이학석사학위논문

# Anterior cingulate cortex에서 일어나는 만성 통증에 의한 시냅스 가시의 구조적인 재편성

**Chronic pain induced structural reorganization of spines in the anterior cingulate cortex**

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# Chronic pain induced structural reorganization of spines in the anterior cingulate cortex

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## Abstract

# Chronic pain induced structural reorganization of spines in the anterior cingulate cortex

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Anterior cingulate cortex (ACC) has known to be involved in pain processing. Changes in spine number and morphology are some of the critical events during long-term synaptic plasticity. We induced chronic pain by neuropathic pain surgery and analyzed the change of dendritic spines after imaging single neuron in z-stack. Spine volumes, head diameters and straightness which reflected the overall structures of spines were not altered by chronic pain. Many other parameters of spines were also not altered by chronic pain, but only spine length, especially spine neck length decreased by chronic pain. Anisomycin treatment blocked chronic pain induced morphological changes of spines. From

these results, we could conclude that chronic pain signal induced by nerve ligation in hind limb evoked structural plasticity of spines only in a minor level and it was related with protein synthesis.

Key words: Anterior cingulate cortex, chronic pain, spine, structural plasticity, protein synthesis, Imaris, synaptic activity

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# Introduction

## Anterior cingulate cortex (ACC) functions and structures

The anterior cingulate cortex (ACC) is part of a larger matrix of structures that form the rostral limbic system and include the amygdala, periaqueductal grey, ventral striatum, orbitofrontal and anterior insular cortices. ACC contributes to obsessive-compulsive behaviors and aberrant social behavior. Reduced ACC activity induced by legion can also contribute to behavioral disorders like akinetic mutism, diminished self-awareness and depression, motor neglect and impaired motor initiation and reduced responses to pain. Overall, ACC have roles in decision-making, reward anticipation, motivation, emotion and goal-directed behaviors. Among the many functions related with ACC is the role of anterior cingulate cortex in pain responsiveness that was revealed by legion studies and functional imaging studies during noxious stimulation. Pain perception can be divided into two categories, affective pain and cognitive pain. The affective division of pain is related with autonomic activity and emotional responses, while the cognition division is related with physiological responses to noxious stimuli and skeletomotor activity (Devinsky *et al.*, 1995).

The fact that the ACC is critically involved in pain processing is well known (Iannetti *et al.*, 2013; Shackman *et al.*, 2011; Sikes & Vogt, 1992; Wager *et al.*, 2013). ACC neuronal activity increased in direct response or anticipation to noxious stimuli (Hutchison *et al.*, 1999; Iwata *et al.*, 2005; Koyama *et al.*, 2001), but did not affect the ability to sense unpleasant stimulus itself. According to pain relief treatment which surgically excised cingulate cortex, many pain symptoms related with diseases including intractable cancer pain,

reflex sympathetic dystrophy, upper abdominal/lower thoracic pain, low back pain, and neuropathic pain were successfully rescued. Brain imaging studies also consistently showed that ACC neuronal activity was detected preceding and during the noxious stimuli (Cifre *et al.*, 2012; Yuan *et al.*, 2013). Hypnotic therapy showed ACC was involved in the processing of affective pain by the indirect evidence of changed regional cerebral blood flow (rCBF) in the ACC, but not in the somatosensory cortex (Rainville *et al.*, 1997).

## Chronic pain animal model

The standard definition of chronic pain suggested by the International Association for the Study of Pain is persistent pain that lasts past the healing phase after an injury (Merskey and Bogduk, 1994). To study mechanisms of chronic pain systematically, there have been diverse animal models that caused persistent pain. Wall's autotomy model (Wall *et al.*, 1979), dorsal rhizotomy model (Lombard *et al.*, 1979), and Bennett's injury model (Bennett & Xie, 1988) constituted pioneering model. Since then, new models are continually being suggested that are becoming more sophisticated. Our experiments used the spared nerve injury model (Decosterd & Woolf, 2000) that gives rise to neuropathic pain. This model cuts tibial and peroneal branches of the sciatic nerve and shows peak hyperalgesia and mechanical allodynia at 2 weeks after the injury (Apkarian *et al.*, 2009).

## Spine structures

Dendritic spines are specialized structures on neuronal dendrites for synaptic transmission and the majority of excitatory synapses are localized on the spines.

Spines are highly dynamic structures which are influenced by synaptic activities. Experience-dependent brain alteration and information storage based on synaptic activity-dependent regulation of spine structures (Lai & Ip, 2013).

In the spine structure, neurotransmitter receptors are largely located to the surface and this zone is called the postsynaptic density (PSD), a membrane-associated disc of electron dense material which consists of receptors, channels and signaling systems (Peter *et al.*, 1991). Representative structure of dendritic spine is spherical head shape connected to the narrow neck. The head contains the PSD and concentrated actin microfilaments (Capani *et al.*, 2001; Fifková & Delay, 1982; Matus *et al.*, 1982), but mitochondria and microtubules are excluded (Peter *et al.*, 1991). Some pyramidal cell spines contain a peculiar structure called the spine apparatus which consists of two or more smooth endoplasmic reticulums (Gray & Guillery, 1963). Polyribosomes are frequently found 82% of spines in the visual cortex, in 10% of dentate granule cell spines (Steward & Falk, 1985; Steward & Reeves, 1988), and in 13% of Purkinje cell spines but free ribosomes are rarely found in spines (Spacek, 1985).

Dendritic spines, especially in pyramidal neurons, have morphological diversity and they can be divided into several morphologic categories. The most commonly used category was introduced by Peters & Kaiserman-Abramof in 1970 and it divided spines into three categories based on the sizes of the spine head and neck (Peters & Kaiserman-Abramof, 1970). Stubby spines have no neck part, mushroom spines have a large head and a narrow neck and thin spines have a small head and a long neck. Filopodium which is additionally categorized in some cases have hairlike morphology and are found mostly during development (Skoff & Hamburger, 1974). Even though spine classification criteria are qualitative and make some difficulty, these class categories have been gradually refined (Nimchinsky *et al.*, 2002).

## Morphological development of spines

Spine composition alters with development. The stubby spines, very long spines and filopodia are major spine types in early development. Among them were stubby spines that were the most abundant. During the next developmental stage, average spine length, filopodia density and spine motility decreases and total density increases (Dunaevsky *et al.*, 1999; Nimchinsky *et al.*, 2001).

Related with the way a mature spine is formed, there are three general views. In the first view, dendritic filopidia robustly find synaptic partners and after they are met, filopodium shortens and axon part is drawn to dendrite of filopodia. At there, mature synapse is formed. Spine head enlarges and synapse structure is stabilized (Dailey & Smith, 1996; Ziv & Smith, 1996).

In the second view, dendritic filopodia also find synaptic partners but synapses are not necessarily formed at the apical part. Next, filopodium shortens completely so that postsynaptic part is diminished. From there, spine emerges and head enlarges to form mature spine (Fiala *et al.*, 1998; Harris, 1999).

In the third view, spines are constantly dynamic throughout lifetime. Spines are constantly formed whenever spines and synaptic partners meets, stabilizing into functional spines of any structure. Depending on synaptic plasticity, spines are change in morphology and even disappear. From this view of spine formation, spine structure is not the indication of maturation but the simply established structure by possibility (Lendvai *et al.*, 2000).

Common ideas between the three views are that premature spines all undergo from filopodia, thin spines, and stubby spines to mushroom spines (Nimchinsky *et al.*, 2002).

## Synaptic activity-dependent structural plasticity

According to numerous studies testing relationship between neuronal excitability and spine formation (Annis *et al.*, 1994; Drakew *et al.*, 1996; Gutierrez & Heinemann, 1999; Muller *et al.*, 1993; Papa & Segal, 1996), moderate activation of synapse can induce spine formation, but excessive and unrestrained activation can cause loss of spines. There were diverse evidences supporting this idea. First, in cultured hippocampal neurons, short pulses of glutamate or NMDA receptor activating medium induced new spine formations, whereas long pulses of glutamate or high concentration of NMDA caused spines to shrink (Goldin *et al.*, 2001; Halpain *et al.*, 1998; Korkotian & Segal, 1999a). Second, moderate increase in intracellular $[Ca^{2+}]$(200–400nM)causedelongationofspines(Korkotian & Segal, 1999b).

In cultured neuron, spine density was not altered by inhibiting action potential-induced neurotransmitter release, but significantly decreased by blocking AMPA receptors or inhibiting presynaptic vesicle release. Additionally, spine density was not altered by only spontaneous release of glutamate which gave rise to miniature excitatory postsynaptic currents (mEPSC) (McKinney *et al.*, 1999). Taken together, spines are maintained by only small portion of synaptic activity. Depending on synaptic activity strength, spines plastically change their structures (Nimchinsky *et al.*, 2002).

## Structural plasticity of spines Associated with Long-Term Potentiation

Long-term potentiation (LTP) is one of the characteristic forms of change in synaptic efficacy and there have been numerous studies regarding the relationship

between LTP and structural plasticity of spine. In one study, spine volumes increased after LTP inducible stimulation was given to hippocampal neurons. The increases of spine volumes persisted over 23 h after stimulation and peaked at between 10 and 60 min after stimulation (Fifkova & Van Harreveld, 1977). Also, only stimulated spines were structurally changed. Specific brain region which received synaptic input showed structural changes of spines, but the other regions which did not receive synaptic input did not. Taken together, structural plasticity of spine and LTP are closely related. Moreover, LTP did not alter overall synapse density, indicating that only structures of spines were changing by LTP (Chang & Greenough, 1984; Sorra & Harris, 1998). To induce LTP in almost all synapses analyzed, chemically induced form of LTP was used and also did not show changes in spine density or length (Hosokawa *et al.*, 1995). Overall, the LTP effect is working only in local target and makes changes in spine structure.

# Methods

## 1. Subjects

Total 12 male mice aged between 6 and 8 weeks had C57BL/6NCrljBgi genetic background. All animals were purchased from Orient Bio and housed in standard laboratory cages on a 12-hour light-dark cycle. All mice had access to food and water *ad libitum*. All experiments were conducted according to the guidelines of the Institutional Animal Care and Use committee of Seoul National University.

## 2. Surgery

*Drug infusion.* Mice were anesthetized with a ketamine/xylazine mixture and placed on Kopf stereotax. The head was shaved and sterilized with alcohol. Small craniotomies were made above the ACC and guide cannulas (24 gauge) were bilaterally implanted (AP +0.7 mm, ML ±0.4 mm, DV -1.7 mm). After at least 1 week of recover, we infused 0.5 μl anisomycin (100 μg/μl in aCSF) or vehicle (aCSF) bilaterally into the ACC within 2 minutes using pump. Injection cannulas (30 gauge) were inserted through guide cannulas into the final target (AP +0.7 mm, ML ±0.4 mm, DV -1.9 mm). After drug infusion, injection cannulas were remained additionally 2 minutes.

*Inducing neuropathic pain.* The mice were anesthetized with ketamine/xylazine mixture and lubricating ointment (Artificial tear jelly) was applied to the eyes. The left thigh of each mouse was shaved and sterilized with alcohol and povidone iodine liquid. About 1 cm region of left thigh skin was cut and muscle was incised with scissors and sterilized with saline. The common

peroneal nerve (CPN) was ligated with a wax coated braided suture 4-0 without disturbing blood vessel. The ligature was tightened until the dorsiflexors of the foot were twitching and knot was made. The skin was sutured and applied with povidone iodine.

## 3. Biocytin labeling

Mice were anesthetized with isoflurane and decapitated. The brain was quickly extracted and coronally sectioned (300 μm) with VT1000S (Leica microsystems). ACC slices were incubated in room temperature for 1 hr and transferred to chamber (32~34°C) perfused with oxygenated aCSF containing 124 mM NaCl, 2.5 mM KCl, 1 mM $NaH_2PO_4$, 25 mM $NaHCO_3$, 10 mM Glucose, 2 mM $CaCl_2$ and 2 mM $MgSO_4$ at a flow rate of 2 mL/min. The recording pipettes (3~5 MΩ) were filled with internal solution containing 145 mM K-Gluconate, 5 mM NaCl, 0.2 mM EGTA, 10 mM HEPES, 2 mM MgATP, 0.1 mM $Na_3GTP$, 1 mM $MgCl_2$ and 2 mg/ml Biocytin (pH 7.2 with KOH, 280~290 mOsm). The bath solution contained 124 mM NaCl, 2.5 mM KCl, 1 mM $NaH_2PO_4$, 25 mM $NaHCO_3$, 10 mM Glucose, 2 mM $CaCl_2$, 2 mM $MgSO_4$ saturated with 95% $CO_2$, 5% $O_2$. 1~2 pyramidal neurons in ACC Layer II/III were labeled by voltage clamping at –70 mV for 15 min. ACC slices were fixed with 4% paraformaldehyde (PFA) overnight at 4°C.

We washed fixed slices 3 times with PBS shaking in 150 rpm for 5 min at room temperature. Next, slices were blocked and permeablized with 5 % goat serum, 0.2 % Triton-X 100 in PBS shaking in 80 rpm for 1 hr at room temperature. Streptavidin, Alexa Fluor® 488 conjugate (Life Technologies) diluted blocking solution (1:2000) was treated for overnight at 4°C. Next day, slices were washed 3 times with PBS shaking in 150 rpm for 5 min at room

temperature and mounted on slide glasses.

## 4. Spine analysis

Biocytin labeled neurons were imaged by Zeiss LSM700 with 488 laser by 100x oil immersion lens. 100-300 μm dendrites in total length were imaged in Z-stack. Stack interval was 0.2 μm and imaging scale was one airy unit. Secondary/tertiary apical dendrites were imaged and analyzed.

By using Imaris Filament Tracer (Bitplane), Z-stack images were reconstructed in 3D models. Spines were traced automatically according to their fluorescence intensity and then manually refined. All the parameters including spine head width, spine length, spine volume, etc were measured for each spine by automatically. Spine head part was spheric apical region and neck part was rod-like root region.

## 5. Statistics

All structural analysis data of spines except maximum, mean, minimum head diameter comparison were analyzed by two-way ANOVA with Bonferroni's *post hoc* test. Maximum, mean, minimum head diameter comparison data were analyzed by one-way ANOVA with Bonferroni's *post hoc* test. P-values are as indicated. All data are presented as mean ± SEM.

# Results

## Dendritic spines stimulated by chronic pain were analyzed

ACC was well known hub region dealing with affective pain processing. Chronic pain induced by nerve ligation (NL) surgery treated at hind limb would stimulate neurons in ACC, making them activated constantly. Continuously applied synaptic activities induced by pain signal caused diverse changes in neuronal functions and structures. One of characteristic changes was structural plasticity of spines and to test it, we analyzed morphological dynamics of spines (Fig 1A).

To make chronic pain animal model, mice were first implanted with guide cannulas. The guide cannulas were used for drug infusions later. Then, mice received NL surgery which induced neuropathic persisting pain or just sham surgery at hind limb. By comparing two groups which received surgery, chronic pain induced changes in spines were analyzed. Protein synthesis inhibitor anisomycin or vehicle was treated to test chronic pain induced structural changes of spines were dependent on protein synthesis (Fig 1B).

Because usually there were too many neurons in brain, it was very hard to analyze neurons or spines individually. Single neurons were specifically selected and then labeled by staining (Fig 1B). Spines in apical parts of secondary or tertiary dendrites were analyzed by measuring diverse parameters like spine length and volume (Fig 2).

### Overall structures of spines were not altered by chronic pain

To see the changes of overall structures of spines, spine volumes and areas were first analyzed. Spines had specific structures and their structure was divided into head and neck part. According to spine structures, various parameters, including spine volume, area, length and even head volume, length and neck volume, length respectively, could be utilized and analyzed. Among all the parameters, spine volume and area were selected as marker of general spine dynamics.

Spine volumes were not altered by nerve injury and anisomycin treatment (Fig. 3A). Spine areas, spine head volume and spine straightness were also not altered by nerve injury and anisomycin treatment (Fig. 3B, C, D, Sham-Veh=11, Shan-Ani=13, NP-Veh=17, NP-Ani=13, total 12 mouse). Chronic pain did not change overall structure of spines. Spine straightness reflected the angle between spine and dendrite.

## Spine neck length was altered by chronic pain

To analyze spines in more detail, next, spine length is selected as a criterion. When spines are maturing by synaptic stimulation, spine length and head size are especially changing. We see the influence of pain stimuli on spine maturation.

Spine lengths decreased by nerve injury and increased back to level of not treated with pain stimuli by anisomycin treatment (Fig. 4A, Sham-Veh=11, Shan-Ani=13, NP-Veh=17, NP-Ani=13, total 12 mouse). Spine neck lengths also showed similar results. Spine neck lengths decreased by nerve injury and increased back to level of not treated with pain stimuli by anisomycin treatment (Fig. 4B, Sham-Veh=11, Shan-Ani=13, NP-Veh=17, NP-Ani=13, total 12 mouse).

Interestingly, spine head lenths were not altered by nerve injury or anisomycin treatment (Fig. 4C). From these results, it was suggested that chronic pain induced structural changes of spines, especially in neck part length. Protein synthesis inhibition prevented the chronic pain induced increase of spine length.

## Spine head diameters were fluctuating based on measurement criterion

Spine head diameter is important parameter to classify spine type. Spines are dynamically created and diminished and during that process, spines showed active change of morphology. They are changing from thin filopodia-like shape to mushroom structure and due to head part enlargement, spine head diameters increase.

Before comparing spine head diameter alteration by chronic pain or anisomycin treatment, we considered the criterion to measure head diameter first. Cause spine heads were not perfect sphere, one of maximum, mean, or minimum head diameter could be a measuring criterion. In a control group which did not treated with nerve injury and anisomycin, maximum, mean and minimum spine head diameter showed significant difference between them (Fig 5, Sham-Veh=11, total 2 mouse). From minimum to maximum spine head diameter, there were gradual increases. When analyzing the change of spine head diameter, adequate consideration is needed according to the situation.

## Mean spine head diameter was not altered by chronic pain

To see the influence of chronic pain on spine head diameter, we analyzed mean spine head diameters. There's no obvious reason to choose mean spine

head diameter as a representative parameter, but we assumed that spine head would be almost perfect sphere shape.

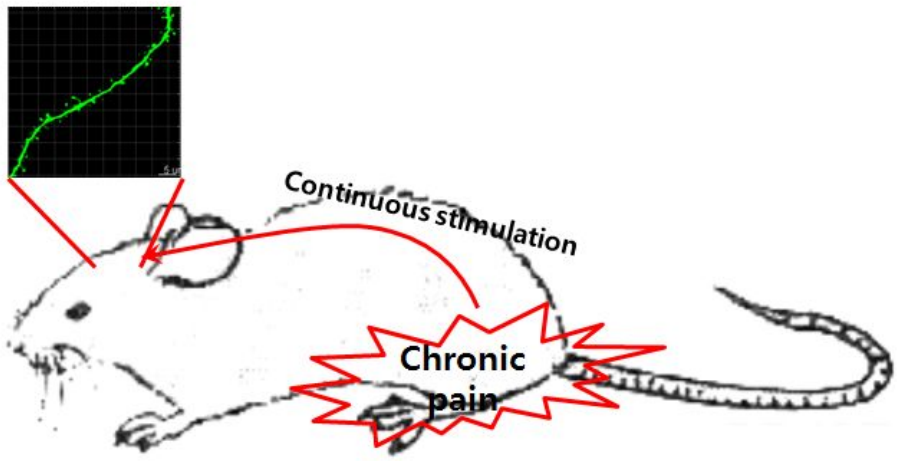
Mean spine head diameter was not altered by nerve injury and anisomycin treatment (Fig. 6, Sham-Veh=11, Shan-Ani=13, NP-Veh=17, NP-Ani=13, total 12 mouse). Spine heads were not enlarged by chronic pain stimuli, suggesting that spine types may not be changed. But several parameters are additionally needed to classify spine types.

# Figures

Figure 1.

Chronic pain model and experimental scheme. (**A**) Nerve ligation (NL) induced chronic pain which started from hindlimb continuously stimulated anterior cingulate cortex (ACC). Spines stimulated by pain signal in ACC were analyzed. (**B**) Experimental scheme. Guide cannulas were implanted, NL surgery was done, anisomycin (50 ìg) or vehicle (0.5 ìL) was injected and then neuron labeling and spine analysis was done. Ani : anisomycin, Veh : vehicle.

A


Continuous stimulation
Chronic pain

B

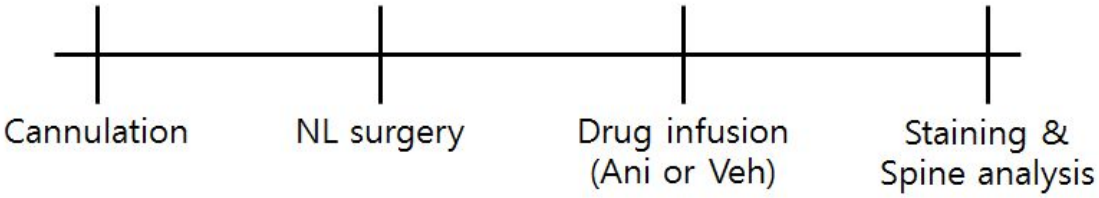

Cannulation
NL surgery
Drug infusion
(Ani or Veh)
Staining &
Spine analysis

**Figure 2.**

Spines of secondary/tertiary apical dendrites were analyzed. (**A**) Representative biocytin labeled neuron sample image. Scale bar represents 5 ìm.

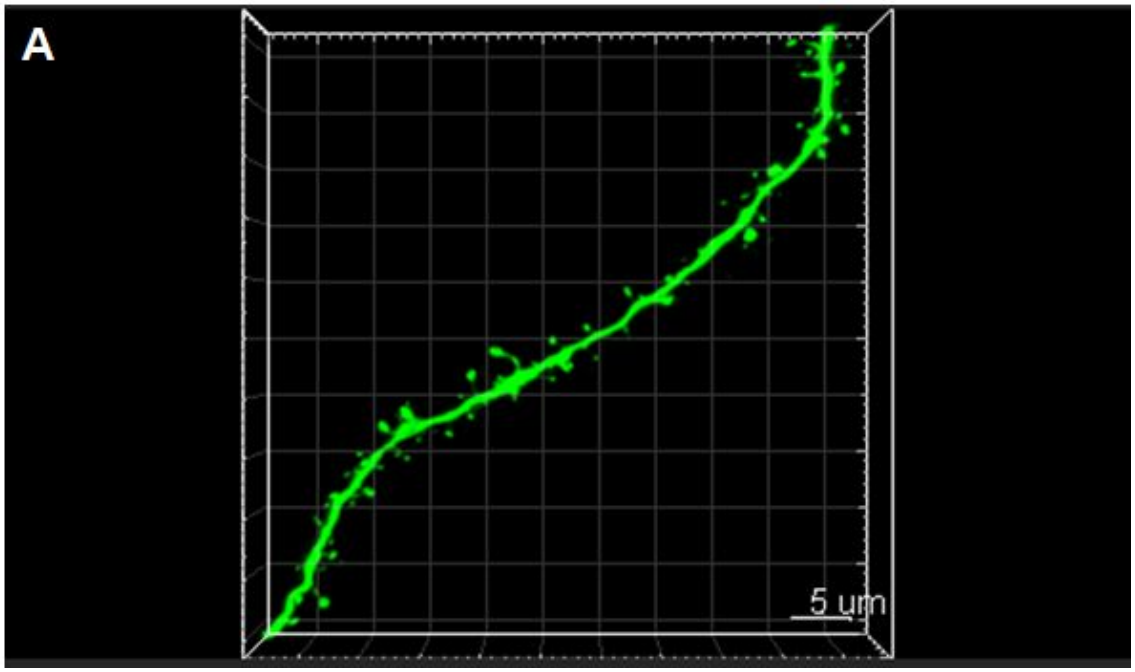

A
5 µm

**Figure 3.**

The effect of chronic pain and protein synthesis on overall structures of spines. (**A**) Spine volumes were not affected by chronic pain or anisomycin treatment. (**B**) Spine head volumes were not affected by chronic pain or anisomycin treatment. (**C**) Spine areas were not affected by chronic pain or anisomycin treatment. (**D**) Spine straightness was not affected by chronic pain or anisomycin treatment. Two-way ANOVA, followed by Bonferroni's *post hoc*. Data are presented as mean ± s.e.m.

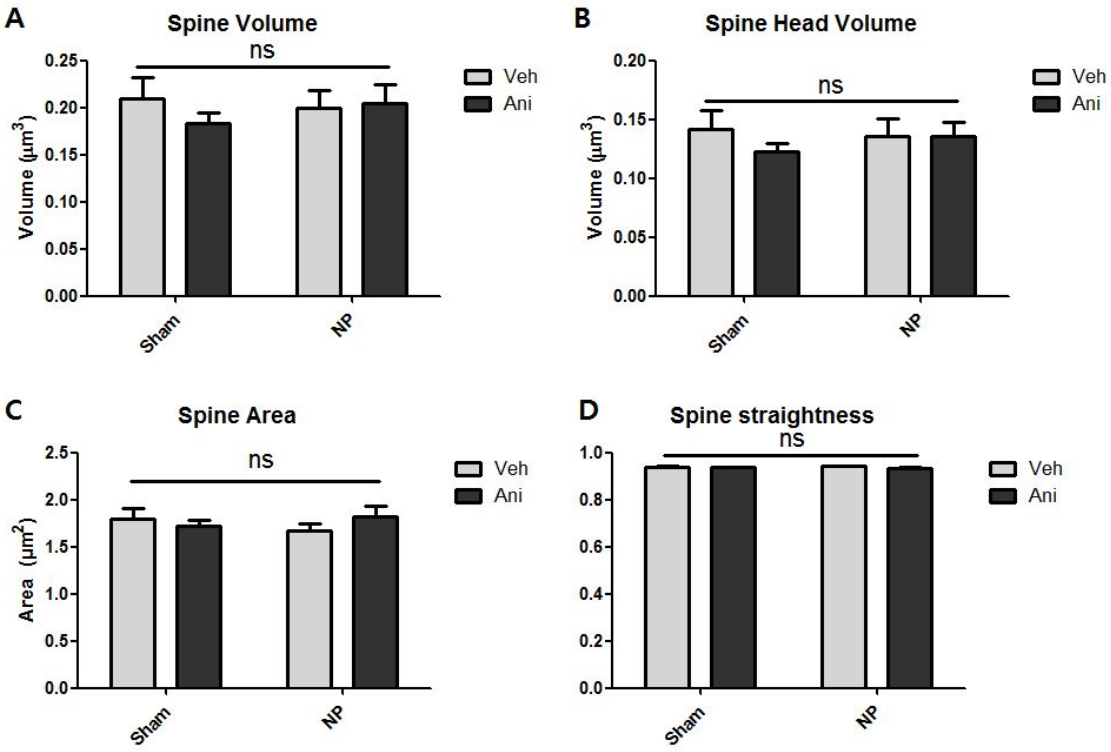

A
Spine Volume
ns
Volume (μm3)
0.25
0.20
0.15
0.10
0.05
0.00
Sham
NP
Veh
Ani
B
Spine Head Volume
ns
Volume (μm3)
0.20
0.15
0.10
0.05
0.00
Sham
NP
Veh
Ani
C
Spine Area
ns
Area (μm2)
2.5
2.0
1.5
1.0
0.5
0.0
Sham
NP
Veh
Ani
D
Spine straightness
ns
1.0
0.8
0.6
0.4
0.2
0.0
Sham
NP
Veh
Ani

**Figure 4.**

The effect of chronic pain and protein synthesis on spine lengths. (**A**) Spine lengths significantly decreased by chronic pain and increased by anisomycin treatment. (**B**) Spine neck lengths significantly decreased by chronic pain and increased by anisomycin treatment. (**C**) Spine part head lengths were not affected by chronic pain or anisomycin treatment. Two-way ANOVA, followed by Bonferroni's *post hoc*. $^{**}P<0.01$, $^{***}P<0.001$. Data are presented as mean ± s.e.m.

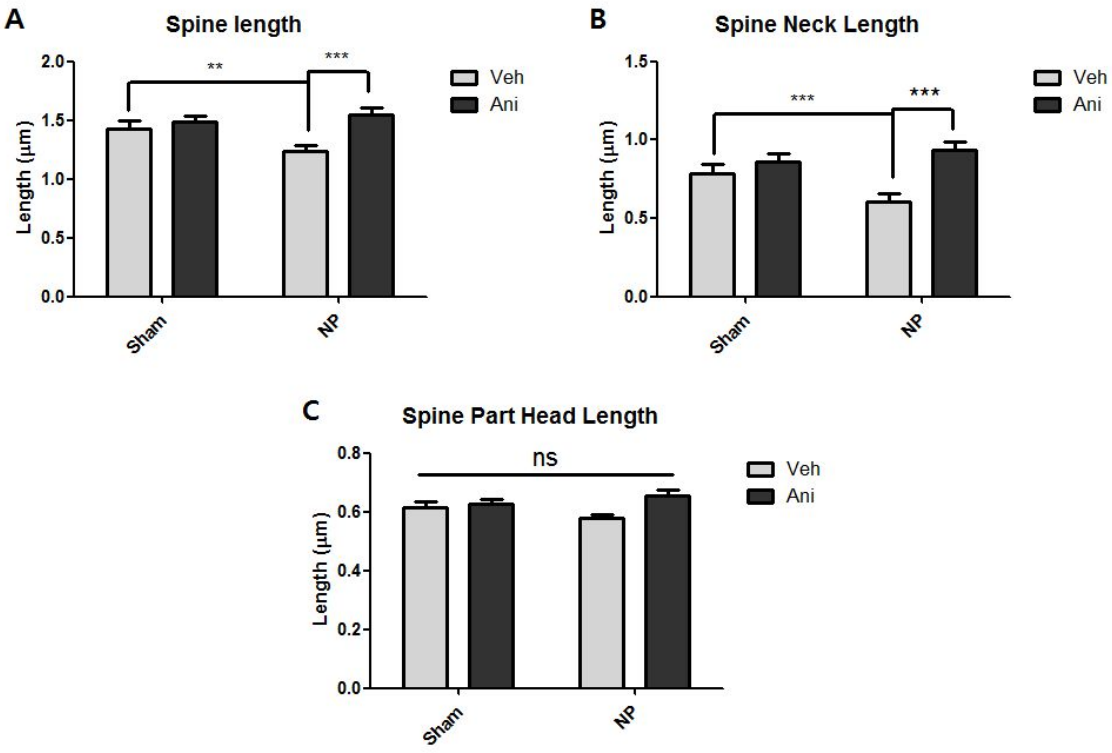

A
Spine length
Length (μm)
2.0
1.5
1.0
0.5
0.0
**
***
Sham
NP
Veh
Ani
B
Spine Neck Length
Length (μm)
1.5
1.0
0.5
0.0
***
***
Sham
NP
Veh
Ani
C
Spine Part Head Length
Length (μm)
0.8
0.6
0.4
0.2
0.0
ns
Sham
NP
Veh
Ani

**Figure 5.**

The differences of spine head diameters based on measurement criterion. (**A**) Minimum, mean, maximum spine head diameters showed significant difference. Maximum spine head diameters were significantly longer than mean spine head diameters and mean spine head diameters were significantly longer than minimum spine head diameters. One-way ANOVA, followed by Bonferroni's *post hoc* $F/R^2$: 15.73/0.5119. $^{*}P<0.05$. Data are presented as mean ± s.e.m.

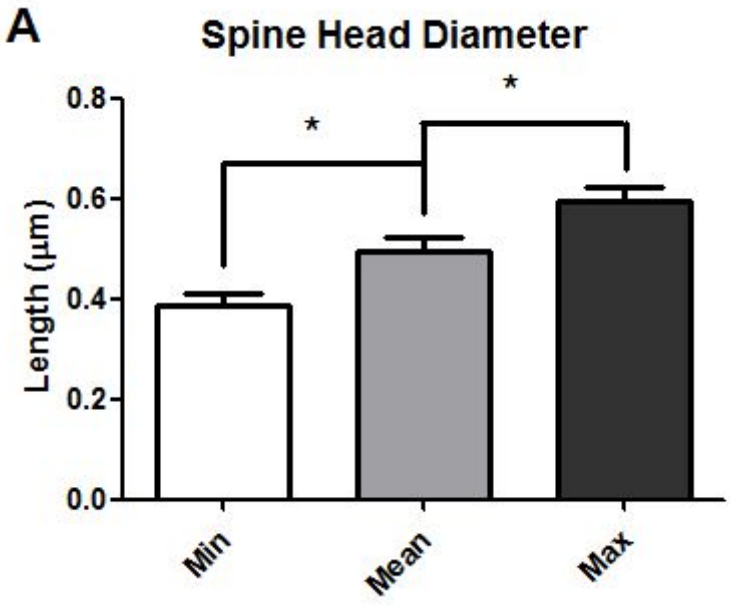
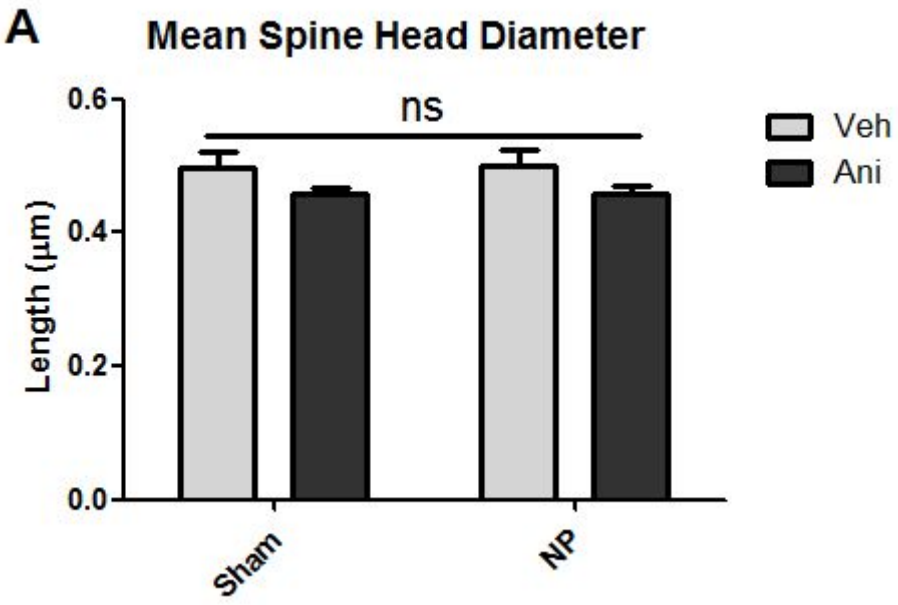

A
Spine Head Diameter
Length (µm)
0.8
0.6
0.4
0.2
0.0
*
*
Min
Mean
Max

**Figure 6.**

The effect of chronic pain and protein synthesis on mean spine head diameters.(**A**) Mean spine head diameters were not affected by chronic pain or anysomycin treatment. Two-way ANOVA, followed by Bonferroni's *post hoc*. Data are presented as mean ± s.e.m.


A
Mean Spine Head Diameter
ns
Veh
Ani
Length (µm)
0.6
0.4
0.2
0.0
Sham
NP

# Discussion

We investigated structural changes of dendritic spines by chronic pain stimuli. Spine volumes and straightness which reflected the overall structures of spines were not altered by chronic pain. Many other characteristics of spines including spine head diameter, spine density, and spine head length (Data not shown), were also not altered by chronic pain, but spine length, especially spine neck length decreased by chronic pain. From these results, we could conclude that sensory stimuli induced structural plasticity of spines only in a minor level. In a structural aspect of spines, there were no significant changes of spines on the whole view.

Chronic pain stimulated the related neuronal circuits continuously and in that situation, we could hypothesize that successive sensory stimuli could make neurons to be potentiated and be amenable to receive stimuli. Spine enlargement is the structural characteristics of potentiated dendritic spines and represented synaptic strengthening. But we found that continuous pain stimuli did not induce effective changes of spine structures. There would be two possibilities to explain these results. One is that chronic pain was not sufficient to induce LTP in ACC and actually there were no significant synaptic changes in overall spines. Considering the result that spine neck lengths significantly decreased among the many parameters, it is hard to say that there were no actual changes in spine structures and synaptic strengths. When spines matured from thin spines to mushroom spines, neck part of thin spines became shorter and head part of it became enlarged. From those evidences, chronic pain likely induced some structural changes of spines and our first assumption is unlikely. The other is that chronic pain affected some portion of neurons in ACC and this made only a few spines to be structurally altered. Even though stimulated spines were to be

enlarged and strengthened, general or total structures of spines could not be affected. This hypothesis can explain both aspects of results, decrease of spine neck lengths and unchanged spine head diameters. Unchanged spine head diameters can be explained by our second assumption easily; a little portion of stimulated spines could not make significant impact on total spines. On the other hand, spine neck lengths would be considered in another perspective. Spines can be lengthened enormously to find and make synapses, even from 0.5 μm up to about 6 μm. Spines were stimulated and made synapses by continuous pain stimuli and this resulted in spine neck shortening, being sufficient to affect even average spine neck lengths. While the latter hypothesis is likely to explain the results, there are still the former could be right. To distinguish two possibilities, further studies are required.

Spine neck lengths which were reduced by chronic pain increased by anisomycin treatment and the level was similar with the result of not receiving chronic pain. This result represents that protein synthesis was related with structural plasticity of spines in ACC. Even though the effect was not that dramatic, continuous pain stimuli induced structural changes of spines and it was blocked by protein synthesis inhibitor, anisomycin. During synaptic strengthening, protein synthesis dependent potentiation is important step and once established, persists long time (Sala & Segal, 2014). We consider that successive pain stimuli induced progressive synaptic plasticity and eventually protein synthesis dependent structural maturation of spines. We showed that chronic pain signal induced by nerve ligation in hind limb had significant impact on structural plasticity of spines in ACC and it was related with protein synthesis. Further studies including electrophysiological plasticity and the protein which was target of protein synthesis would improve and support our results.

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## 국문 초록

# Anterior cingulate cortex에서 일어나는 만성 통증에 의한 시냅스 가시의 구조적인 재편성

최 태 혁

Anterior cingulate cortex (ACC)는 고통과 관련된 정보를 처리하는 것으로 잘 알려져 있다. 시냅스 가시의 숫자와 형태가 변하는 것은 장기적인 시냅스 가소성에 의해 발생하는 중요한 변화이다. 이를 참고로 하여 신경병증 통증을 사용하여 만성 통증을 일으킨 다음, z-stack으로 단일 뉴런을 이미징하여 시냅스 가시를 분석하였다. 시냅스 가시의 전체적인 구조를 반영하는 척도인 부피, 머리 지름, 곧은 정도는 만성 통증에 의한 차이를 보이지 않았다. 시냅스 가시를 나타낼 수 있는 다른 여러 척도들도 만성 통증에 의한 차이를 보이지 않았으며, 단지 시냅스 가시의 길이, 특히 목 부분의 길이만이 만성 통증에 의해 감소하였다. Anisomycin을 처리하였을 경우 이러한 만성 통증에 의해 일어났던 시냅스 가시의 구조적인 변화는 사라졌다. 결과들을 종합하면, 뒷다리 신경의 결찰에 의해 만성 통증이 발생하게 되고, 이 만성 통증은 시냅스 가시에 작은 구조적 변화만을 일으키게 된다. 또한, 시냅스 가시의 구조적인 변화는 단백질 합성과 관련되어 있다.

주요어: Anterior cingulate cortex, 만성 통증, 시냅스 가시, 구조적 가소성, 단백질 합성, Imaris, 시냅스 활성

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