregulation of neurotransmitters. Up to now, studies on the mechanisms of neuropathic pain has been conducted mostly at the level of the dorsal root ganglia or the spinal cord. However, according to the latest reports, the gracile nucleus has been suggested to play an important role in neuropathic pain. In this study, the effects of intracisternaly injected galanin and neuropeptide Y (NPY), and iontophoretically applied galanin and NPY on the modulation of neuropathic pain were examined at the level of the medulla. Under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to neuropathic surgery by tightly ligating and cutting the left tibial and sural nerves, leaving the common peroneal nerve intact. For the purpose of intracisternal injection of drugs, a stainless steel cannula was inserted to slide along the inner table of the occipital bone until the tip was just visible in the cistern. Mechanical allodynia was monitored using a von Frey filament. Then galanin or NPY was applied intracisternally. In an electrophysiological experiment, rats were reanesthetized with urethane and the responses of gracile nucleus neurons to mechanical stimulation

were recorded. Galanin or NPY was applied microiontophoretically. Intracisternally administered NPY reduced neuropathic pain behaviors dose-dependently. High doses (20, 40μg) of galanin inhibited neuropathic pain behaviors. At the lowest dose (1μg) of galanin and NPY, neuropathic pain behavior was increased at 30 min after drug injection. Responses of gracile nucleus neurons to mechanical stimulation were inhibited by iontophoretically ejected galanin and NPY. These results suggest that the neuropeptides including galanin and NPY play an important role in the gracile nucleus in the modulation of neuropathic pain.

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Key Words : neuropathic pain, gracile nucleus, neuropeptide Y, galanin

# Modulation of neuropathic pain by neuropeptides at the level of the medulla in rats

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**(Directed by Professor Bae Hwan Lee)**

## I. INTRODUCTION

Neuropathic pain resulting from peripheral nerve injury is characterized by the development of abnormal pain states (spontaneous pain, hyperalgesia and allodynia). Neuropathic pain is dependent on a number of neuroplastic alterations at the level of the spinal cord including upregulation or downregulation of neurotransmitters. Of the neurotransmitters, Neuropeptides especially galanin and neuropeptide Y (NPY) have been regarded to play an important role in the modulation of neuropathic pain.

Galanin, a 29-amino acid peptide, is widely distributed in the central and peripheral nervous system.[1] It has been shown to be involved in the transmission or modulation of nociceptive information at the spinal level.[2] Since its discovery in 1983 by Tatemoto and collaborators,[3] the

function of galanin for the modulation of pain at the spinal level has been studied, and complex functional patterns were shown. In all neuropathic pain animal models with peripheral nerve injury, complete axotomy,[4,5,6] nerve crush,[6] chronic constriction injury (CCI)[7,8,9] and partial nerve ligation,[7,9] galanin in dorsal root ganglia (DRG) neurons was upregulated. Behavioral and electrophysiological studies have shown that galanin produces complex effects with a remarkably inhibitory action on spinal nociception. The antinociceptive effect of intrathecal galanin is usually observed only at high doses (>1μg).[10,11] However, low doses (10ng-1μg) intrathecal (i.t.) galanin caused spinal reflex facilitation[12,13,14,15] and nociceptive hahavior .[16,17,18]

Neuropeptide Y (NPY), a 36-amino acid peptide, is an important regulatory peptide in both the central and peripheral nervous system.[19] Since its discovery in 1982,[20,21] NPY has been linked to numerous homeostatic mechanisms mediated by the increasing number of NPY receptors found in both central and peripheral nervous systems.[24] NPY plays a major role in mediating hyperalgesia and analgesia, and may be involved in the mechanisms of endogenous antinociceptive systems.[23,24,25] In subpopulations of dorsal horn interneurons, NPY-like immunoreactivity (IR) can be observed where it often coexists with the inhibitory transmitter GABA.[26] NPY is not found normally in sensory neurons in the DRG, but it is upregulated primarily in medium to large sized DRG neurons after axotomy.[27] Rats that were intrathecally administered NPY produced antinociception with the hot plate and paw pressure tests.[23] I.t. NPY caused a dose dependent, biphasic facilitation and inhibition of the flexor reflex in rats with intact sciatic nerves in a flexor reflex model in decerebrated, spinalized, unanesthetized rats,

Up to now, studies on neuropathic pain have progressed mostly at

the level of the DRG or spinal cord. According to the latest reports, the gracile nucleus may play an important role in neuropathic pain. Recently, Sung et al.[28] suggested that the mechanical allodynia, after partial peripheral nerve injury, involves the transmission of triggering sensory signals to sites rostal to the L1 spinal segment via the ipsilateral pathway(s). And, galanin-IR fibers and substance P were observed in the ipsilateral gracile nucleus of the neuropathic pain rats with peripheral nerve injury.[7,29] Miki et al.[30] reported that gracile nucleus neurons showed hyperexcitability to mechanical and/or thermal stimulation of the receptive fields in rats with CCI injury. Thus, it seems that supraspinal sites, especially the gracile nucleus, may play an important role in the modulation of neuropathic pain.

In this study, the effects of intracisternaly injected galanin and NPY on mechanical allodynia and iontophoretically ejected galanin or NPY on the activities of gracile nucleus neurons were examined in neuropathic pain rat model to show the specific roles of galanin and NPY in the modulation of neuropathic pain.

# II. MATERIALS AND METHODS

## 1. Subject

Male Sprague-Dawley rats (Daehan Bio Link, Daejeon, Korea) weighing 200-250g at the time of surgery were used. They were housed in groups of four until the time of surgery and provided with food and water ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine.

## 2. Surgery

Neuropathic pain rat model was prepared according to the method described by Lee at al.[31] Under pentobarbital anesthesia (50㎎/㎏, i.p.), a segment of the sciatic nerve was exposed between the midthigh level and the popliteal fossa by skin incision and blunt dissection through the biceps femoris muscle. The three major divisions of the sciatic nerve (tibial, sural and peroneal nerves) were clearly separated by individual perineurium. Neuropathic injury underwent tight ligation and cutting of the left tibial and sural nerves, leaving the common peroneal nerve intact. Complete hemostasis was confirmed and the wound was closed with muscle and skin sutures.

## 3. Behavioral test

A behavioral test for mechanical allodynia were performed using von Frey filament (8mN) for two weeks postoperatively. Rats were placed

on a metal mesh floor under a transparent plastic dome (8x8x18㎝), and innocuous mechanical stimulus was applied with a von Frey filament to the sensitive area of the hind paw. The most sensitive area was first determined by poking various areas of the paw with a von Frey hair and the actual test was conducted by gently poking the spot with the filament. A von Frey filament was applied ten times (once every 3-4 sec) to each hind paw. The frequency of foot withdrawal response expressed as a percentage was used as the index of mechanical allodynia.

**4. Intracisternal injection**

For the purpose of intracisternal injection of drugs, the surgical procedures were performed using methods previously described by Lankhorst et al.[32] and Solomon et al.[33] The rats were anesthetized with pentobarbital (50㎎/㎏, i.p.) and were mounted on a stereotaxic frame (Narishige, Tokyo, Japan). A midline incision of about 4 cm was made above the skull and two burr holes were made lateral of the midline, and two screws were placed on the two burr holes. A third burr hole was made just before the squama occipitalis. The occipital bone was then cleared of the surrounding muscular tissue and the atlanto-occipital membrane was identified and cleaned of extraneous connective tissue. Care was taken not to open the cisterna magna while establishing a translucent membrane through which the contents of the cisterna magna could be viewed. The stainless steel guide cannula of 1.1㎜ o.d. was placed in the third burr hole at an angle of about 60 degrees with the top of the skull. This approach allowed the cannula tip to slide along the inner table of the occipital bone. While the cisterna magna

was being viewed under high magnification of a surgical microscope through the atlanto-occipital membrane, the cannula was lowered until the tip was just visible in the cistern, and dental acrylic was then poured over the exposed surface of the skull to fix the cannula and screw assembly. And then a single dummy cannula was inserted into the guide cannula. After recovery, the rat was returned to a separate cage. This sugery was executed on the same day with neuropathic pain surgery. 2 weeks after surgery, for the intracisternal injection, the internal cannula was connected to a PE-50 tubing, which was connected to a Hamilton syringe that was first filled with saline, 1㎕ of air and then 7㎕ of drugs. The internal cannula was then inserted into the guide cannula and drugs was injected using infusion pump (55-2226, Harvard, Holliston, MA, USA) over a one-minute period. Injection of drugs was verified by the movement of a bubble created by the 1㎕ of air within the PE-50 tubing. Solutions for administration were prepared with sterilized saline, each with a volume of 7㎕ of 1, 10, 20 and 40㎍ of galanin and NPY (Bachem, Prussia, PA, USA). In order to identify if the effects of intracisternal injection of drugs resulted from the medulla, especially the gracile nucleus, laminectomy was carried out on the spinal column after behavioral test and then Evans blue (1%) was ejected intracisternally with volumes identical to the drugs. The flowing time and distance of Evans blue solution was observed for 3 hours.

**5. Microiontophoresis**

Two weeks after neuropathic injury, the rats were reanesthetized with urethane (1.25g/㎏ i.p.) and subjected to the electrophysiological

experiment. The trachea was cannulated for artificial ventilation. A laminectomy was performed at vertebral level of C1 and the rat was fixed in a stereotaxic frame (Narishige, Tokyo, Japan). A hole was drilled into the rat's occipital bone. The rat was paralyzed with a injection of pancuronium bromide (0.6㎎/㎏/㏗, i.v.). Artificial ventilation was adjusted to keep end-tidal $CO_2$ concentration between 3.5 and 4.5% using rodent ventilator (683, Harvard, Holliston, MA, USA) and capnomater (NORMOCAP200, DATEX, Mission Hill, CA, USA). Rectal temperature was monitored and maintained close to 37.5℃ by a servo-controlled electric heating pad (Harvard, Holliston, MA, USA). The exposed spinal cord and cerebellum were covered with a pool of warmed mineral oil (37℃). A 7-barreled microiontophoretic electrode was inserted into the gracile nucleus. At this time, the 7-barreled electrode was inserted at an angle of 30°. The center barrel of the 7-barreled electrode filled with 2M NaCl and 2% pontamine sky blue was used to record extracellularly the single unit activities of gracile nucleus neurons. One of the other six barrels was filled with 0.9% saline for use as a current balance, whereas the other outer barrels were used for iontophoretic ejection of substances. Action potentials of the gracile nucleus neurons were characterized based on their responses to mechanical stimulation by a von Frey filament applied to the peripheral receptive field. For the iontophoresis, the following substances were applied to the single neuron through multibarreled microelectrode: galanin (1㎜, 80-110㎁) and NPY (1㎜, 80-110㎁). Any changes in the single unit activities were monitored. Action potentials were amplified with an AC-coupled amplifier (DAM-80i, WPI, Sarasota, FL, USA) and drive amplifier (AM502, Tektronix, Beaverton, OR, USA). Amplified signals were led to an oscilloscope (5115, Tektronix,

Beaverton, OR, USA) for display. They were also led to a window discriminator (121 WPI, Sarasota, FL, USA), whose output was used to drive a speaker and to construct peristimulus time histogram (PSTH) by a data acquisition system (1401 plus, CED, Cambridge, UK) and personal computer (Fig. 1).

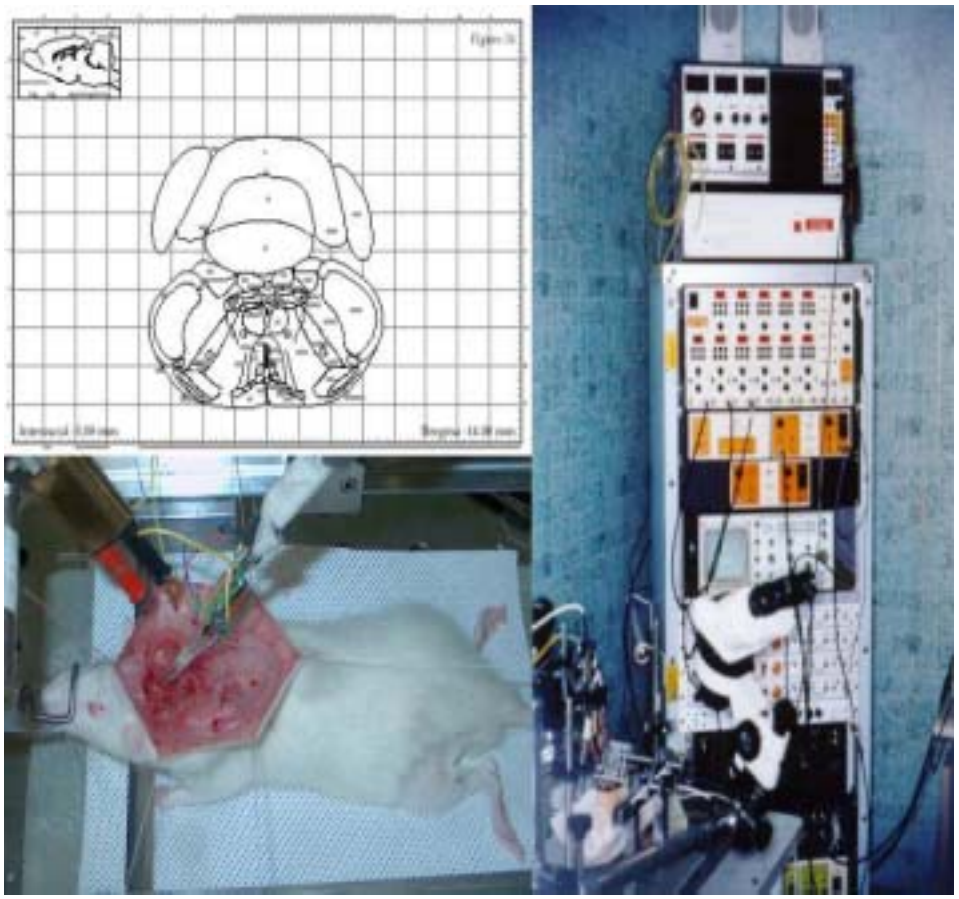

**Fig. 1.** Microiontophoresis setup

**6. Statistical analysis**

The discharge frequency was presented as mean ± standard error of the mean (S.E.M.). Differences in the changes of neuropathic pain behavior following intracisternal injection of galanin or NPY were tested using the one sample t-test. Differences in the changes of neuropathic pain behavior between saline treated group and drug injection group were tested using the one way ANOVA followed by Dunn's post hoc pairwise comparisons. Differences in changes of neuronal activity after drug ejection by iontophoresis were tested using the one sample t-test.

# III. RESULTS

## 1. The effects of intracisternally applied galanin on mechanical allodynia

Rats, which had neuropathic pain, received intracisternal injection of 1 (n=16), 10 (n=16), 20 (n=17) and 40μg (n=19) of galanin, and 0.9% saline for comparison (n=11). The results are shown in Fig. 2. The behavioral data from the rats in which Evans blue did not reach the area of the gracile nucleus were excluded. The statistical difference was evaluated by one sample t-test.

20 and 40μg of galanin reduced mechanical allodynia only at 5min after intracisternal injection (Fig. 2D and E; $p<0.05$). However, the lowest dose (1μg) of galanin increased mecanical allodynia at 30min after drug injection (Fig. 2B; $p<0.05$). There were no effects on mechanical allodynia after injection of 10μg galanin and saline (Fig. 2A and C).

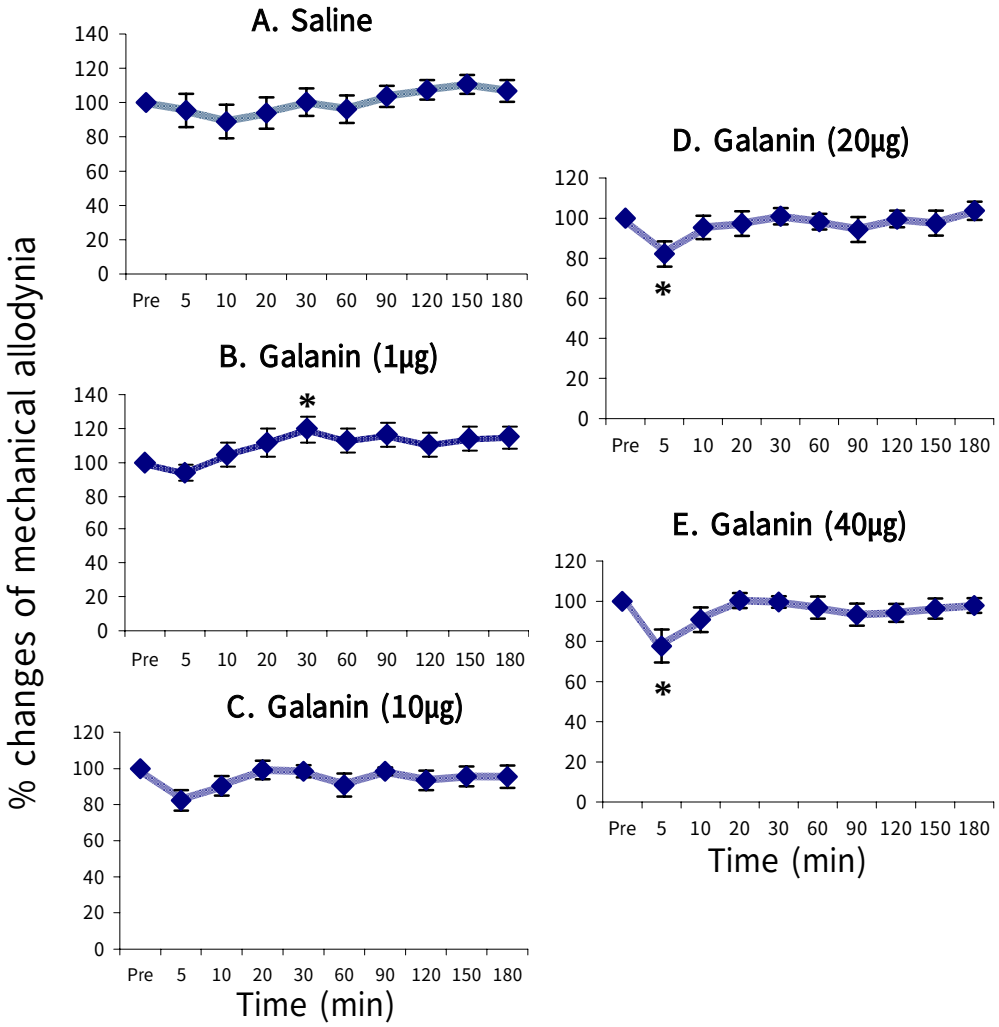


**Fig. 2.** Effects of intracisternal injection of galanin on mechanical allodynia. Mechanical allodynia was monitored using a von Frey filament (8mN). A: Intracisternal injection of 7㎕ of 0.9% saline as a control group, B: Intracisternal injection of galanin 1㎍, C: Intracisternal injection of galanin 10㎍, D: Intracisternal injection of galanin 20㎍, E: Intracisternal injection of galanin 40㎍. The data are expressed as mean ± S.E.M. Analysis was done by one sample t-test, *p<0.05.

## 2. The effects of intracisternally applied neuropeptide Y on mechanical allodynia

Rats, which had neuropathic pain, received intracisternal injection of 1 (n=20), 10 (n=16), 20 (n=19) and 40μg (n=18) of NPY, or 0.9% saline for comparison (n=16). The results are shown in Fig. 3. The behavioral data from the rats in which evans blue did not reach the area of the gracile nucleus were excluded. The statistical difference was evaluated by one sample t-test.

Intracisternally applied NPY inhibited mechanical allodynia 2-3 weeks after nerve injury. 1 μg of NPY inhibited mechanical allodynia at 5 min after intracisternal injection (Fig. 3B; $p<0.01$). However, facilitated effect was also shown at 30 min and 90 min after intracisternal injection of 1 μg of NPY (Fig. 3B; $p<0.05$). 10, 20 and 40 μg of NPY inhibited mechanical allodynia after intracisternal injection and the inhibitory effects lasted for 10 to 60 min (Fig. 3C, D and E; $p<0.01$). There were no effects on mechanical allodynia after injection of saline (Fig. 3A).

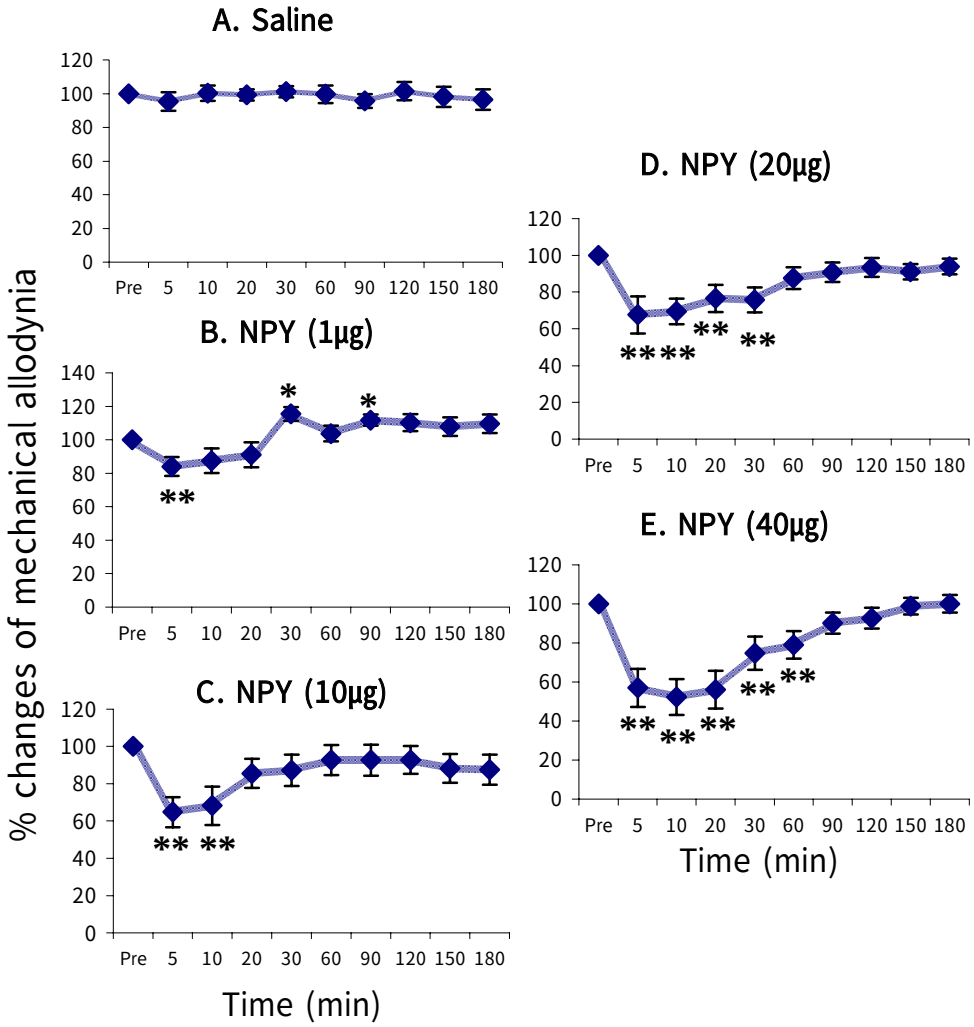


**Fig. 3.** Effects of intracisternal injection of neuropeptide Y on mechanical allodynia. Mechanical allodynia was monitored using a von Frey filament (8mN). A: Intraciternal injection of 7μℓ of 0.9% saline as a control group, B: Intracisternal injection of NPY 1μg, C: Intracisternal injection of NPY 10μg, D: Intracisternal injection of NPY 20μg, E: Intracisternal injection of NPY 40μg. The data are expressed as mean ± S.E.M. Analysis was done by one sample t-test, *p>0.05, **<0.01.

### 3. Comparing the effects of different doses of galanin and neuropeptide Y applied intracisternally on mechanical allodynia

The effects of different doses of galanin and NPY applied intracisternally on mechanical allodynia were compared. The results are shown in Fig. 4.

All doses of galanin were not shown to inhibit mechanical allodynia significantly compared to the saline treated group (Fig. 4A; $p>0.05$). However, intracisternally injected NPY inhibited mechanical allodynia dose-dependently. 10μg of NPY inhibited mechanical allodynia at 10min after intracisternal injection compared to the saline treated group. 20μg of NPY inhibited mechanical allodynia at 10 min and 30 min after intracisternal injection. At the highest dose of NPY (40μg), mechanical allodynia was reduced significantly at 5 to 30 min after drug injection compared to the saline treated group (Fig. 4B; $p<0.05$). The statistical difference was evaluated by one way ANOVA followed by Dunn's post hoc pairwise comparisons at each time point.

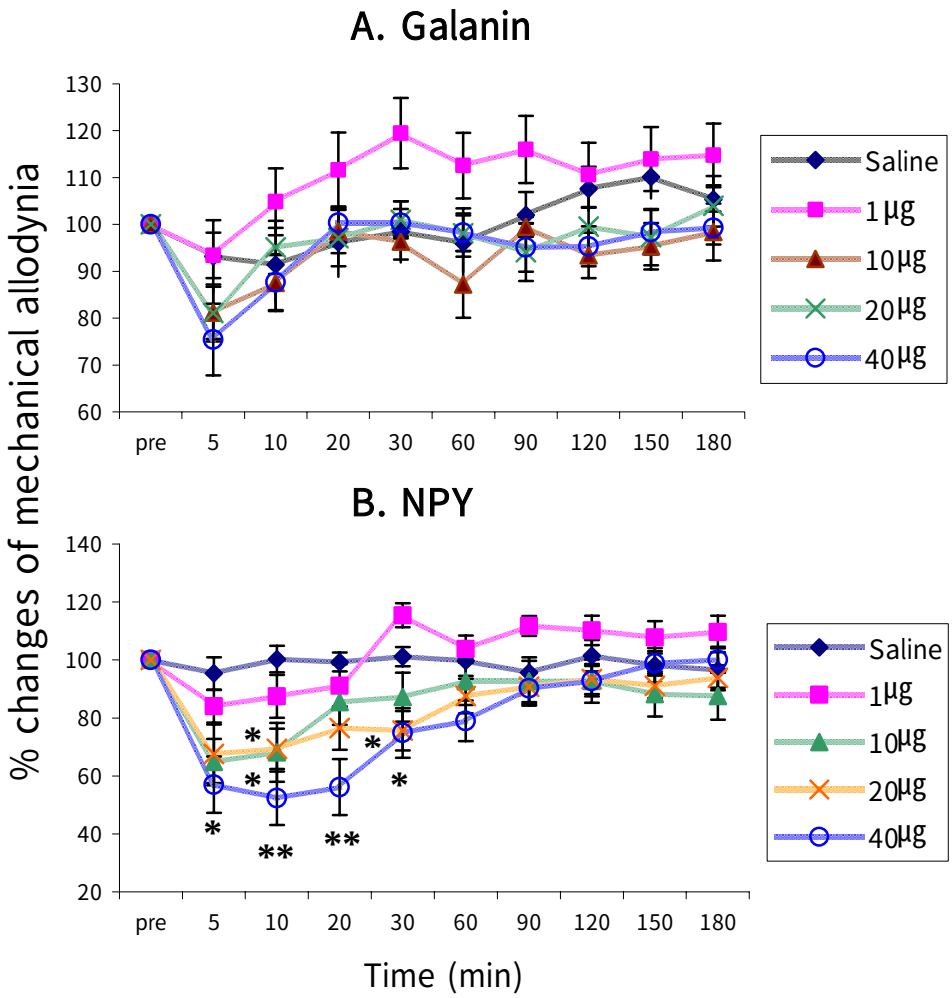


**Fig. 4.** Comparing the effects of different doses of galanin and neuropeptied Y on mechanical allodynia. A: galanin injection group with different doses, B: NPY injection group with different doses. Neuropathic pain behavior to mechanical stimulation was inhibited dose dependently after intracisternal injection. The data are expressed as mean ± S.E.M. Analysis was done by one way ANOVA followed by Dunn's post hoc pairwise comparisons, *p<0.05, **<0.01 compared with the saline treated group.

## 4. Effects of iontophoretically applied galanin and neuropeptide Y on the responses of gracile nucleus neurons to mechanical stimulation

Galanin (1㎜, 80-110㎁) and NPY (1㎜, 80-110㎁) were ejected for 2min microiontophoretically into the gracile nucleus while recording neuronal activities.

A representative response of a gracile nucleus neuron to mechanical stimulation following microiontophoretic ejection of neuropeptides is shown in Fig. 5. As Shown in Fig. 5. iontophoretically ejected galanin and NPY inhibited the response of a gracile nucleus neuron to mechanical stimulation. Fig. 6. shows the effects of microiontophoretically applied galanin and NPY on the response of gracile nucleus neurons to mechanical stimulation. The statistical difference was evaluated by one sample t-test.

Galanin inhibited neuronal responses to mechanical stimulation during drug ejection ($p<0.05$) but the inhibitory effect was recovered immediately after ejection (Fig. 6A). Responses of gracile nucleus neurons to mechanical stimulation were reduced during NPY ejection ($p<0.05$) and the inhibitory effects lasted for 90s after ejection (Fig. 6B).

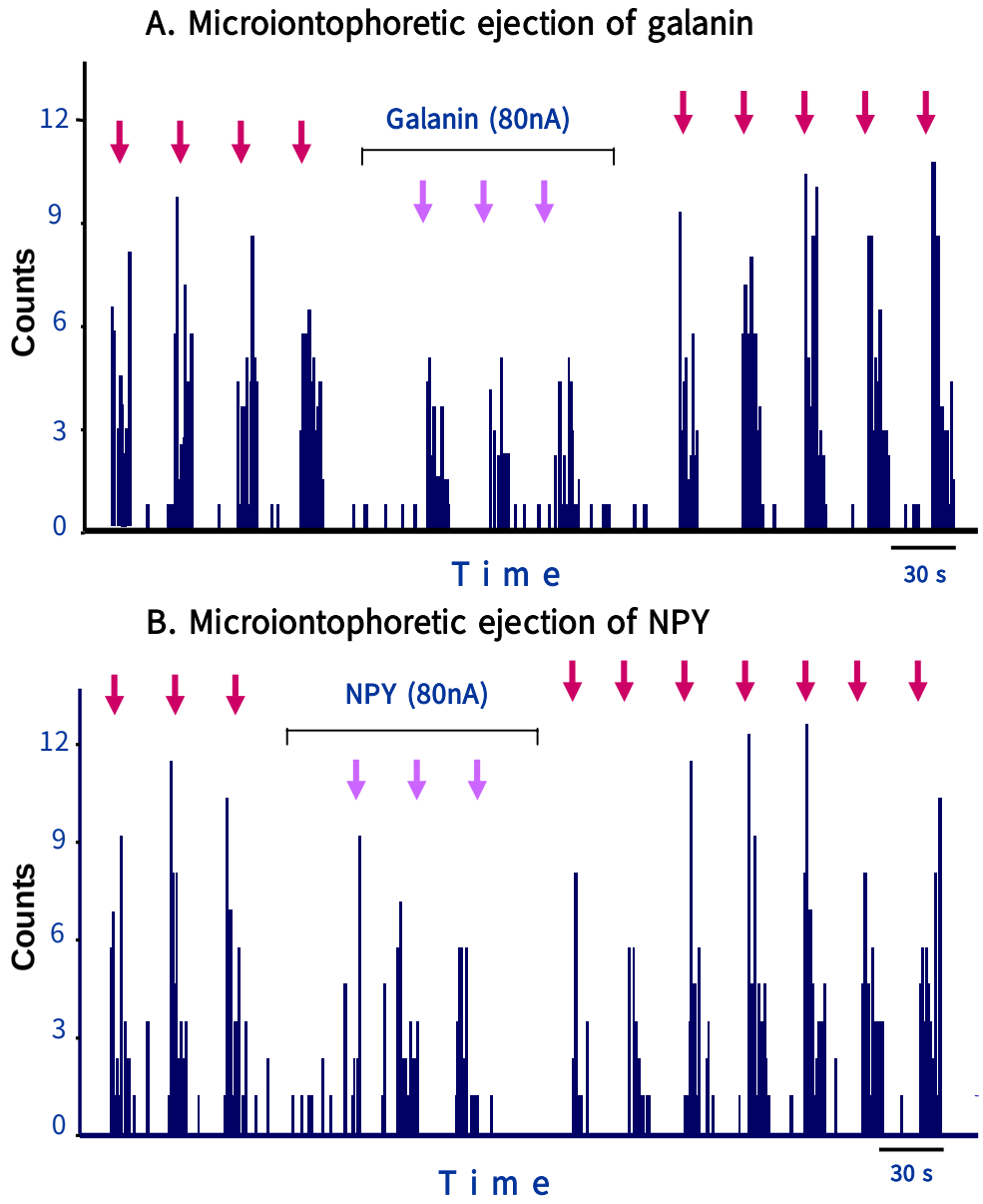


**Fig. 5.** A representative response of a gracile nucleus neuron to mechanical stimulation following the applications of galanin and NPY. Arrows indicate the mechanical stimulation using von Frey filament. A: Galanin ejection, B: NPY ejection.

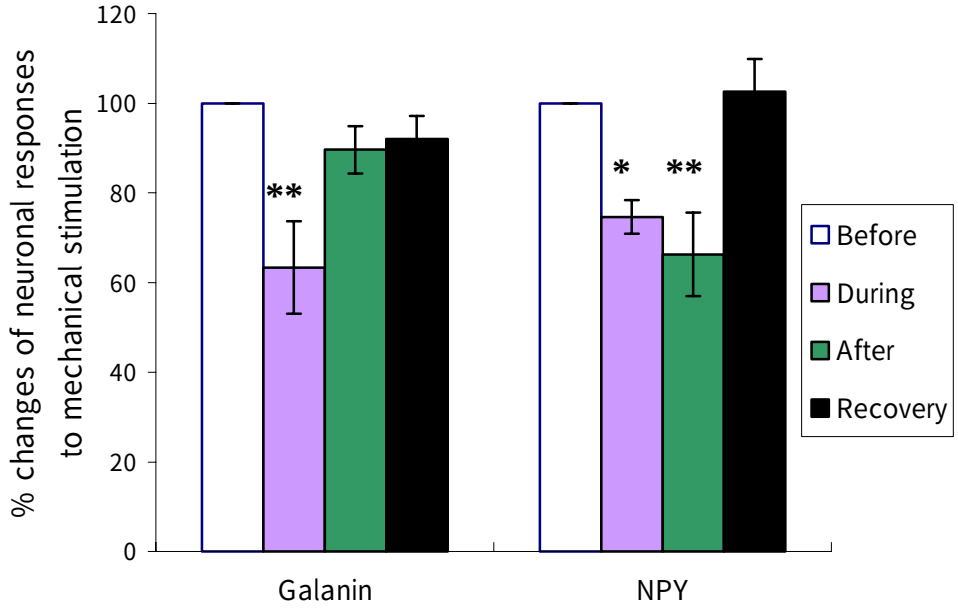


**Fig. 6.** Effects of iontophoretically applied galanin and neuropeptie Y on the responses of gracile nucleus neurons to mechanical stimulation. Galanin and NPY were ejected for 2min into gracile neucleus neurons. Before: before drug ejection, During: during drug ejection, After: after drug ejection but before recovery, Recovery: after recovery. The data are expressed as mean ± S.E.M. Analysis was done by one sample t-test, *p<0.05, **<0.01.

# IV. DISCUSSION

## 1. Inhibitory effects of neuropeptides on mechanical allodynia

In the present study, intracisternally injected NPY into the gracile nucleus inhibited mechanical allodynia dose-dependently in a neuropathic pain rat model. However, galanin reduced neuropathic pain behavior only 5 min after injection, but had no effect on controlling neuropathic pain at any time point compared with the saline treated group.

In order to identify if neuropeptides were injected into the vicinity of the gracile nucleus, laminectomy was carried out through the vertebral column after behavioral test. Then Evans blue was injected intracisternally with volumes identical to the those of the drugs. The flowing time and distance of Evans blue were observed for 3 hours. In 60% of the rats, Evens blue flowed to the vertebral level of C3-4 immediately, but did not flow over the C level. In 30% of the rats, flow stayed around the vertebral level of C1, but some Evans blue solution spread toward the cerebellum. In 10% of the rats, Evans blue did not reach the area of the gracile nucleus, so the data of these rats were discarded. During 3 hour observation, Evans blue didn't reach the thoracic level in any rat. Therefore, it was considered that the drugs exerted their effects on the gracile nucleus in the medulla.

In the electrophysiological study, galanin reduced responses of gracile nucleus neurons to mechanical stumuli during drug ejection, but the inhibitory effect was recovered immediately after ejection. However, NPY inhibited responses of gracile nucleus neurons to mechanical stimuli and the inhibitory effects lasted for around 90s after ejection.

The inhibitory effects of galanin did not last long compared with those of NPY. Thus, in the behavioral study, it can be regarded that high doses of galanin could reduce mechanical allodynia only at 5 min after intracisternal injection. On the other hand, high doses of NPY could inhibit mechanical allodynia for 60 min after intracisternal injection.

**2. Role of galanin in neuropathic pain**

Up to now, most studies on neuropeptides for the modulation of neuropathic pain has been concentrated on the mechanisms at the level of DRG or spinal cord. Recently, however, there have been many studies that certified the effects of neuropeptides in the supraspinal site. It has been hypothetized that neuropeptides, as a part of their response to peripheral axotomy, have a function in large and medium-sized DRG neurons projecting to the gracile nuclei.[34]

In few small-sized sensory neurons in rat DRG, which also contain substance P (SP) and calcitonin gene-related peptide (CGRP), galanin was expressed.[35] In all neuropathic pain animal models with peripheral nerve injury, complete axotomy,[4,5,6] nerve crush,[6] chronic constriction injury (CCI)[7,8,9] and partial nerve ligation,[7,9] galanin in DRG neurons was upregulated. In agreement, enhanced immunoreactive galanin release was found in the ipsilateral superficial dorsal horn to the sciatic nerve injury.[36] Unstimulated galanin release was increased in rats following peripheral nerve injury, which was further increased by the electrical stimulation of C-fibers in injured peripheral nerves.[36,37] These neurons containing galanin projected towards contralateral medial posterior thalamic structures.[38] In addition, following partial sciatic nerve injuries at the high thigh level, galanin immunoreactivites were significantly

increased in the ipsilateral gracile nucleus.[7]

In this study, intracisternally administered galanin inhibited neuropathic pain behavior only at 5 minutes after injection of high doses (20 and 40 μg) of galanin. In contrast, a low dose (1 μg) of galanin facilitated mechanical allodynia 30 min after injection. Similar results were reported at the spinal level. Endogenous galanin was an important factor in blocking nociceptive input after peripheral axotomy.[39,40,41] The intrathecal administration of high doses (>1 μg) of galanin inhibited the nocifensive reflex in spinalized rats and normal rats.[12] Moreover, very high doses (>30 μg) of galanin inhibited the neuropathic pain behaviors following peripheral nerve injury.[11] In contrast, low doses (10ng-1 μg) of i.t. galanin caused spinal reflex facilitation,[12,13,14,15] and nociceptive behaviors.[16,17,18] Thus, it may be assumed that galanin receptors, which have inhibitory effects, were activated after nerve injury in gracile nucleus. However, galanin recpetor may not be activated enough to control mechanical allodynia in the gracile nucleus as those activated in the DRG and spinal cord after nerve injury.

In the electrophysiological study, galanin inhibited the responses of gracile nucleus neurons to mechanical stimulation during drug ejection. Also, iontophoretically applied galanin (1mM) may not act at a low dose as in the behavioral study in the gracile nucleus neurons. Therefore, galanin may have inhibitory effects on neural responses to mechanical stimulation. According to the previous studies, exogenous galanin elicits a remarkable inhibition of all neuronal responses, electrically evoked, mechanical and thermal responses in neuropathic rats.[42] Also, galanin inhibited the activity of spontaneous discharge of dorsal horn neurons in the CCI neuropathic pain model.[43] However, exogenous spinal galanin administration caused a facilitation of the neuronal responses to electrical and natural stimulation in normal rats. Reeve et al.[44] have

reported similar findings in vivo with exogenous galanin facilitating of the initial C-fiber response in rats.

In recent years three galanin receptor subtypes (GalR1, GalR2, GalR3) have been cloned.[45] GalR1 is expressed mainly in large to medium sized neurons and deeper layers.[46] GalR2 is expressed in small neurons and superficial laminae I and II.[46] GalR3 is not as prevalent as GalR1 and GalR2 in the spinal cord,[47] and as of now, its effect after nerve injury are not know. In DRG neurons, GalR1 mRNA and GalR2 mRNA are down-regulated following axotomy.[48,49] A loss of putative inhibitory and excitatory galanin receptors in the spinal cord may occur due to this down-regulation.[50] Galanin probably caused antinociception, through the selective plasticity of GalR1 and GalR2 receptor activation, caused antinociception. [51]

**3. Role of neuropeptide Y in neuropathic pain**

NPY is involved in the transmission of assumed nociceptive information at the spinal cord[23] and supraspinal level.[52,53] NPY is significantly expressed in many large and medium-sized neurones in laminae III-IV of the lumbar spinal dorsal horn and in the gracile nucleus.[54,55,56,57] NPY-IR can be observed in a subpopulation of dorsal horn interneurons, where NPY-IR coexists with the inhibitory transmitter GABA,.[26] Following complete transection, galanin and NPY were colocalized in large DRG neurons.[58] Axotomy upregulates the synthesis of NPY primarily in medium to large sized DRG neurons where it is not normally found in sensory neurons at the DRG neurons.[29] NPY fibers and terminals were widely present ipsilateral to the injury in the lumbar spinal cord and gracile nucleus after complete sciatic nerve transection,

CCI and partial nerve injury.[7,34,59,60,61,62] Delicate NPY-IR axonal fibers were present in the gracile nucleus of young rats after nerve axotomy whereas NPY-IR swollen dystrophic axons were in the gracile nucleus of aged rats.[63] The changes of NPY-IR fibers within the gracile nucleus suggest that many of the large DRG neurons increased NPY synthesis through the dorsal column to the gracile nucleus.[64,65]

Hua et al.[23] were the first to report that intrathecally administered NPY produced antinociception in rats with the hot plate and paw pressure tests. The inhibitory effect of i.t. NPY was substantial and prolonged, but reversible.[66] By using a flexor reflex model in decerebrated, spinalized, unanesthetized rats, i.t. NPY caused a dose-dependent, biphasic facilitation and inhibition of the flexor reflex in rats with intact sciatic nerves. In electorphysiological studies, the induction of outward current was seen after ejection of NPY into the DRG.[57] Increased NPY produced the excitability of DRG cells.[67] Duggan et al.[24] using the antibody microprobe technique, reported that following electrical stimulation of unmyelinated afferent fibers, microinjection of NPY in the region of substantia gelatinosa reduced the in vivo release of SP.

In the present study, intracisternally administered NPY reduced neuropathic pain behavior and showed prolonged inhibitory effects dose-dependently. And the responses of gracile nucleus neurons to mechanical stimuli were inhibited by iontophoretically ejected NPY. Similar effects were reported at the brain level. NPY produced a dose-dependent antinociception in the nucleus raphe magnus (NRM) of rats and Y1 receptors were involved in the antinociceptive effect induced by NPY in the NRM.[68] The injection of NPY into the PAG resulted in prolonged withdrawal latencies to thermal and mechanical stimuli.[69]

Thus, it is reasonable to assume that NPY receptors which have inhibitoy effects were activated after nerve injury in gracile nucleus, and the bindings between NPY and NPY receptors are important to controlling neuropathic pain.

Five different NPY receptor subtypes (Y1, Y2, Y4, Y5 and Y6) have been cloned.[70] Y1 receptors are expressed and kept within DRG neurons, whereas Y2 receptors can be found numerously in the dorsal horn.[71,72] Y2 receptors are mainly expressed within medium and large sized neurons of normal rats, but, after axotomy, Y2 receptor mRNA increases predominately in small DRG neurons.[72] It has been hypothetized that Y2 receptors account for the majority of NPY binding sites in the dorsal horn, but Y1 receptor agonists can also partially reduce NPY binding in the dorsal horn.[71,73] Also, NPY binding sites, primarily the Y2 type, are increased in the dorsal horn.[74] The Y1 receptor-positive interneurons have not been fully distinguished. However, there is a possibility that they correspond to enkephalinergic neurons, which receive synaptic input from GABAergic neurons,[75] many of these neurons contain NPY.[76] Thus, NPY in conjunction with GABA, could cause disinhibition by inhibiting these enkephalinergic neurons.[75] Activation of the Y1 receptors in the dorsal horn may inhibit an additional subset of excitatory interneurons. Thus, after axotomy, it may relate to the increase in Y1 receptor binding.[74] Reduced antinoception was reported in mice lacking a neuropepetide Y1 receptor.[77] The inhibitory effects mediated by NPY receptors are very complex and need further study.

## 4. Conclusion

There are many reports that galanin and NPY is upregulated after peripheral nerve injury at the DGR, spinal cord, and gracile nucleus. Also, in previous studies, galanin knock-out mice were shown to be slightly hyperalgesic to mechanical and thermal stimulation,[78] and transgenic mice that overexpress galanin had decreased thermal nociception compared to wild type controls.[79] Although there were no reports using the NPY knock out model or overexpressed NPY as with galanin, antinoception was reduced in mice lacking a neuropepetide Y1 receptor. Thus it can be assumed that NPY can reduce neuropathic pain as shown above. Therefore, upregulated galanin and NPY after nerve injury appear to activate endogenous defense mechanism against excess pain. Thus, exogenously injected galanin and NPY can inhibit neuropathic pain. In the present study, mechanical allodynia was inhibited by the injection of galanin and NPY. This is a good agreement with previous studies.

In conclusion, neuropeptides including galanin and NPY in the gracile nucleus may play an important role in the modulation of neuropathic pain. Also, the inhibitory effects of the NPY was stronger than that of galanin in the gracile nucleus with equal dosages. However, more studies on galanin, NPY and their subtypes at the supraspinal level are needed to understand the modulation of neuropathic pain in detail.

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국문요약

# 연수 수준에서 neuropeptide에 의한 neuropathic pain의 조절 작용

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정 세 정

말초신경 손상에 의한 신경병리성 통증(neuropathic pain)은 비정상적인 통증의 발달로 특징지을 수 있다. 이러한 신경병리성 통증은 척수 수준에서의 신경전달물질들의 이상 조절작용을 포함한 신경가소성의 변화를 유발한다고 알려져 왔다. 현재까지 신경병리성 통증에 관한 연구는 주로 척수 혹은 척수신경절 부위를 중심으로 연구되어 왔다. 그러나 최근 보고에 따르면 신경병리성 통증을 조절하는데 있어 연수의 박속핵(gracile nucleus)이 중요한 역할을 수행한다. 따라서 본 연구에서는 연수 수준에서의 신경펩타이드(neuropeptide)에 의한 신경병리성 통증의 조절 작용을 알아보기 위해 galanin과 neuropeptide Y(NPY)를 거대수조(cisterna magna)내에 주입한 후 von Frey 세사를 이용하여 행동검사를 실시하고, 이온영동적 주입법을 이용하여 박속핵 뉴런에 이들 신경펩타이드를 가하면서 전기생리학적 연구를 수행하였다.

흰쥐를 pentobarbital로 마취시킨 후 좌골신경 분지 중 경골신경(tibial nerve)과 장딴지신경(sural nerve)을 결찰한 후 미세 수술가위로 절단하고 총비골신경(common peroneal nerve)은 남겨둠으로써 신경병리성 통증 모델을 제작하였다. 행동적 연구에서는 약물을 거대수조내 주입하기 위해 후두골에 구멍을 낸 후 스테인레스 스틸 관(stainless steel cannula)을 안쪽 면을 따라서 삽입하였다. 이때 관의 끝이 거대수조에서 관찰될 때까지 삽입하였다. 행동적으로 기계적 이질통은 von Frey 세사를 이용하여 측정하였다. 전기생리학적 연구에서는 galanin과 NPY의 이온영동적 주입을 위해 통증이 뚜렷하게 나타나는 쥐를 우레탄(urethane)으로 마취시키고 기계적 자극에 대한 박속핵 뉴런들의 반응을 기록하였다. 거대수조내 주입된 NPY는 신경병리성 통증 반응을 농도 의존적으로 감소시켰다. Galanin은 고농도(20, 40μg)로 거대수조내에 주입했을 때 신경병리성 통증 반응을 억제시켰다. NPY와 galanin 모두 저농도(1μg)에서는 약물을 주입하고 30분 후에 기계적 이질통이 오히려 증가하였다. 이온영동적으로 주입된 galanin과 NPY는 기계적 이질통에 대한 박속핵 뉴런의 반응을 억제시켰다. 이러한 결과는 galanin과 NPY와 같은 신경펩타이드가 신경병리성 통증을 조절하는데 있어서 연수 수준 특히 박속핵에서 중요한 역할을 하는 것으로 생각된다.

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핵심되는 말 : 신경병리성 통증, 박속핵, neuropeptide Y, galanin