이학박사 학위논문

Non-Opioid Alternative therapeutics for Chronic Pain: Validation of Analgesic effects of Naloxone and Refeeding

마약성 진통제 사용의 대체 치료법: 나록손과 섭식조절을 이용한 진통효과 검증

2021년 8월

서울대학교 대학원

자연과학대학 뇌인지과학과 전공

Grace Joy Lee

Non-opioid alternative therapeutics for chronic pain: Validation of analgesic effect of naloxone and refeeding

Seog Bae Oh, D.D.S., Ph.D.

Submitting a Ph.D. Dissertation of Natural Sciences

June 2021

Graduate School of Natural Sciences
Seoul National University
Brain and Cognitive Sciences Major

Grace Joy Lee

Confirming the Ph.D. Dissertation written by Grace Joy Lee
June 2021

Chair	정 천 기
Vice Chair	오 석 배
Examiner	이 인 아
Examiner	김 상 정
Examiner	우 충 완

Abstract

Non-Opioid Alternative therapeutics for Chronic Pain: Validation of Analgesic effects of Naloxone and Refeeding

Chronic pain is a major public health problem worldwide that is highly prevalent. Among the available pharmacological treatments, opioids are widely used to treat moderate-to-severe chronic pain. Although these drugs are important tools for treating acute postoperative and traumatic pain, their comparative effectiveness and usefulness for chronic pain are questioned. Also, the misuse and addiction to opioids is a serious national crisis that affects public health as well as social and economic welfare. Surprisingly, in 2019, nearly 50,000 people in the United States died from opioid-involved overdoses. Therefore, exploring alternative effective treatments for chronic pain is important to decrease or eliminate opioid use. Hence, in this thesis, I aimed to explore the non-opioid treatments for chronic inflammatory pain.

In the first chapter, I focused on non-opioid pharmacological management. Alteration of the opioid system in the brain is known to exacerbate chronic pain. Hence, I focused on reversing the altered brain opioid system with pain chronification. In this chapter, using the Complete Freund's adjuvant-induced (CFA) chronic inflammatory pain animal model, I showed that the kappa opioid system was enhanced, and the mu-opioid system was suppressed within the nucleus accumbens

(NAc) and the central nucleus of the amygdala (CeA), but not in other brain areas. Systemic administration of a general opioid antagonist (naloxone, 10 mg/kg), or a kappa opioid receptor antagonist (norbinaltorphimine, nor-BNI 10 mg/kg) reduced spontaneous pain behavior in the CFA model. Furthermore, microinjection of both naloxone and nor-BNI into the NAc and CeA also significantly reduced spontaneous chronic pain behavior.

Since chronic pain has emotional and cognitive features, there is a limit to control pathological pain through pharmacological treatment. Therefore, I aimed to study non-pharmacological treatment of chronic pain in chapter 2. Lifestyle factors such as physical inactivity, stress, poor sleep, and unhealthy diet are associated with chronic pain. Among them, feeding behavior modification is accessible for every patient. Hence, I focused on managing chronic pain via feeding behavior modification in this chapter. Accumulating evidence showed analgesic effect of fasting and refeeding after 24 hr fasting. However, while brain circuits mediating fasting-induced analgesia have been identified, the underlying mechanism of refeeding-induced analgesia is still elusive. Hence, I sought to explore the underlying mechanism of refeeding-induced analgesia in this chapter. I demonstrated that the neural activities in the nucleus accumbens shell (NAcS) and anterior insular cortex (aIC) were increased in chronic inflammatory pain conditions, which was reversed by refeeding. I also found that refeeding reduced the enhanced excitability of aIC – $NAcS^{D2R}$ projecting neurons in this CFA model. Besides,

chemogenetic inhibition of aIC–NAcS^{D2R} neural circuit suppressed chronic pain behavior while activation of this circuit.

Altogether, my thesis provides an alternative non-opioid treatment option for managing chronic pain without causing significant side effects, which may allow the reduction of opioid use.

Key Words: chronic pain; dopamine; insular cortex; nucleus accumbens; opioid; refeeding

Student Number: 2017-21571

Table of contents

List of figures

Background

Chapter 1

Chapter 2

Background

1. Brain pain processing system

Noxious stimuli are transmitted from nociceptors by primary afferent Aδ and C fibers and synapse with neurons in the spinal dorsal horn [12]. Projection neurons from the dorsal horn decussate at the ventral commissure and ascend in the lateral spinothalamic tract to the ventral posterolateral nuclei of the thalamus. Finally, the information is transmitted to the brain [9,13]. Nociceptive information is transmitted to not only somatosensory but also brain areas involved in memory and affective aspects of pain, such as the amygdala, hypothalamus, periaqueductal grey (PAG), and nucleus accumbens (NAc) through the spinoreticular and spinomesencephalic tracts [9,14] **(Fig. 1)**. Descending pain modulatory systems involve the PAG and rostral ventral medulla (RVM). The RVM is the major output node in the descending modulation of nociception. It receives input from the PAG and sends diffuse bilateral projections to the dorsal horn, terminating at multiple levels [19].

2. Chronic pain and central nervous system

Chronic pain is a common and complex problem, which has a significant impact on society and individuals. In 2019, 20.4% of U.S. adults had chronic pain and 7.4% of adults had chronic pain that limits activities . The types of chronic pain are vary depending on the etiology. However, there are common mechanisms underlying the generation of chronic pain. The perception of pain arises through a complex peripheral signaling, central processing, cortical activation, and finally behavioral response. Interestingly, numerous recent animal and clinical studies show functional and structural changes in the central nervous system (CNS) under chronic pain condition **(Fig. 2)**. Especially, functional imaging studies show abnormalities in the brain regions, often referred to as the "pain matrix," even with different types of chronic pain. The development of chronic pain is associated with synaptic plasticity, structural and functional changes in corticolimbic brain regions such as the prefrontal cortex, ACC, amygdala, hippocampus, NAc, and PAC .

3. Limbic system and chronic pain

The limbic system are associated with affective aspects of pain and regulate emotional and motivational responses . Changes in emotional and motivational cues can affect the intensity and degree of pain experience. Since the limbic system is involved in emotion, motivation, and reward-related behaviors, this suggests that the processing of pain perception can be influenced by changes in these functional connections. The nucleus accumbens (NAc) is a part of mesolimbic dopaminergic system which plays important role in reward, motivation and addiction. The NAc is a neuroanatomically and functionally heterogeneous structure, made up primarily of two main subregions, a core (NAcC) and a shell (NAcS) **(Fig. 3A)**. It is composed with 97% of GABAergic medium spiny neurons (MSN) and 3% of interneurons. MSNs are segregated into those expressing dopamine receptor type 1 (D1R) or dopamine receptor type 2 (D2R) **(Fig. 3B)**. D1R expressing neurons are known to encode positive valence/reward, whereas D2R expressing neurons encode negative/aversive responses. Recently, the NAc has been investigated as having a critical role in modulating chronic pain in humans and animal models. Several animal studies show release of dopamine in the mesolimbic system with noxious stimuli and pain relief, but the dopamine release is decreased under chronic pain condition . Also, in clinical studies, patients with chronic back pain demonstrate reduced dopamine release in response to noxious challenges . Although fMRI studies show decrease in volume in the NAc in chronic pain, the activity in this region collectively increases .

4. Opioid management in chronic pain

The opioid is widely used for pain management. Opioids exert their pharmacological actions through three opioid receptors, mu, delta and kappa **(Fig. 4A)** in both the central and peripheral nervous systems. Within the central nervous system, opioids have effects in many areas, including the spinal cord. The opioid drugs produce analgesia by actions at several levels of the nervous system, in particular, inhibition of neurotransmitter release from the primary afferent terminals in the spinal cord and activation of descending inhibitory controls in the midbrain **(Fig. 4B)**. Opioids are good analgesics for acute pain, but evidence indicates that opioids are less effective for chronic pain than acute pain. Opioids have a high potential for causing misuse and it became a serious national crisis that affects public health as well as social and economic welfare. The number of drug overdose deaths increased by nearly 5% from 2018 to 2019 and has quadrupled since 1999 and over 70% of the 70,630 deaths in 2019 involved an opioid .

5. non-pharmacological management of chronic pain

Lifestyle factors such as physical inactivity, sedentary behavior, stress, poor sleep, unhealthy diet, and smoking are associated with chronic pain **(Fig. 5A)**. For example exercises attenuate and prevent chronic pain via activation of mesolimbic dopaminergic system and dietary pattern is known to influence the most the occurrence, maintenance, and perception of chronic pain. Therapeutic fasting techniques include intermittent fasting (e.g., 60% energy restriction every other day), partial fasting (e.g., 5-day diet providing 750–1100 kcal), and time-restricted feeding (limiting the daily period of food intake to ≤8 h). Therapeutic fasting provides advantages on the peripheral system such as reducing the proinflammatory state and oxidative stress, consecutively increasing cellular metabolism and promoting stem cell-based regeneration . Also, accumulating evidences report that fasting improves brain functions **(Fig. 5B)**.

Figure 1.

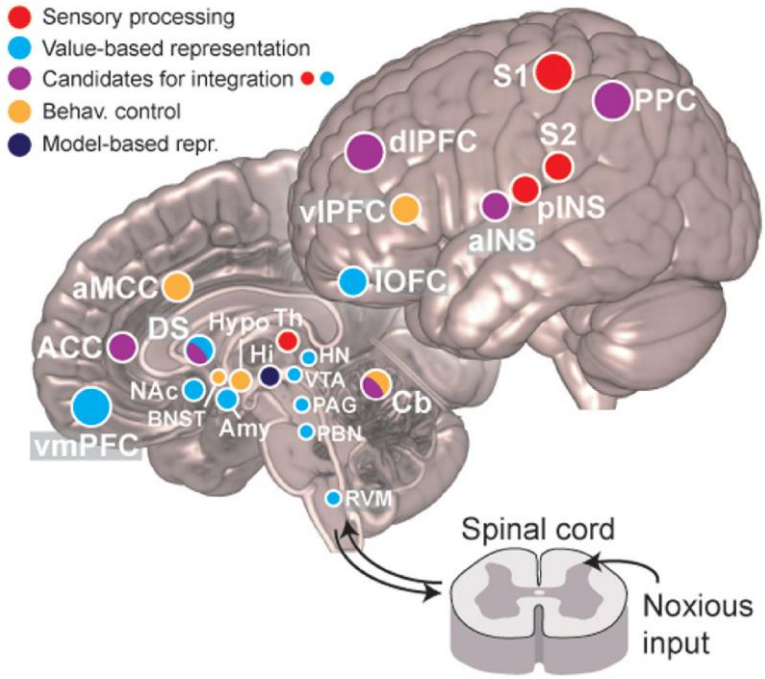

Sensory processing
Value-based representation
Candidates for integration
Behav. control
Model-based repr.
S1
PPC
S2
dlPFC
vlPFC
pINS
aINS
lOFC
aMCC
DS
Hypo Th
ACC
Hi
HN
VTA
NAc
PAG
Cb
BNST
Amy
PBN
vmPFC
RVM
Spinal cord
Noxious
input

Figure 1.

Several brain areas such as cortical and subcortical sensory, limbic, and associative areas are activated with pain. These include the primary somatosensory cortex (S1) and secondary somatosensory cortex (S2) ventrolateral prefrontal cortex (vlPFC), thalamus (Th), amygdala (Amy), and periaqueductal gray (PAG). These regions receive ascending nociceptive inputs both directly and via the parabrachial nucleus (not shown). The effects of attention and emotion on pain sensation are ultimately mediated by descending pathways from the vlPFC, dorsal anterior cingulate cortex (dACC), and Amy to the PAG. The rostral ventromedial medulla (RVM) involves functional connections between the ventromedial PFC and the nucleus accumbens (NAc).

Fig. 1 *adopted from Seymour, 2020*

Figure 2.

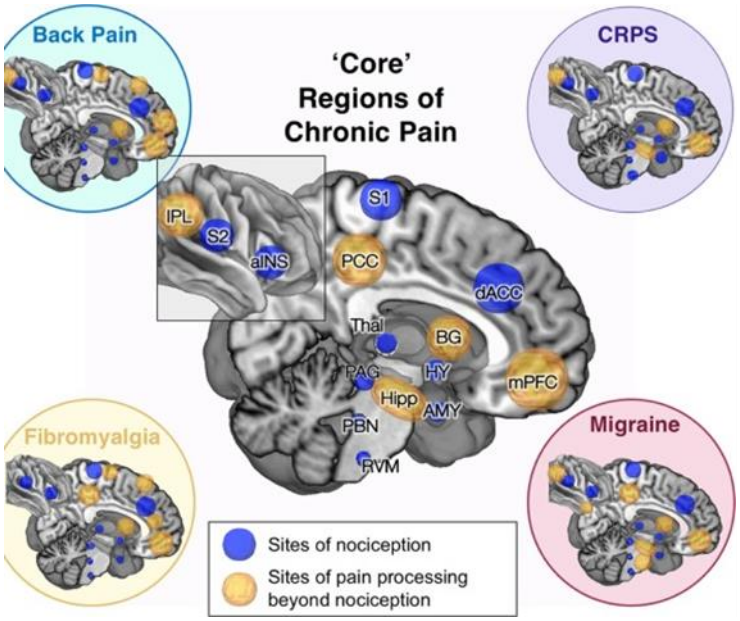

Back Pain
'Core'
Regions of
Chronic Pain
CRPS
IPL
S2
aINS
S1
PCC
dACC
Thal
BG
PAG
HY
mPFC
Hipp
AMY
PBN
RVM
Fibromyalgia
Migraine
Sites of nociception
Sites of pain processing
beyond nociception

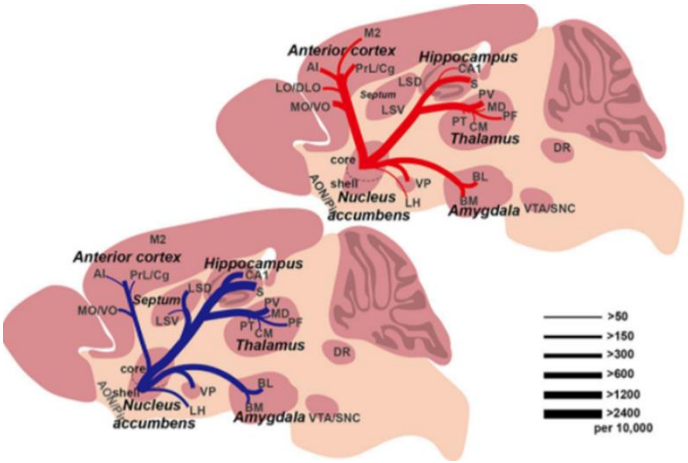
Figure 2

'Core' Regions of Chronic Pain. Despite the different chronic pain pathologies, some regions are consistently implicated across diseases. These include, sites of nociception (dark grey) such as the Thal, dACC, SI, SII, aINS, as well as sites of pain-related pain processing beyond nociception (light grey) such as the Hipp, mPFC, BG, PCC, and IPL. Thal thalamus, dACC dorsal anterior cingulate cortex, SI somatosensory I, SII somatosensory II, aINS anterior insula, hipp hippocampus, mPFC medial prefrontal cortex, BG basal ganglia, PCC posterior cingulate cortex, and IPL inferior parietal lobule.

Fig. 2A *adopted from Marianne C.ReddanTor D.Wager, 2018*

Figure 3.

A


M2
Anterior cortex
AI
PrL/Cg
LO/DLO
MO/VO
Hippocampus
CA1
S
LSD
Septum
LSV
PV
MD
PF
PT
CM
Thalamus
DR
core
shell
Nucleus accumbens
AON/PrCx
VP
LH
BL
BM
Amygdala
VTA/SNC
>50
>150
>300
>600
>1200
>2400
per 10,000

B

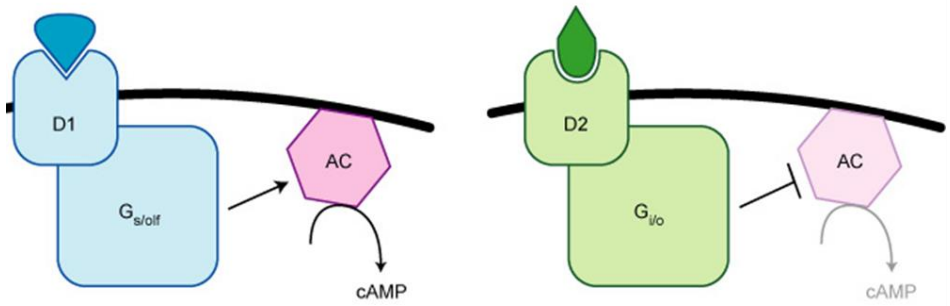

D1
$G_{s/olf}$
AC
cAMP
D2
$G_{i/o}$
AC
cAMP

Figure 3. Nucleus accumbens

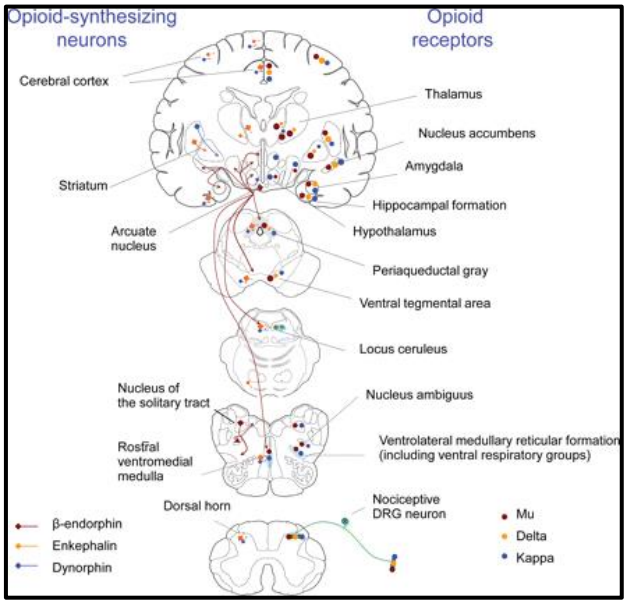
(A) Summary of monosynaptic inputs to the NAc core (top, red) and shell (bottom, blue), considering D1R-MSNs and D2R-MSNs together. Line thickness represents the number of inputs. (B) Types of medium spiny neurons in the nucleus accumbens. D1 and D2 dopamine receptors exert opposing effects on the same signaling pathway mediated by heterotrimeric GTP-binding proteins. D1 receptor activation positively affects adenylyl cyclase, and D2 receptor activation is either uncoupled from adenylyl cyclase.

Fig. 3A *adapted from Zhao Li, 2018*

Fig. 3B *adapted from Alexia Pollack, 2004*

Figure 4.

A


Opioid-synthesizing neurons
Opioid receptors
Cerebral cortex
Thalamus
Nucleus accumbens
Amygdala
Striatum
Hippocampal formation
Arcuate nucleus
Hypothalamus
Periaqueductal gray
Ventral tegmental area
Locus ceruleus
Nucleus of the solitary tract
Nucleus ambiguus
Rostral ventromedial medulla
Ventrolateral medullary reticular formation (including ventral respiratory groups)
Dorsal horn
Nociceptive DRG neuron
β-endorphin
Enkephalin
Dynorphin
Mu
Delta
Kappa

B

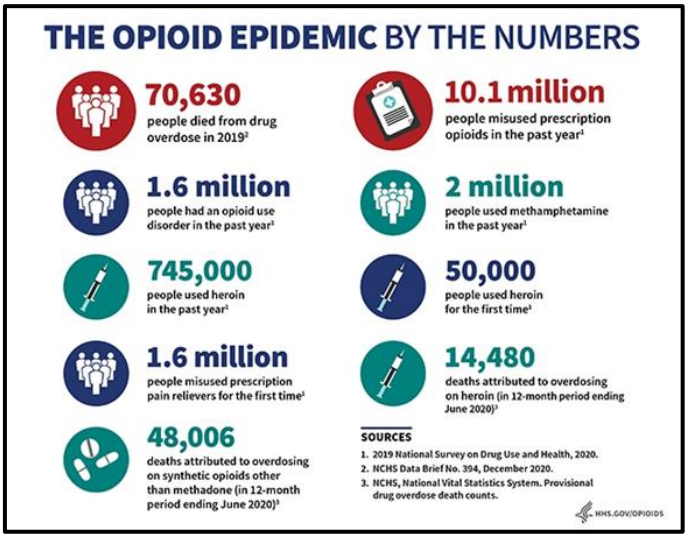

THE OPIOID EPIDEMIC BY THE NUMBERS
70,630
people died from drug overdose in 2019[2]
10.1 million
people misused prescription opioids in the past year[1]
1.6 million
people had an opioid use disorder in the past year[1]
2 million
people used methamphetamine in the past year[1]
745,000
people used heroin in the past year[1]
50,000
people used heroin for the first time[1]
1.6 million
people misused prescription pain relievers for the first time[1]
14,480
deaths attributed to overdosing on heroin (in 12-month period ending June 2020)[3]
48,006
deaths attributed to overdosing on synthetic opioids other than methadone (in 12-month period ending June 2020)[3]
SOURCES
1. 2019 National Survey on Drug Use and Health, 2020.
2. NCHS Data Brief No. 394, December 2020.
3. NCHS, National Vital Statistics System. Provisional drug overdose death counts.
HHS.GOV/OPIOIDS

Figure 4. Opioid management

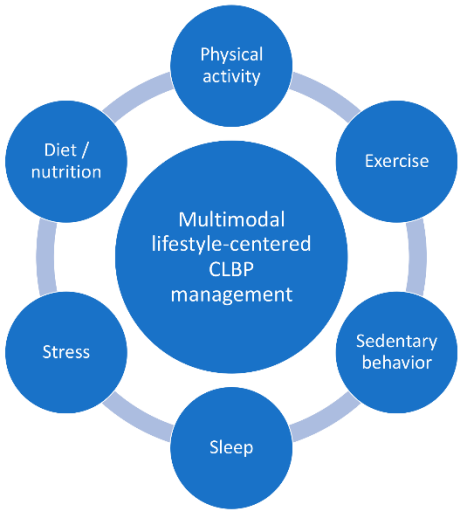
(A) Distribution of mu-, delta-, kappa-opioid receptors in the central nervous system (B) Opioid crisis epidemics in numbers.

Fig. 4A *adapted from Benarroch, 2012*

Fig. 4B *adapted from Trang, 2015*

Figure 5.

A


Physical activity
Exercise
Sedentary behavior
Sleep
Stress
Diet / nutrition
Multimodal lifestyle-centered CLBP management

B

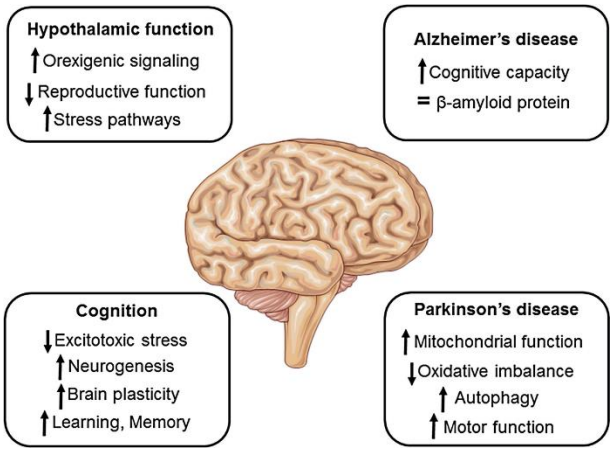

Hypothalamic function
↑Orexigenic signaling
↓Reproductive function
↑Stress pathways
Alzheimer's disease
↑Cognitive capacity
= β-amyloid protein
Cognition
↓Excitotoxic stress
↑Neurogenesis
↑Brain plasticity
↑Learning, Memory
Parkinson's disease
↑Mitochondrial function
↓Oxidative imbalance
↑Autophagy
↑Motor function

Figure 5. Effect of time restrict feeding on the CNS.

(A) Lifestyle factors such as physical (in)activity, sedentary behavior, stress, poor sleep, unhealthy diet, and smoking are associated with chronic pain severity and sustainment. This applies to all age categories, that is, chronic pain across the lifespan. (B) Intermittent fasting (IF) improvements in cognitive capacity are supported by increased neuronal resistance to excitotoxic stress, neurogenesis, and brain plasticity, resulting in preserved learning and memory. IF can delay Alzheimer's disease progression independently of changes in neuronal β-amyloid peptide accumulation. IF also prevents Parkinson's disease development through enhancements in mitochondrial function and autophagy, along with reduction in oxidative imbalance.

Fig. 5A *adapted from Nijs et al., 2019*

Fig. 5B *adapted from* Cerqueira et al., *2019*

Purpose

Despite of high prevalence and use of excessive opioids, chronic pain tends to difficult to treat. Moreover, the misuse and addiction to opioids are serious national crisis. Therefore, alternative effective treatments for chronic pain needs to be explored.

- Explore pharmacological treatment for chronic inflammatory pain management (validate analgesic effect of opioid antagonists in chronic pain)
- Explore non-pharmacological treatment for chronic inflammatory pain management (identify underlying mechanism of refeeding-induced analgesia)

Chapter 1

Paradoxical analgesia of opioid antagonists in the chronic inflammatory pain condition

*This chapter includes reproductions from an article published by Grace J. Lee, Shin Ae Kim, Yae Jin Kim, Seog Bae Oh 2021. Naloxone-induced analgesia mediated by central kappa opioid system in chronic inflammatory pain. Brain research, 2021, 1762, 147445. Doi: https://doi.org/10.1016/j.brainres.2021.147445

Abstract

Opioids are widely used for the treatment of pain, but their comparative effectiveness and usefulness for chronic pain are questioned despite its frequent use. Hence, this study is aimed to understand altered brain opioid system in chronic pain conditions and identify the alternative effective non-opioid treatment for chronic pain. I used modified complete Freund's adjuvant (CFA)-induced chronic inflammatory pain model throughout the study. First, I analyzed the density of prodynorphin (ligand of the kappa-opioid receptor, KOR) and mu-opioid receptor expression in the whole brain of the CFA model. The result showed that prodynorphin was significantly enhanced, while mu-opioid expression was significantly suppressed in the central nucleus of the amygdala (CeA) and nucleus accumbens (NAc) but not in other brain areas. Interestingly, systemic administration of non-specific opioid antagonist (naloxone, 10 mg/kg) induced paradoxical analgesia in spontaneous pain measure but not mechanical allodynia in the CFA group. Similarly, systemic KOR antagonist (norbinaltorphimine, nor-BNI 10 mg/kg) alleviated spontaneous pain behaviors but not mechanical allodynia. Furthermore, microinjection of naloxone and nor-BNI into the NAc and CeA significantly reduced spontaneous chronic pain behavior. Taken together, our results suggest that both non-specific opioid antagonists and kappa-opioid antagonists may suppress spontaneous chronic inflammatory pain behavior via blocking facilitated kappa-opioid systems in the NAc and CeA.

Key words: chronic pain; dynorphin; naloxone; spontaneous pain

Abbreviations

ACC, anterior cingulate cortex; CeA, central amygdala; CFA, complete Freund's adjuvant; IL-6, interlukin-6; i.p., intraperitoneal; KOR, kappa opioid receptor; MOR, mu opioid receptor; NAc, nucleus accumbens; nor-BNI, nor-Binalorphimine dihydrochloride; TNF-α tumor necrosis factor.

Introduction

Chronic pain is a growing public health concern worldwide. Major pathological manifestations of chronic pain include spontaneous pain, hyperalgesia, and allodynia. In the clinical study, about 96% of chronic pain patients answer to have spontaneous pain while less than 25% have allodynia. Spontaneous pain is the main reason for subjects seeking medical care and is the primary complaint of chronic pain patients. Such conditions diminish the quality of life and increase anxiety and depression.

Opioids are routinely prescribed to manage moderate to severe pain. Opioids are effective against both spontaneous pain and mechanical allodynia, but their comparative effectiveness and usefulness for chronic pain are questioned. The opioid can be effective for short-term use, but a lack of evidence supporting longer-term (>four months) effects or superiority of opioids compared with other analgesics such as anti-inflammatory and anti-depressant drugs. Especially, long-term use is associated with addiction, misuse, depression, and hyperalgesia as opioid receptors are expressed not only throughout the nociceptive pain pathway but also regions involved in reward and emotion in the central nervous system. Interestingly, reward and emotion-related brain regions are known to be functionally disrupted in chronic pain, and these changes might be related to the pharmacological action of opioids on spontaneous pain and affective pain. Indeed, the clinical effects of opioids can be very variable depending on receptor subtypes which are differentially expressed and targeted by the drugs in the brain and spinal cord.

Systemic kappa opioid receptor (KOR) agonist induces analgesic effects via direct inhibition of pain pathways in the spinal cord. However, the supraspinal KOR system contributes to the production of affective pain rather than analgesia. The prototypical kappa opioid agonist, ethylketocyclazocine, only produces analgesia following co-administration into the periaqueductal gray and the locus coeruleus that was blocked by the mu1-selective antagonist, naloxonazine.

The KOR system is also known to be facilitated within the limbic system in chronic pain, resulting in suppression of the morphine-induced rewarding effect in rats. Also, blocking KOR recovered mu opioid-mediated dopamine release in the formalin-induced pain model, which suggests the potential involvement of interactions between mu-opioid receptor (MOR) and KOR in pain modulation produced by opioids.

Given the facilitation of KOR in the limbic system under chronic pain conditions, we hypothesized that the activated KOR system may exacerbate pain behavior, which might underlie decreased analgesic effects of opioids under chronic pain conditions. In this study, we aimed to further elucidate the association between the altered brain opioid system and the effect of analgesics by using complete Freud's adjuvant (CFA)-induced chronic inflammatory pain model.

Material and methods

Mice

6 weeks old male C57BL/6 mice were purchased from DooYeol Biotech (Korea). The mice were housed 5 per cage at a temperature-controlled room (23 ± 1 ℃, 12 h/12 h light/dark cycle with lights on at 08:00) and maintained with standard lab chow (pellet diet) and water ad libitum. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Seoul National University (SNU-180518-1-1). All experiments were performed in accordance with relevant guidelines and regulations that were confirmed by IACUC.

Measurement of pain behaviors in CFA (complete Freund's adjuvant)-induced pain model

20 μl of undiluted CFA (Sigma-Aldrich, St. Louis, MO, USA) was injected into the plantar surface of the left hind paw. To extend the length of CFA-induced inflammatory pain behavior, a second 20 μl of CFA injection was given 7 days after the first. The CFA-induced spontaneous pain behavior was video recorded and analyzed as previously described . To assess mechanically evoked pain, the 50% paw withdrawal threshold measured using von Frey filaments (North Coast Medical, Morgan Hill, CA, USA) as previously described .

Administration of drug

Naloxone (TOCRIS Bioscience), a non-selective opioid antagonist

was diluted in 0.9% saline. KOR antagonist nor-Binalorphimine dihydrochloride (nor-BNI) (TOCRIS Bioscience) were dissolved in 0.9% saline. Systemic administration of naloxone (10 mg/kg), nor-BNI (10 mg/kg) were delivered via intraperitoneal (i.p.) injection (10 ml/kg). For direct brain infusion, nor-BNI (5 μg/μl) and naloxone (1 μg/μl) was dissolved in 0.9% saline and unilaterally microinjected (0.5 μl over 5 min) into the contralateral NAc and CeA to achieve a dose of 2.5 μg for nor-BIN and 0.5 μg for naloxone. Injectors were left in place for an additional 2 min before removal.

Surgical procedures for microinjection

C57BL/6 mice were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with guide cannula (26-gauge, 4.6 mm of length, Plastics One) aimed at the NAc (coordinates: anteroposterior (AP) 1.4 from bregma, mediolateral (ML) 0.5 from midline, dorsoventral (DV) −4.6 from skull surface) and CeA (AP −1.22, ML + /− 3.0, DV −4.1). When inserted, microinjector tips extended 0.5 mm beyond the guide. Microinjector for drug delivery was left in place for 2 min.

Immunohistochemistry

Following administration of pentobarbital (50 mg/kg), the animals were transcardially perfused, first with PBS and then with a 4% paraformaldehyde solution. Brains were post fixed at 4°C in paraformaldehyde for 1 d following the extraction and cryoprotected in 30% sucrose for 3 days. The brains were then sectioned into 40 μm slices and preserved in PBS. Free-floating sections were rinsed in PBS,

incubated for 30 min in 0.3% hydrogen peroxide PBS solution to quench endogenous peroxidase activity, rinsed several times in PBS, and incubated in a blocking solution that contained 5% normal goat serum diluted in 0.3% Triton X-100 in PBS for 60 min. The sections were incubated in rabbit anti-prodynorphin polyclonal antibody (1:1000 dilution, ab11137 Abcam, UK) diluted in PBS that contained 0.5% Tween 20 and 5% normal goat serum for 48 h at 4°C. After incubation in the primary antibody, the sections were rinsed three times for 10 min in PBS and incubated for 2 h in biotinylated secondary antiserum made from goat anti-rabbit antibody (1:200 dilution; Vector laboratories, USA) in PBS. Then the sections were processed with ABC kit (PK-6100, Vectastain ABC kit, Vector laboratories) for an hour and visualized with DAB kit (DAB substrate kit for peroxidase, Vector laboratories). After several rinses in PBS, the sections were mounted on coated glass slides, air dried, dehydrated through a series of graded ethanols, and clearing agents. Then permounted (Sigma-Aldrich, Germany) and examined under the bright-field microscope (DM5000B, Leica). Images were all taken at 10× magnification at one time to maintain identical lighting intensity and color balance. Averaged number of prodynorphin positive neurons was determined using inverted color images in ImageJ software (National Institutes of Health). To detect MOR expression, sections were incubated overnight in blocking buffer (0.3% Triton X-100, 2% bovine serum in PBS) at room temperature. The primary antibodies guinea pig anti-mu opioid receptor (1:200 dilution, nb100-1618, Novus biologicals) was diluted in the blocking solution, and the sections were incubated for 48 h at 4 °C.

The slices were washed three times for 10 min each in 0.1 M PBS. The secondary antibody, Cy3 donkey anti-guinea pig (1:200 dilution, 706-165-148, Jackson ImmunoResearch Laboratories), and DAPI (1:5000 dilution, D9542, Sigma Aldrich) were diluted in the secondary antibody buffer (0.1% Triton X-100, 2% BSA) and incubated 2 h at room temperature. The sections were then washed three times with 1x PBS for t10 min each, and mounted using Vectashield Mounting media (Vector Laboratories). Mounted slices were imaged using a confocal microscope (LSM 700, Carl Zeiss). Images were all taken at 200× magnification at one time to maintain identical lighting intensity and color balance.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, USA). Comparison between two groups was made using the unpaired Student's *t*-test. For multiple comparisons, data were analyzed using two-way ANOVA followed by the post hoc Bonferroni test. Detailed statistics for each experiment were shown in the figure legend. Data are presented as mean ± SEM. Differences with $p < 0.05$ were considered significant.

Results

Prodynorphin level enhanced, and MOR expression is suppressed in the NAc and CeA under chronic inflammatory pain condition

To confirm the contribution of the KOR system in the brain to chronic inflammatory pain, we checked the level of prodynorphin, the precursors that are endogenous ligands for KOR, by immunohistochemical analysis in the whole brain. We used modified complete Freund's adjuvant (CFA)-induced chronic inflammatory pain model. Since structural and functional changes of the brain due to pain chronification are known to develop 14 days after the pain induction in rodents , we tried to extend pain behavior with a booster. First, with a booster at 4 days after the first when the pain behavior maximizes, the spontaneous pain behavior lasted only for 10 days **(Fig. 6A)**. However, with a booster at 7 days after the first when the pain behavior starts to decline **(Fig. 6A)**, we observed that spontaneous pain behavior significantly increased from day 4 to day 22 (**Fig. 6B**; ***$p < 0.0001$, two-way ANOVAs followed by Bonferroni, n=7 each group) and pain behavior lasts longer than a booster injection on day 4 (+++$p < 0.0001$, two-way ANOVAs followed by Bonferroni, n=7 each group). Also, the mice developed mechanical allodynia in the ipsilateral paw, which lasted more than 16 days, while a booster on day 4 lasted only for 14 days (**Fig. 6C**; +++$p < 0.0001$, two-way ANOVAs followed by Bonferroni, n=8 each group). Hence, we used a model with a booster injection on day 7 throughout this study.

Using a prodynorphin antibody, we compared prodynorphin levels of the CFA group with the naïve group **(Fig. 6D**; n=4 each group). Among various brain regions, we observed a profound increase of prodynorphin density in the contralateral NAc and CeA in the CFA group **(Figs. 6D, 6E)**. Conversely, density within the anterior cingulate cortex (ACC) in the CFA group was significantly decreased compared to naïve mice **(Fig. 6D)**.

As preclinical and human subject research have shown strong correlations between dysfunction of KOR and MOR system under chronic pain conditions , we then examined the expression of MOR in the NAc and CeA via immunohistochemistry. MOR was decreased in the NAc and CeA of the CFA group compared to the naïve group (**Figs. 8C-E**; ***$p < 0.001$, unpaired t-test, two-tailed, n=4 each group).

Systemic administration of high dose naloxone reduced spontaneous chronic inflammatory pain behavior

Given the enhanced kappa opioid system and suppressed mu-opioid system within the NAc and CeA, we examined whether KOR and MOR alteration affect chronic inflammatory pain behavior by systemic administration of naloxone (non-specific opioid antagonist, 10 mg/kg, i.p.). With the same doses and route used in our previous study , naloxone (10 mg/kg, i.p.) was injected 15 days from the 1st CFA injection **(Fig. 7A)**. We found that spontaneous pain behavior decreased with the naloxone injection (**Fig. 7C**; ***$p < 0.001$, unpaired t-test, two-tailed, n=8 each group) whereas mechanical allodynia was not affected (**Fig. 7D**; $p=0.4505$, unpaired t-test, two-tailed, n=8 each group).

Systemic administration of kappa opioid antagonist reduced spontaneous chronic inflammatory pain

We confirmed that blockage of MOR and KOR alleviates spontaneous chronic inflammatory pain behavior. Then we sought to determine the involvement of the KOR system in chronic inflammatory pain. A kappa-opioid antagonist, nor-BNI (10 mg/kg, i.p.), was administrated into the CFA-treated mice **(Fig. 8A)**. The dose used was determined based on the results of the previous report . The systemic injection of nor-BNI significantly decreased spontaneous in the CFA-treated mice **(Fig. 8C**; ***p < 0.001, unpaired t-test, two-tailed, n=8 each group). However, mechanical allodynia was not affected by systemic nor-BNI (**Fig. 8D**, p=0.1361, unpaired t-test, two-tailed, n=8 each group).

Nor-BNI injection into the NAc and CeA relieved spontaneous chronic inflammatory pain

To further investigate whether an action of naloxone limited to the NAc and CeA would be sufficient to produce analgesia, naloxone was microinjected directly into these areas. The pain behavior was measured 5 min after the naloxone administration for 30 min. Microinjection of 0.5 μl of naloxone (1 μg/μL) into the NAc (**Fig. 9C**; ***p < 0.001, paired *t*-test, two-tailed, vehicle n = 7, naloxone n = 11) and CeA (**Fig. 9F**; ***p < 0.001, paired *t*-test, two-tailed, n = 6 each group) also reduced spontaneous pain compared to the microinjection of the vehicle.

Given the enhanced prodynorphin content within the NAc and CeA, we tested whether blocking KOR in the NAc and CeA would be

sufficient to reduce spontaneous chronic inflammatory pain behavior. The nor-BNI (5 μg/0.5 μL) was microinjected into the contralateral NAc and CeA separately. The dose used was determined based on the results of the previous report . Microinjection of nor-BNI into NAc (**Fig. 9D**; ***$p < 0.001$, unpaired t-test, two-tailed, vehicle n=5, nor-BNI n=7) and CeA (**Fig. 9G**; ***$p<0.001$, unpaired t-test, two-tailed, vehicle n=7, nor-BNI, n=11) alleviated spontaneous pain compared to the vehicle group.

Discussion

The aim of this study was to investigate non-opioid pharmacological treatment for chronic inflammatory pain. We found that prodynorphin expression was enhanced while MOR expression was decreased in the NAc and CeA under chronic pain conditions. We revealed that systemic injection of non-specific opioid receptor antagonist and kappa receptor antagonist unexpectedly decreases only spontaneous pain behavior but not mechanical allodynia in the chronic inflammatory pain model. In addition, microinjection of both kappa receptor antagonist and naloxone into NAc and CeA reduced spontaneous pain behavior. These findings provide evidence that inhibition of KOR facilitation in the NAc and CeA contributes to reduction of spontaneous pain behavior in chronic pain states.

Chronic pain in humans is represented by changes in nociceptive thresholds and ongoing pain conditions. It has been suggested that spontaneous pain, rather than stimulus-evoked pain, maybe the more significant clinical problem. However, measurement of spontaneous ongoing pain in rodents is challenging. Also, it is well known that alteration of the central nervous system in chronic pain occurs 14 days after inflammation or surgery in mice. Hence, we made a booster injection to observe both spontaneous pain and mechanical allodynia for a longer period (Fig. 6B). With this model, we found that naloxone alleviates only spontaneous pain, but not mechanical allodynia, in chronic inflammatory conditions.

KOR is abundant in the medial pain pathway, which is associated

with the affective component of pain. Considerable evidence suggests that the KOR system is facilitated in spontaneous pain. For instance, PET scan imaging in rodents showed increases in dynorphin tone in the NAc under inflammatory pain conditions. The NAc is a brain region well known to mediate reward-driven behaviors. Human imaging studies have consistently shown that chronic pain undergoes a change in the NAc. The amygdala, especially its CeA, has also emerged as a key element of the pain matrix . These findings indicate that the KOR system in the brain areas related to affective pain is facilitated under chronic pain conditions. Consistent with these reports, we found that chronic pain increases dynorphin expression in the NAc and CeA **(Fig. 8A, B)**.

A microinjection of kappa-opioid antagonist into the CeA of neuropathic pain model eliminated the aversiveness of ongoing pain , and KOR agonist-mediated conditioned place aversion in chronic neuropathic pain model . Likewise, we observed decreased spontaneous pain behavior with systemic KOR antagonist injection as systemic naloxone injection did **(Fig. 7)**. Thus, our results are compatible with these previous studies and suggest that blockage of KOR alleviates spontaneous pain behavior under chronic pain conditions.

The prodynorphin immunoreactivities were decreased in ACC under chronic inflammatory pain conditions in this study **(Fig. 7A)**. Numerous animal studies, as well as human imaging, support the role of the ACC in the evaluation of the aversiveness of pain. There was a study reporting increased prodynorphin mRNA and decreased KOR gene in ACC under neuropathic pain conditions. The role of ACC can be different in

pain aversiveness associated with inflammatory versus neuropathic pain. Also, the discrepancies between mRNA and protein expression can be caused by posttranscriptional regulation, as well as differences in mRNA and protein turnover rates.

The activation of MOR represents the gold standard for pain relief. Endogenous MOR and KOR systems can be physiologically balanced under a normal state. However, inflammatory pain facilitates the endogenous KOR system and downregulation of MOR function resulting in a decrease in dopamine release in the NAc. Similarly, we confirmed decreased MOR expression under chronic pain conditions in both NAc and CeA **(Fig. 8C, D, E)**. Likewise, microinjection of naloxone into these areas reversed spontaneous pain as microinjection of kappa antagonist did **(Fig. 9B, D)**. Our results may indicate that naloxone alleviates spontaneous pain via suppressing facilitated KOR in NAc and CeA. Also, we assume that blocking KOR in the NAc and CeA is more important than enhancing MOR to suppress spontaneous pain. There could be an interrelationship between KOR and MOR, but further studies are required to better understand how KOR, and MOR interact under chronic pain conditions.

The possibility of the peripheral effect of KOR cannot be ruled out. However, the analgesic effect of the peripheral KOR system is known to be mediated through activation of KORs with agonists rather than the antagonists. Hence the result from the present study emphasizes the antagonistic effect of naloxone targeting the brain KOR in alleviating spontaneous pain.

There can be a sex difference in naloxone-induced analgesia. There are several studies comparing the effect of kappa opioid in male and female mice. Interestingly, a study reports that while the prodynorphin gene in chronic pain condition is increased only in the male group, KOR antagonist alleviates affective pain in both sex. Similarly, our study was conducted in chronic pain model and the changes in the KOR expression and pain behavior in the male group were consistent with this study. However, further study is needed to confirm whether the pain behavior is indeed similar in the female group.

Emerging experimental evidence suggests that opioid and kappa-opioid antagonist combination provides pain relief in chronic pain patients with opioid dependence or addiction and prevents side effects of opioids. Our results strongly support the use of naloxone and nor-BNI as a therapeutic adjuvant to alleviate spontaneous pain, which is the most significant component of chronic pain.

Figure 6.

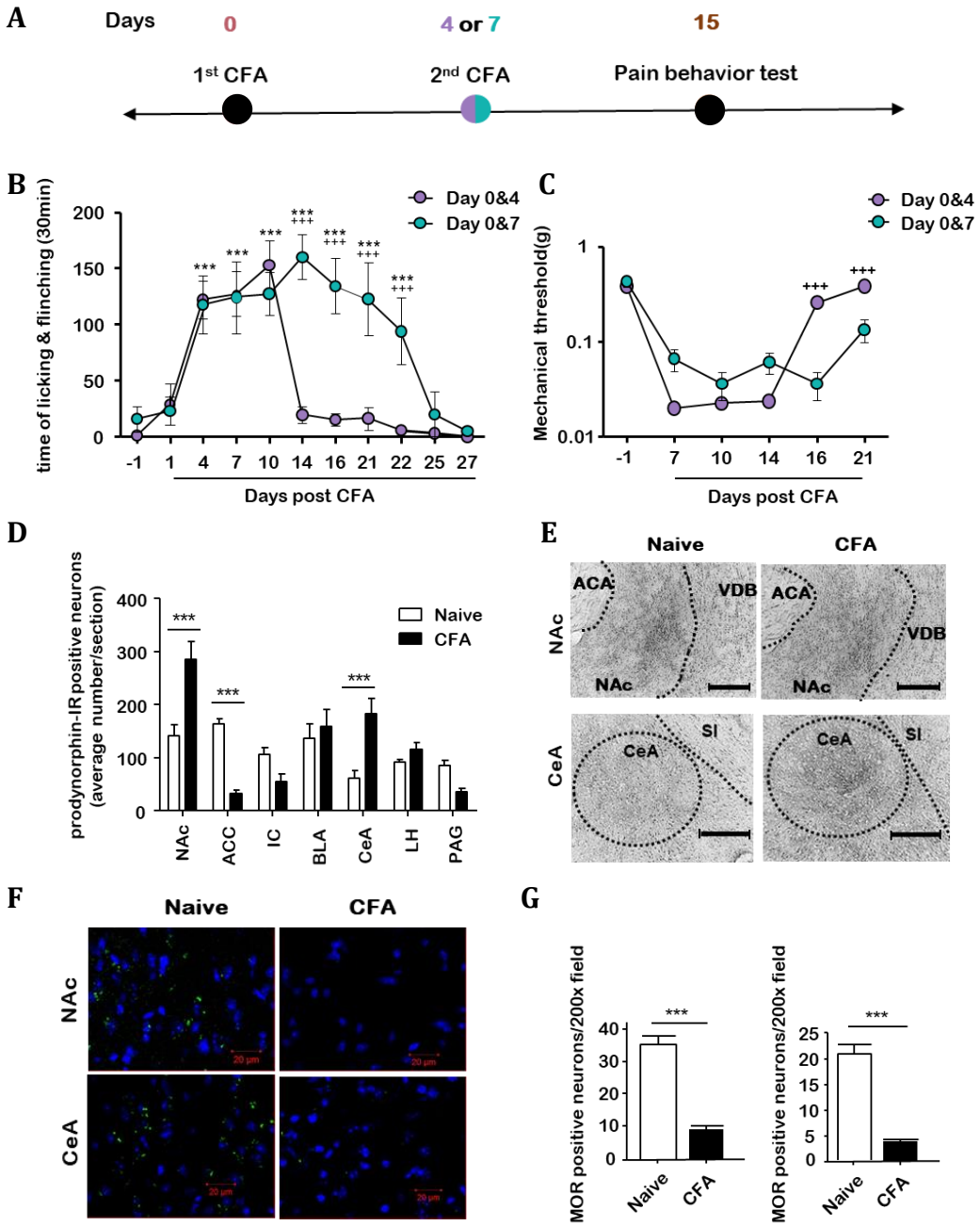

A
Days
0
4 or 7
15
1st CFA
2nd CFA
Pain behavior test
B
time of licking & flinching (30min)
Day 0&4
Day 0&7
Days post CFA
C
Mechanical threshold(g)
Day 0&4
Day 0&7
Days post CFA
D
prodynorphin-IR positive neurons (average number/section)
Naive
CFA
NAc
ACC
IC
BLA
CeA
LH
PAG
E
Naive
CFA
ACA
VDB
NAc
CeA
SI
F
Naive
CFA
NAc
CeA
G
MOR positive neurons/200x field
Naive
CFA

Figure 6. Immunohistochemical analysis for prodynorphin content and MOR expression in the NAc and CeA in chronic inflammatory pain model. (A) Schematic representation of CFA-induced chronic inflammatory pain model. (B) Time course of spontaneous pain behavior following CFA injection with a booster on day 4 and 7. (C) Time course of mechanical allodynia following CFA injection with a booster on day 4 and 7. (D) Comparison of prodynorphin immunoreactivity between naïve group and CFA-induced chronic pain model in whole brain. The number of prodynorphin-ir neurons (in a 500 × 500 μm area) is expressed on the Y axis and brain area written below the graph columns on the X axis. Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; IC, insular cortex; LH, lateral hypothalamus; NAc, nucleus accumbens; PAG, periaqueductal gray. (E) Representative microscopic (10 x) prodynorphin staining samples of the naïve and CFA group in the NAc and CeA. Scale bar 200 μm. Abbreviations: ACA, anterior commissure; SI, substantia innominata; VDB, nucleus of the vertic7l limb of the diagonal band. (F) Representative microscopic (200x) images showing MOR expression in NAc and CeA of naïve and CFA group. The number of MOR-ir neurons is expressed on the Y axis. (G) Comparison of number of MOR positive neurons between naïve and CFA-induced chronic pain model in NAc (left) and CeA (right). Data are presented as mean ± SEM.

Figure 7

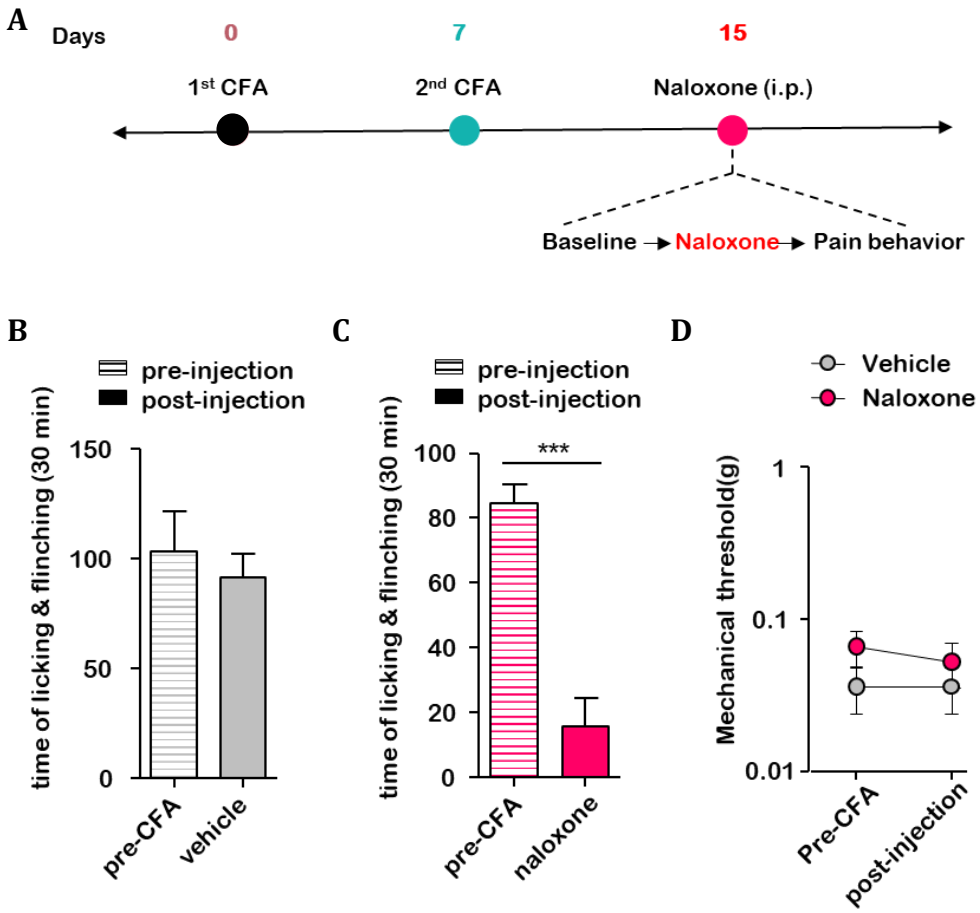

A
Days
0
7
15
1st CFA
2nd CFA
Naloxone (i.p.)
Baseline
Naloxone
Pain behavior
B
pre-injection
post-injection
time of licking & flinching (30 min)
150
100
50
0
pre-CFA
vehicle
C
pre-injection
post-injection
time of licking & flinching (30 min)
***
100
80
60
40
20
0
pre-CFA
naloxone
D
Vehicle
Naloxone
Mechanical threshold(g)
1
0.1
0.01
Pre-CFA
post-injection

Figure 7. Effect of systemic naloxone administration on the pain behaviors. (A) Schematic representation of i.p. injection of naloxone (opioid receptor antagonist, 10 mg/kg) timelines for CFA-induced chronic inflammatory model. Naloxone and vehicle were administered 30 min before behavior test. (B) The effect of systemic vehicle on spontaneous pain. (C) The effect of systemic naloxone on spontaneous pain. (D) The effect of systemic naloxone on mechanical allodynia in chronic inflammatory pain. The asterisk denotes significance levels in comparison with pre-injection. Crosses denote significance levels in comparison with a booster injection on day 4 and 7. Data are presented as mean ± SEM.

Figure 8

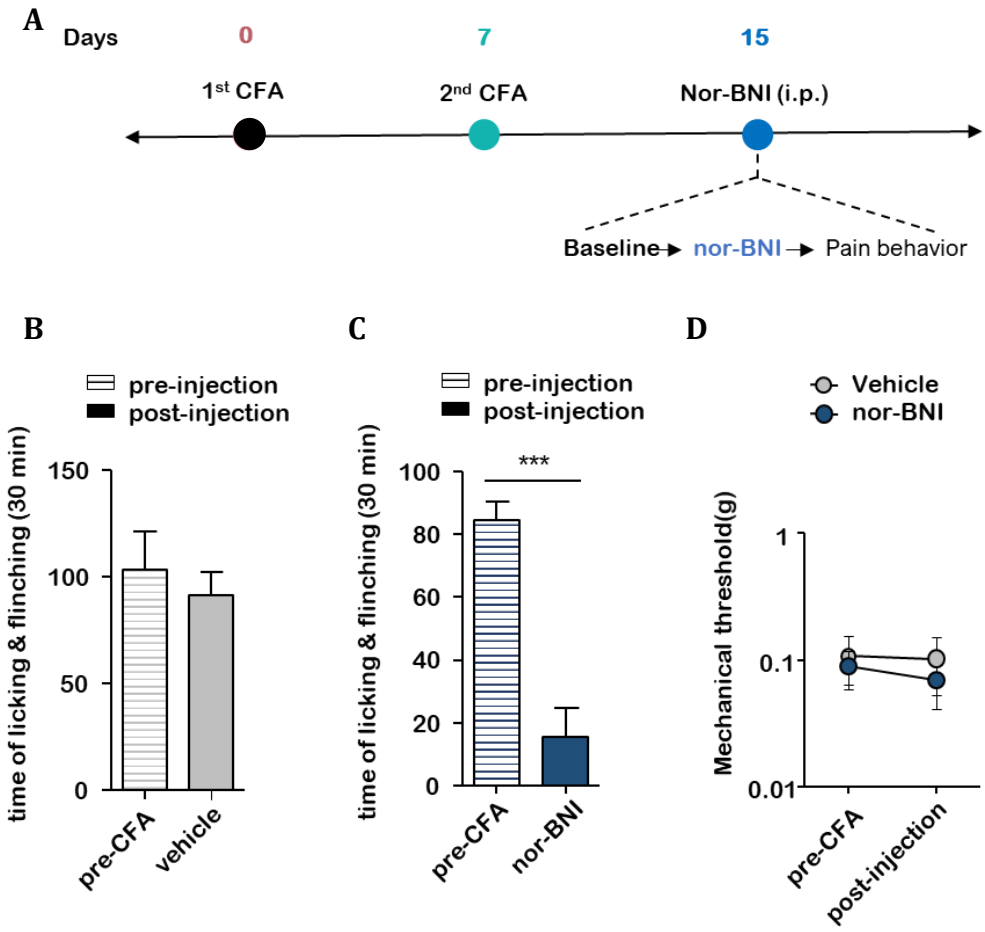

A
Days
0
7
15
1st CFA
2nd CFA
Nor-BNI (i.p.)
Baseline
nor-BNI
Pain behavior
B
pre-injection
post-injection
time of licking & flinching (30 min)
150
100
50
0
pre-CFA
vehicle
C
pre-injection
post-injection
***
time of licking & flinching (30 min)
100
80
60
40
20
0
pre-CFA
nor-BNI
D
Vehicle
nor-BNI
Mechanical threshold(g)
1
0.1
0.01
pre-CFA
post-injection

Figure 8. Effect of systemic nor-BNI administration on the pain behaviors. (A) Schematic representation of CFA-induced spontaneous pain behavior study in naloxone injected mice. (B) Effects of systemic vehicle on the CFA-induced spontaneous pain behavior. (C) Effects of systemic nor-BNI (10 mg/kg) on CFA-induced spontaneous pain behavior. (D) Effects of systemic nor-BNI and vehicle on mechanical allodynia in chronic inflammatory pain model. Data are presented as mean ± SEM.

Figure 9

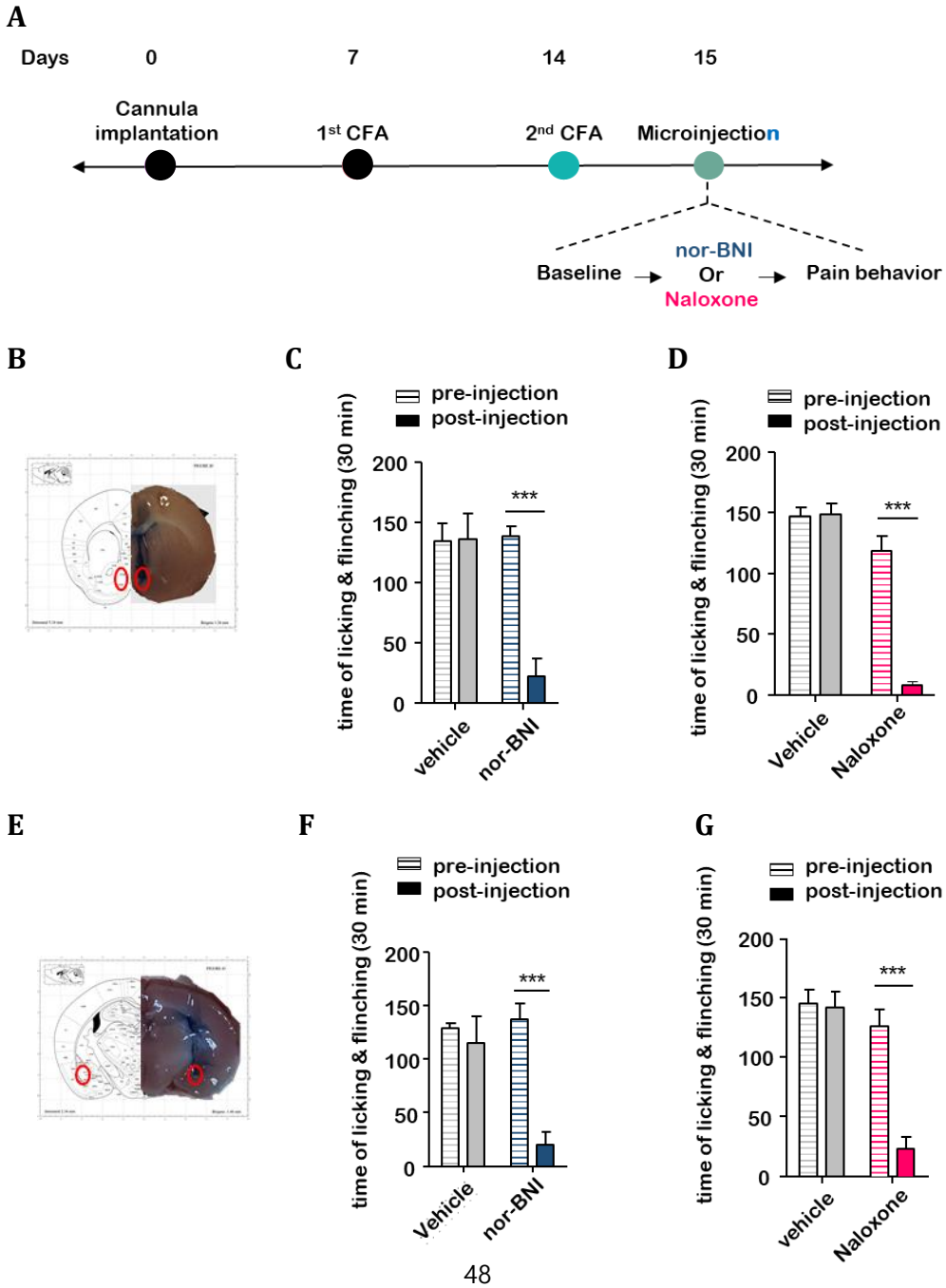

A
Days
0
7
14
15
Cannula implantation
1st CFA
2nd CFA
Microinjection
Baseline
nor-BNI
Or
Naloxone
Pain behavior
B
C
pre-injection
post-injection
time of licking & flinching (30 min)
200
150
100
50
0
***
vehicle
nor-BNI
D
pre-injection
post-injection
time of licking & flinching (30 min)
200
150
100
50
0
***
Vehicle
Naloxone
E
F
pre-injection
post-injection
time of licking & flinching (30 min)
200
150
100
50
0
***
Vehicle
nor-BNI
G
pre-injection
post-injection
time of licking & flinching (30 min)
200
150
100
50
0
***
vehicle
Naloxone

Figure 9. The effect of microinjection of nor-BNI and naloxone into NAc and CeA on spontaneous chronic inflammatory pain behavior. (A) Schematic representation of CFA- induced spontaneous pain behavior study in naloxone and nor-BNI microinjected mice. (B) Location of microinjection site in NAc with atlas from Paxinos, George, and Keith B.J. Franklin. (C) Changes in time spent licking behavior in CFA-induced inflammatory pain model following microinjection of nor-BNI (2.5 μg) into the NAc. (D) Changes in time spent licking behavior in CFA-induced inflammatory pain model following microinjection of naloxone (0.5 μg) into the NAc. (E) Location of microinjection site in CeA with atlas from Paxinos, George, and Keith B.J. Franklin. (F) Changes in time spent licking behavior of CFA-induced inflammatory pain model following microinjection of nor-BNI (2.5 μg) into the CeA (G) Changes in time spent licking behavior of CFA-induced inflammatory pain model following microinjection of naloxone (0.5 μg) into the CeA. Data are presented as mean ± SEM.

Chapter 2

Anterior insular-nucleus accumbens pathway controls refeeding-induced analgesia under chronic inflammatory pain condition

Abstract

Feeding behaviors are closely associated with chronic pain in adult rodents. Our recent study revealed that 2 hours of refeeding after 24 hours fasting (i.e. refeeding) attenuates pain behavior under chronic inflammatory pain conditions. However, while brain circuits mediating fasting-induced analgesia have been identified, the underlying mechanism of refeeding-induced analgesia is still elusive. Herein, we demonstrate that the neural activities in the nucleus accumbens shell (NAcS) and anterior insular cortex (aIC) were increased in a modified complete Freund's adjuvant (CFA)-induced chronic inflammatory pain condition, which was reversed by refeeding. We also found that refeeding reduced the enhanced excitability of aIC–NAcSD2R projecting neurons in this CFA model. Besides, chemogenetic inhibition of aIC–NAcSD2R neural circuit suppressed chronic pain behavior while activation of this circuit reversed refeeding-induced analgesia. The present study suggests that aIC-NAcS neural circuit mediates refeeding-induced analgesia, thereby serving as a potential therapeutic target to manage chronic pain.

Introduction

Since chronic pain has emotional and cognitive features, there is a limit to control pathological pain through pharmacological treatment. Recently, numerous evidence reported close relation between chronic pain and lifestyle. Lifestyle factors such as physical inactivity, sedentary behavior, stress, poor sleep, unhealthy diet, and smoking are associated with chronic pain. Amon them, it has become increasingly clear that feeding behavior affects pain perception. Interestingly, it has been identified that both fasting and food consumption after fasting (i.e. refeeding) suppress pain behavior. A recent study reported that hunger-sensitive Agouti-related protein (AgRP) neurons projecting to the parabrachial nucleus (PBN) mediates fasting-induced analgesia in inflammatory pain. Despite of accumulating evidence supporting for the relationship between fasting and pain reduction, the underlying mechanisms of how refeeding ameliorate pain is still elusive. Since refeeding-induced analgesia includes a variety of factors such as calorie recovery, satiety and eating behavior, the brain circuit mediating refeeding-induced analgesia could be different from that of fasting-induced analgesia. Given refeeding fulfills physical needs from hunger and provide satisfaction, the brain circuits mediating reward and satisfaction might be implicated in the refeeding-induced analgesia.

The nucleus accumbens (NAc) is a crucial component of the brain reward system. Recent human and animal imaging observations indicate that the dopaminergic system in the NAc is an important neural substrate of feeding behavior and pain perception. Indeed, food

consumption following 24 hours fasting elevates dopamine release within the NAc for more than two hours but dopamine antagonists cause serious feeding deficit. Also, systemic administration of analgesics enhance dopamine release in the NAc and dopaminergic agonists inhibits nociception. Moreover, several investigations with pharmacological manipulations revealed that dopamine type 2 receptor (D2R) in the NAc plays a critical role in the modulation of pain perception.

The insular cortex (IC) is also highly implicated in feeding behavior and pain perception. The IC has two distinct pain networks, which are the posterior sensory circuit and the anterior emotional network. Tracing studies in rodents have shown that the aIC has abundant connectivity with the limbic system where is known to process affective components of pain. The NAc, a part of the limbic system, receives glutamatergic input from the aIC . Functional neuroimaging studies in human also show a correlated activity between the aIC and NAc with noxious stimuli. Furthermore, change in functional connectivity between the aIC and NAc has been also demonstrated along with pain chronification. Given these observations, we hypothesized that the aIC-NAc circuit may underlie refeeding-induced analgesia in the chronic inflammatory pain condition.

In this study, we thus examined whether the aIC-NAc circuit is essential for the refeeding-induced analgesia under chronic inflammatory pain condition by employing pharmacological and tract-specific manipulations in a modified complete Freund's adjuvant (CFA)-induced chronic inflammatory pain model.

Experimental animals and pain model

Male C57BL/6 mice and Fos-tTA-eGFP transgenic mice (Jackson Laboratory, stock #: 018306) weighing 20-28 g were used for the experiment. The mice were housed 4-6 per cage at a temperature-controlled room (23 ± 1 ℃, 12 h/12 h light/dark cycle) and maintained with pellet diet and tap water *ad libitum* except fasting periods. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC, SNU-191001-9-1) at Seoul National University. The experiments were also designed and performed in accordance with the International Association for the Study of Pain (IASP) guidelines. All efforts were made to reduce animal suffering and decrease the number of animals used. For 24 hr fasting, food was removed from the cage but free access to water. For refeeding, mice were given normal chow for 2 hours following 24 hours fasting. We used a modified CFA-induced chronic inflammatory pain model to prolong pain behaviors more than 20 days, as previously described. 20 μL of undiluted CFA (Sigma-Aldrich) was injected subcutaneously into the plantar surface of the left hind with a 0.3 mL insulin syringe. A booster injection was given 7 days after the first injection. For electrophysiological recordings, the mice were injected with same amount of CFA into both hind paws. All experiments were performed by researcher blinded to treatment condition.

Pain behavior tests

The behavior tests were conducted as described previously. For spontaneous pain measurement, mice were acclimated in cage at least for

a week and then adapted in an acrylic observation chamber (size ranges 12×12×12 cm) before the experiment at least three times for three hours. A mirror was located at 45° angle below the chamber to observe the paws. Mice were video recorded using a video camera for a period of 30 min. The time spent licking or biting was measured by an observer who was blinded to the treatment.

To assess mechanical allodynia, 50% paw withdrawal threshold was measured using von Frey filaments (North Coast Medical). Mice were acclimated in cage at least for a week and then adapted in an acrylic cylinder (6.5 cm diameter, 17 cm height) on the metal mesh floor before the experiment at least three times. All animals left to an acrylic cylinder on the metal mesh floor for 30 min prior to mechanical test. The 50% paw withdrawal threshold was determined based on the up-down method with an ascending series of von Frey filaments (0.16 g, 0.4 g, 0.6 g, 1 g, and 2 g).

Administration of drugs

Sulpiride and SR 141716 were purchased from Tocris. For systemic administration, sulpiride 100 mg/kg was diluted in 10% DMSO (Sigma-Aldrich), and 2 μL of acetic acid glaciel (Duksan) was added, then pH was adjusted with NaOH (pH 7.4). For direct brain infusion, sulpiride (5 μg/uL) was dissolved in 0.9% normal saline and unilaterally microinjected (0.5 μL over 5 min) into the contralateral NAcS. Injectors were left in place for an additional 2 min before removal. SR 141716 (10 mg/kg) was diluted in 0.9% normal saline with 10% DMSO and 1% tween

80.

Clozapine-N-oxide (CNO) was purchased from Tocris. For systemic injection, CNO 10 mg/kg was dissolved in 0.9% normal saline and *intraperitoneally* injected 30 min prior to pain behavior test. For microinjection, CNO (3 μM) was dissolved in the 0.9% saline and administrated into the NAcS 5 min prior to the pain behavior test. For electrophysiology, CNO (3 μM) was dissolved in the bath solution.

Stereotaxic surgeries

C57BL/6 mice were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with guide cannula (26-gauge, 4.6 mm of length, Plastics One) aimed at the NAcS (coordinates: anteroposterior (AP) 1.4 from bregma, mediolateral (ML) 0.5 from midline, dorsoventral (DV) −4.6 from skull surface). When inserted, microinjector tips extended 0.5 mm beyond the guide.

The following vectors were used for single-site Designer receptors exclusively activated by designer drugs (DREADD) and cre-EGFP expression: pAAV-hSyn-DIO-hM4D(Gi)-mCherry, pAAV-CaMKIIa-hM3D(Gq)-mCherry, pENN-AAV-CaMKIIa-HI.GFP-Cre-WPRE-SV40 (Addgene). Viral vectors were injected at the rate of 0.2 μL/min, with a 10-min diffusion time. The injection volume for the single-site injections was 0.5 μL. Misplaced cannula or virus injection were excluded from analysis. DREADD expression was allowed to accumulate for 2 weeks before systemic/local vehicle and CNO injections.

Immunohistochemistry

Following administration of pentobarbital (50 mg/kg), the animals were transcardially perfused, first with PBS and then with a 4% paraformaldehyde solution. Brains were post fixed at 4°C in paraformaldehyde for 1 d following the extraction and cryoprotected in 30% sucrose for 3 days. The brains were then sectioned into 40 μm slices and preserved in PBS. For the DAB staining, free-floating sections were rinsed in PBS, incubated for 30 min in 0.3% hydrogen peroxide PBS solution to quench endogenous peroxidase activity, rinsed several times in PBS, and incubated in a blocking solution that contained 5% normal goat serum diluted in 0.3% Triton X-100 in PBS for 60 min. The sections were incubated in rabbit anti-c-Fos polyclonal antibody (1:1000 dilution, ab11137 Abcam) diluted in blocking serum for 48 hr at 4°C. After incubation in the primary antibody, the sections were rinsed three times for 10 min in PBS and incubated for 2 hr in biotinylated secondary antiserum made from goat anti-rabbit antibody (1:200 dilution; Vector laboratories) in PBS. Then the sections were processed with ABC kit (PK-6100, Vectastain ABC kit, Vector laboratories) for an hour and visualized with DAB kit (DAB substrate kit for peroxidase, Vector laboratories). After several rinses in PBS, the sections were mounted on coated glass slides, air dried, dehydrated through a series of graded ethanols, and clearing agents, then permounted (Sigma-Aldrich).

To detect D1R, D2R and CaMKII expression, sections were incubated overnight in blocking buffer (0.3% Triton X-100, 2% bovine serum in PBS) at room temperature. The primary antibodies rabbit anti-D1R (1:200 dilution, nb100-1618, Novus biologicals), mouse anti-D2R

(1:50 dilution, b-10, Santa Cruz) and mouse anti-CaMKII (1:100 dilution, sc-5306, Santa Cruz) were diluted in the blocking solution, and the sections were incubated for 48 hr at 4°C. The slices were washed three times for 10 min each in 0.1 M PBS. To visualize D2R expression, sections were incubated for 2 hr in biotinylated anti-mouse IgG (Vector, PB-9200). Then washed with 0.1 M PBS three times for 10 min each. The secondary antibody, FITC donkey anti-guinea pig (1:200 dilution, 706-095-148, Jackson ImmunoResearch Laboratories), Alexa 488 donkey anti-mouse (1:200 dilution, 715-545-150 Jackson ImmunoResearch Laboratories), Streptavidin (1:200 dilution, s11223, Invitrogen) and DAPI (1:5000 dilution, D9542, Sigma Aldrich) were diluted in the secondary antibody buffer (0.1% Triton X-100, 2% BSA) and incubated 2 hr at room temperature. The sections were then washed three times with 1×PBS for 10 min each and mounted using Vectashield Mounting media (Vector Laboratories).

Image analysis

Quantification of c-Fos was performed on slices from bregma +2 to +1.6 mm (4-6 sections per mouse, n=3 each group) for aIC and bregma +1.4 to +1.0 mm (4-6 sections per mouse, n=3 each group) for NAc. Mounted slices were examined under the bright-field microscope (DM5000B, Leica) and images were all taken at 10X magnification at one time to maintain identical lighting intensity and color balance. Averaged number of c-Fos positive neurons was determined using inverted color images in ImageJ software (National Institutes of Health).

All immunofluorescent-stained sections were imaged on a confocal microscope (LSM 700, Carl Zeiss). We collected 4 sections per mouse (n=3 mice each group) and images were all taken at 200X magnification. Image analysis was performed manually by identifying and counting D1R+, D2R+, CaMKII+ in same area.

Electrophysiology

Mice were sacrificed by cervical dislocation and decapitated. The brain was immediately transferred to ice-cold sucrose cutting solution (189 mM sucrose, 10 mM D-glucose, 26 mM $NaHCO_3$, 3 mM KCl, 10 mM $MgSO_4.7H_2O$, 1.25 mM NaH_2PO_4, and 0.1 mM $CaCl_2$), which was bubbled continuously with 95% O_2 and 5% CO_2. The brain was sagittal dissected in a Petri dish containing an ice-cold cutting solution, the brain was glued onto a brain holder which was placed in a buffer tray containing ice-cold cutting solution. Subsequently, 250-μm sagittal sections were obtained using a vibrating microtome (Leica, VT-1200). The sections (slices containing anterior commissure) were then transferred to a chamber on a nylon mesh containing external solution (in mM: 124 NaCl, 3 KCl, 1 $MgSO_4.7H_2O$, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, , and 2 $CaCl_2$, pH 7.4, osmolality 320-330 mOsm/kg H_2O) bubbled with 95% O_2 and 5% CO_2 at 33°C. It was incubated for 1 hr. The slices could be maintained in a healthy state for up to 8 h and were transferred to the recording chamber as required.

The slices were continuously perfused with external solution (the same as above) at a rate of 1.0–2.0 ml/min. The recordings were made at room temperature. The neurons were visualized using infra-red

differential interference contrast (IR-DIC) microscopy with a 40x water immersion objective and video imaging camera (BX51WI, Olympus). Electrophysiological recordings were obtained with a Multiclamp 700B amplifier, Digidata 1330A converter and pClamp 10.3 software (Molecular Devices), sampled at 20 kHz, and filtered at 2 kHz.

Patch pipettes were pulled from borosilicate glass capillaries (OD 1.5 mm, I.D. 0.86 mm; Harvard Apparatus, Edenbridge, United Kingdom) on a horizontal puller (P-97, Sutter Instruments). The pipette tip resistance was 6–8 MΩ. The pipette offset potential was adjusted with the amplifier. The pipettes were filled with an internal solution containing the following components: 105 mM potassium gluconate, 20 mM KCl, 10 mM HEPES-Na^+, and 0.1 mM EGTA (pH 7.25 adjusted with KOH, osmolality 280 mOsm/kg H_2O). Recordings usually began >5 min after obtaining access to the cell. Only one neuron in each slice was exposed to a tested compound a single time, and the slice was replaced after one test on a single cell was performed. The recordings were analyzed with Clampfit 10.7 (Molecular Devices).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, USA). Comparison between two groups was made using the unpaired Student's t-test. For multiple comparisons, data were analyzed using two-way ANOVA followed by the post hoc Bonferroni test. Detailed statistics for each experiment were shown in the figure legend. Data are presented as mean ± SEM. Differences with $p < 0.05$ were considered significant.

Results

Systemic D2R antagonist administration reverses refeeding-induced analgesia in chronic inflammatory pain model

Brain dopamine, endocannabinoid and opioid signaling systems play key roles in pain and feeding behavior. We first examined involvement of these neurotransmitters in refeeding-induced analgesia under chronic inflammatory pain condition via pharmacological approach with sulpiride (D2R antagonist, 100 mg/kg) and SR 141716 (cannabinoid receptor antagonist 10 mg/kg). The doses were determined based on the previous studies and drugs were intraperitoneally injected after 2 hours of refeeding. The chronic inflammatory pain was induced by injecting CFA twice (D0 initial injection and D7 booster injection) into the hind paw and behavior tests were conducted at 16 days after the first injection (**Fig. 10A**). The von Frey test and spontaneous pain behavior were measured 30 min after drug injection. Result showed that sulpiride reversed refeeding-induced analgesia in both von Frey test and spontaneous pain behavior test (**Figs. 10B, 10C**). By contrast, SR141716 did not reverse refeeding-induced analgesia (**Fig. 10D**). We did not examine involvement of opioid system in this work, because we previously observed that naloxone, non-specific opioid antagonist, induced paradoxical analgesic effect in the free-fed group in chronic inflammatory pain condition. Taken together, our result demonstrated that dopamine signaling is involved in the refeeding-induced analgesia under chronic inflammatory pain condition.

To determine the sex difference, we repeated behavior tests in the female mice. Similar to the male group, refeeding-induced analgesia was reversed by sulpiride in both von Frey test (**Fig. 10E**) and spontaneous pain behavior test (**Fig. 10F**).

Refeeding reverses D2R upregulation of the NAcS neurons under chronic inflammatory pain condition

Based on the involvement of dopamine system in refeeding-induced analgesia (**Fig. 10**), we next examined brain area involved in the refeeding-induced analgesia. Since the dopamine release in the NAc is essential for pain and feeding behavior, we monitored immunoreactivity (IR) of c-Fos, a marker for neuronal activation, in the two subregions of the NAc, which are core (NAcC) and shell (NAcS) (**Fig. 11A**). Interestingly, the level of c-Fos-IR positive neurons was increased in the NAcS under CFA-induced free fed group compared to the naïve group, which was significantly decreased by 2 hours of refeeding (**Fig. 11B**). The level of c-Fos expression returned to the similar level of the CFA-induced free fed group at 24 hours after refeeding (**Fig. 11B**). In contrast, there was no difference in the level of c-Fos expression within the NAcC between naïve and CFA groups. Also, feeding status did not affect the level of c-Fos expression in the NAcC of CFA group (**Fig. 11B**). Taken together, these results demonstrated the involvement of the NAcS in chronic inflammatory pain behavior.

Given that the NAcS contains dopamine receptor type 1 (D1R) and dopamine receptor type 2 (D2R) expressing neurons, we next determined

c-Fos expressing neurons in the NAcS using Fos-tTA-eGFP transgenic mice. As shown in **Figure 11C**, we found that the D2R-expressing neurons are highly co-labeled with c-Fos rather than D1R-expressing neurons. Also, when we compared the number of D2R IR neurons in the CFA-induced free fed and refed group, D2R IR was significantly decreased in the CFA-induced refed group compared to CFA-induced free fed group (**Figs. 11D, 11E**). Together, our results indicate that chronic inflammatory pain led to activation of the $NAcS^{D2R}$ neurons which was reversed by 2 hr refeeding.

Sulpiride microinjection into the NAcS reverses refeeding-induced analgesia in chronic inflammatory pain model

To verify involvement of $NAcS^{D2R}$ in refeeding-induced analgesia, D2R antagonist (sulpiride, 5 μg/uL) was locally infused into the NAcS after 2 hr refeeding (**Figs. 12A, 12B**). The dose was determined based on a previous study . Sulpiride infusion reversed refeeding-induced analgesia in both von Frey test (**Fig. 12C**) and spontaneous pain behavior test (**Fig. 12D**). Taken together, these results imply a critical role of $NAcS^{D2R}$ in refeeding-induced analgesia.

The aIC^{CaMKII} neurons projecting to $NAcS^{D2R}$ neurons are activated with chronic inflammatory pain

The NAcS receives projection from the anterior insular cortex (aIC) which also mediates pain perception and feeding behavior. We hence determined whether the aIC is involved in refeeding-induced analgesia. We repeated c-Fos analysis in the aIC (**Fig. 13A**) and found that changes in c-Fos expression in the aIC is comparable to that of NAcS. Similarly, the

level of c-Fos expression within the aIC, was enhanced in the CFA group compared to the naïve group, which was decreased by 2 hr refeeding (**Figs. 13A, 13B**). The level of c-Fos expression returned to the similar level of the CFA-induced free fed group at 24 hr after refeeding. Overall, these results suggest that the aIC also contribute to refeeding-induced analgesia.

It has been shown that the NAcS receives glutamatergic input from the aIC. Thus, we confirmed expression of CaMKII, a marker for excitatory glutamatergic neurons, in the c-Fos positive neurons within the aIC. As shown in the **Figure 13C**, c-Fos positive neurons were co-labeled with CaMKII. Next, we identified target neurons of the aIC^{CaMKII} projection within the NAcS by examining D1R and D2R expression. Results showed that D2R expressing neurons were co-labeled with mCherry-positive terminals fiber rather than D1R expressing neurons (**Figs. 13E-G**). These results demonstrate a monosynaptic projection from aIC^{CaMKII} neurons to $NAcS^{D2R}$ neurons.

Refeeding suppresses excitability of aIC^{CaMKII}-innervating $NAcS^{D2R}$ neurons under chronic inflammatory pain condition

Next, we confirmed the effect of refeeding on the functional activity of aIC^{CaMKII}-$NAcS^{D2R}$ circuit under chronic pain condition by whole-cell recordings in the brain slice. As shown in the **Figures 14A and 14B**, we injected pAAV-CaMKIIa-hM3D(Gq)-mCherry into the aIC and recorded neural spikes in the mCherry expressing $NAcS^{D2R}$ neurons. In consistent with previous study, spike numbers were increased in the CFA-free fed group (**Figs. 14C, 14D**). However, spike numbers significantly

decreased in the CFA-induced refed group compared to the CFA-induced free fed group (**Figs. 14C, 14D**). Also, application of CNO (3 μM) into the bath solution significantly enhanced firings (**Figs. 14C, 14D**). At the end of each recording, sulpiride (5 μM) was applied to confirm that the neurons recorded express D2R. In consistent with previous study, sulpiride application increased excitability of D2R expressing neurons in the CFA group (**Figs. 14C, 14D**).

Inhibition of aIC^{CaMKII} terminals in NAcS decreases chronic inflammatory pain behavior

Next, we sought to understand the role of aIC^{CaMKII}-NAcS circuit in chronic inflammatory pain behavior. To answer this question, we chemogenetically inhibited aIC^{CaMKII}-NAcS circuit in the CFA group. The aIC was transduced with pENN-AAV-CaMKIIa-HI.GFP-Cre-WPRE-SV40 and retrograde pAAV-hSyn-DIO-hM4D(Gi)-mCherry was injected into the NAcS (**Figs. 15A, 15B**). Expression of GFP and mCherry were confirmed in the aIC via fluorescent imaging (**Figs. 15C, 15D**) and chemogenetic inactivation by CNO was confirmed by level of c-Fos expression after systemic CNO injection (**Fig. 15E**). CNO injection alleviated both mechanical allodynia and spontaneous pain behavior (**Figs. 15F, 15G**). Together, these results imply that activation of aIC^{CaMKII}-NAcS^{D2R} circuit is necessary for chronic inflammatory pain behavior.

Activation of aIC^{CaMKII} terminals in NAcS reverses refeeding-induced analgesia in chronic inflammatory pain model

Lastly, we examined whether aIC^{CaMKII}-NAcS circuit mediates

refeeding-induced analgesia. In this time, we chemogenetically activated aIC^{CaMKII}-NAcS circuit following 2 hr refeeding in the CFA group. Mice were transduced with the hM3Dq receptor via injection of pAAV-CaMKIIa-hM3D(Gq)-mCherry and cannula was placed in the NAcS so that CNO could be directly administered to aIC terminals (**Figs. 16A, 16B**). Virus expression was confirmed via fluorescent imaging in the aIC (**Fig. 16C**) and chemogenetic activation by CNO was confirmed by level of c-Fos expression after CNO microinjection (**Fig. 16D**). CNO infusion reversed refeeding-induced analgesia in both von Frey test (**Fig. 16E**) and spontaneous pain behavior test (**Fig. 16F**). These results indicate that activation of aIC^{CaMKII}- NAcS^{D2R} circuit reverses refeeding-induced analgesia.

Figure 10

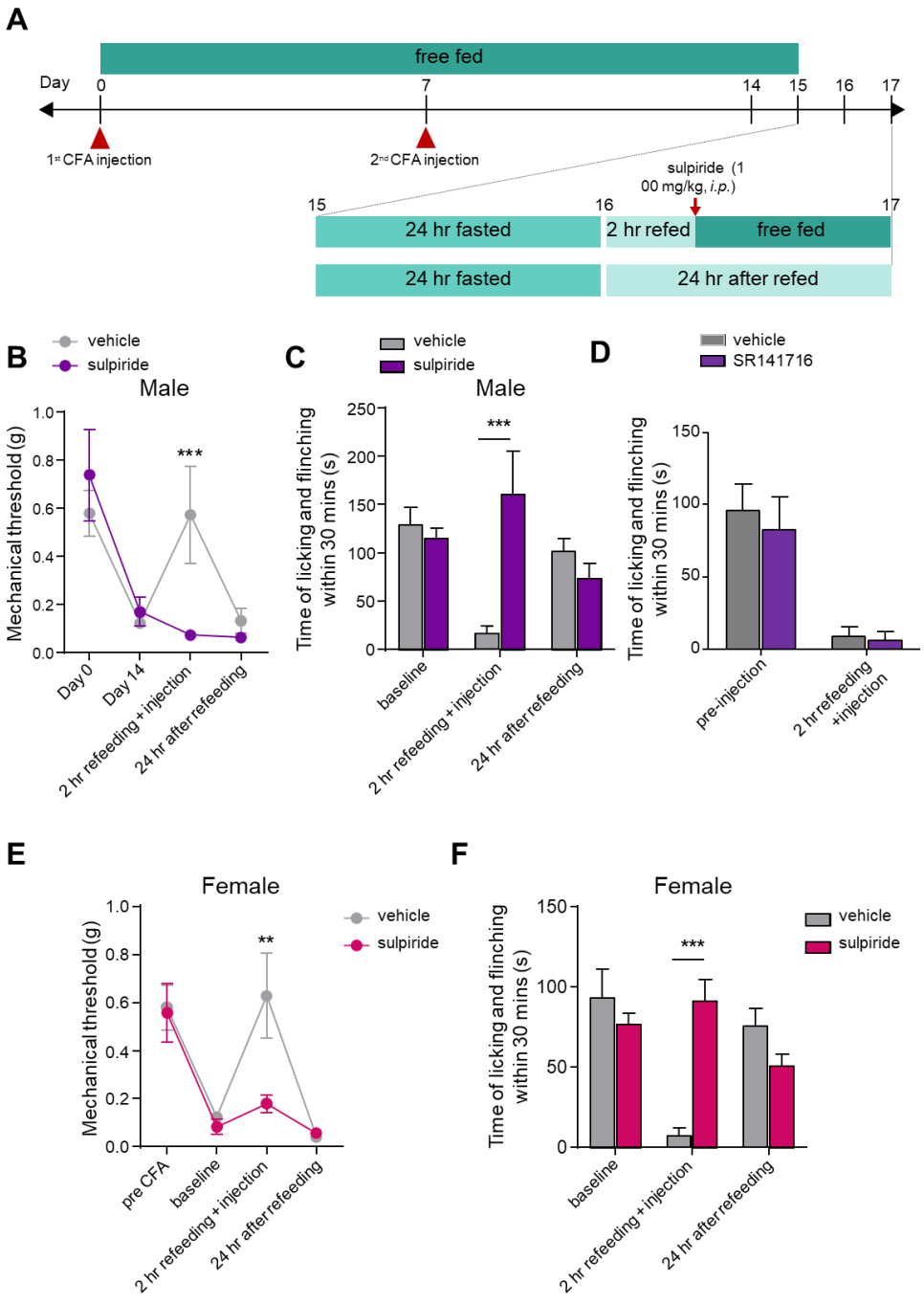

A
free fed
Day
0
7
14
15
16
17
1st CFA injection
2nd CFA injection
sulpiride (100 mg/kg, i.p.)
15
16
17
24 hr fasted
2 hr refed
free fed
24 hr fasted
24 hr after refed
B
vehicle
sulpiride
Male
Mechanical threshold (g)
***
Day 0
Day 14
2 hr refeeding + injection
24 hr after refeeding
C
vehicle
sulpiride
Male
Time of licking and flinching within 30 mins (s)
***
baseline
2 hr refeeding + injection
24 hr after refeeding
D
vehicle
SR141716
Time of licking and flinching within 30 mins (s)
pre-injection
2 hr refeeding +injection
E
Female
vehicle
sulpiride
Mechanical threshold (g)
**
pre CFA
baseline
2 hr refeeding + injection
24 hr after refeeding
F
Female
vehicle
sulpiride
Time of licking and flinching within 30 mins (s)
***
baseline
2 hr refeeding + injection
24 hr after refeeding

Figure 10. The effect of D2R antagonist on refeeding-induced analgesia in chronic inflammatory pain model

(A) Experimental design and schedule. (B) Effect of D2R antagonist (sulpiride, 100 mg/kg) on mechanical allodynia after 2 hr refeeding in the CFA-induced male group. (C) Effect of D2R antagonist (sulpiride, 100 mg/kg, i.p.) on spontaneous pain behavior after 2 hr refeeding in the CFA-induced male group (n=9 and 7 for sulpiride and vehicle, respectively). (D) Effect of D2R antagonist (sulpiride 100 mg/kg) on mechanical allodynia after 2 hr refeeding in the CFA-induced female group (n=9 and 7 for sulpiride and vehicle, respectively). (E) Effect of D2R antagonist (sulpiride, 100 mg/kg, i.p.) on spontaneous pain behavior after 2 hr refeeding in the CFA-induced female group. $^{**}p < 0.01$, $^{***}p < 0.001$ (two-way ANOVA followed by Bonferroni's post hoc test)

Figure 11

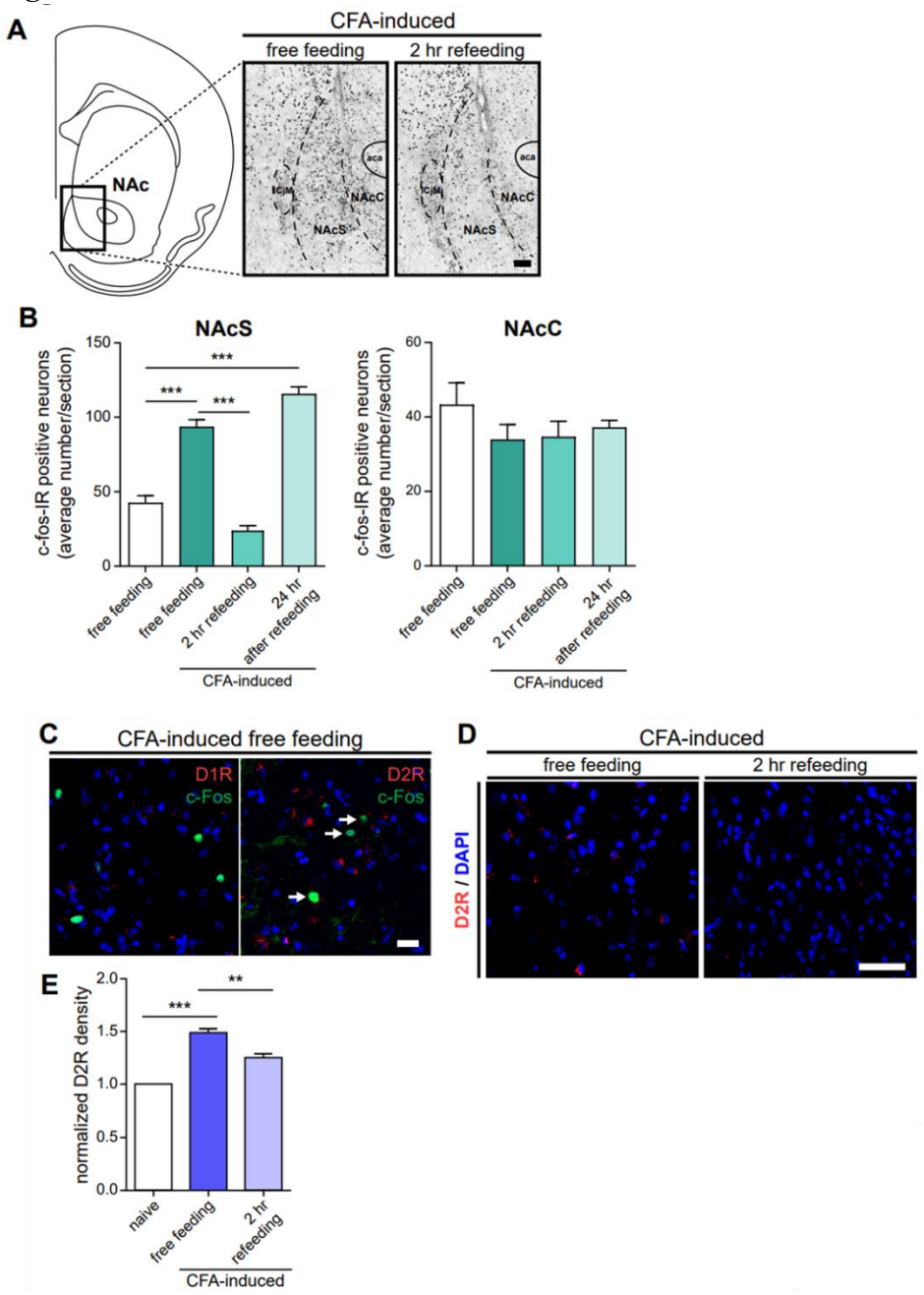
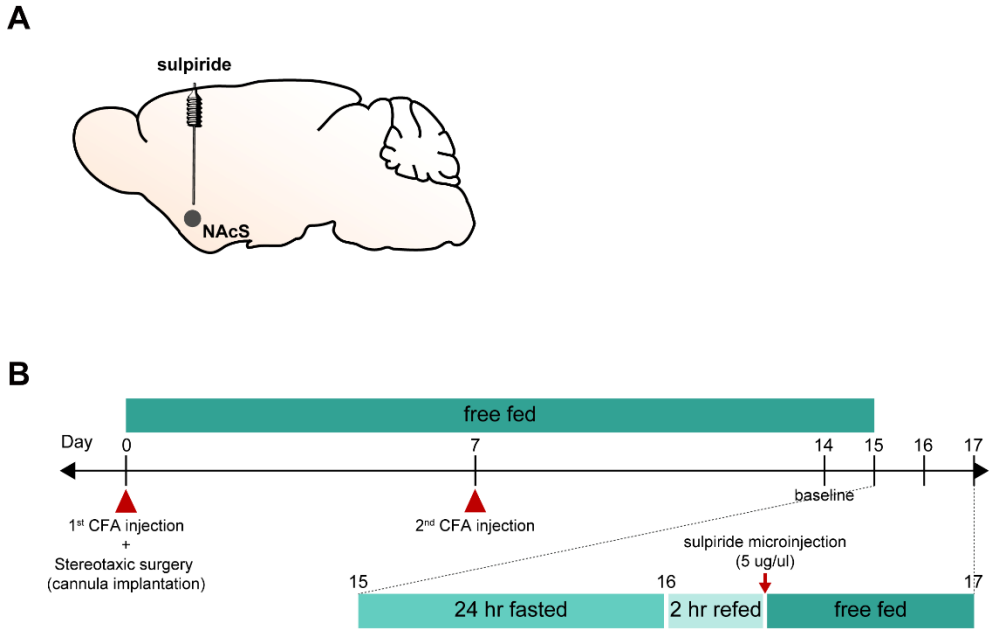

A
CFA-induced
free feeding
2 hr refeeding
NAc
aca
lcjM
NAcC
NAcS
B
NAcS
NAcC
c-fos-IR positive neurons (average number/section)
150
100
50
0
60
40
20
***
free feeding
2 hr refeeding
24 hr after refeeding
CFA-induced
C
CFA-induced free feeding
D1R
c-Fos
D2R
c-Fos
D
CFA-induced
free feeding
2 hr refeeding
D2R / DAPI
E
normalized D2R density
2.0
1.5
1.0
0.5
0.0
**
naive
free feeding
2 hr refeeding
CFA-induced

Figure 11. Involvement of NAcS in refeeding-induced analgesia under chronic inflammatory pain

(A) Regions of interest in coronal sections based on the mouse brain atlas by Paxinos, George, and Keith B.J. Franklin. Representative c-Fos photomicrographs observed in the NAcC and NAcS. Scale bar represents 100 μm; magnification 10X. (B) Bar graphs show mean c-Fos positive cell numbers. Data are presented as mean SEM. ***p < 0.0001 (C) Representative images showing c-Fos (green) neurons co-labeled with D1R (left, red) or D2R (right red) and DAPI (blue) in the NAcS. Scale bar represents 20 μm; magnification 200X (1.5). (D) Representative images showing expression of D2R (red) in free fed (left) and refed (right) chronic inflammatory pain group. Scale bar represents 50 μm; magnification 100X. Normalized density of D2R in the NAcS.

Figure 12


A
sulpiride
NAcS
B
free fed
Day
0
7
14
15
16
17
1st CFA injection
+
Stereotaxic surgery
(cannula implantation)
2nd CFA injection
baseline
sulpiride microinjection
(5 ug/ul)
15
16
17
24 hr fasted
2 hr refed
free fed

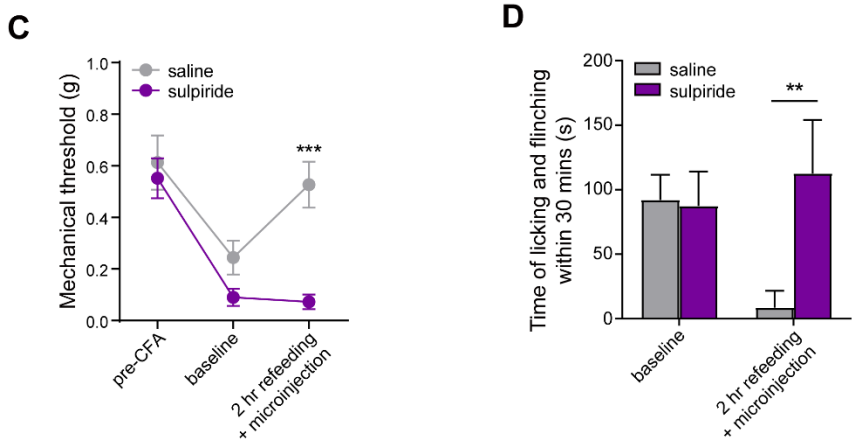

C
Mechanical threshold (g)
saline
sulpiride
***
pre-CFA
baseline
2 hr refeeding
+ microinjection
D
Time of licking and flinching
within 30 mins (s)
saline
sulpiride
**
baseline
2 hr refeeding
+ microinjection

Figure 12. Effect of sulpiride microinjection into the NAcS on refeeding-induced analgesia

A) Sagittal view of cannula implantation above the NAcS. (B) Schematic diagram of experiment schedule. (C) Mechanical threshold after sulpiride or vehicle infusion in the CFA-induced refed group (n=8 and 6 for sulpiride and vehicle, respectively). (D) Time of licking and flinching after sulpiride or vehicle infusion in the CFA-induced refed group (n=8 and 7 for sulpiride and vehicle, respectively). Two-way ANOVA followed by Bonferroni's post hoc test. Data are represented as mean ± SEM, $^{**}p < 0.01$, $^{****}p < 0.0001$.

Figure 13

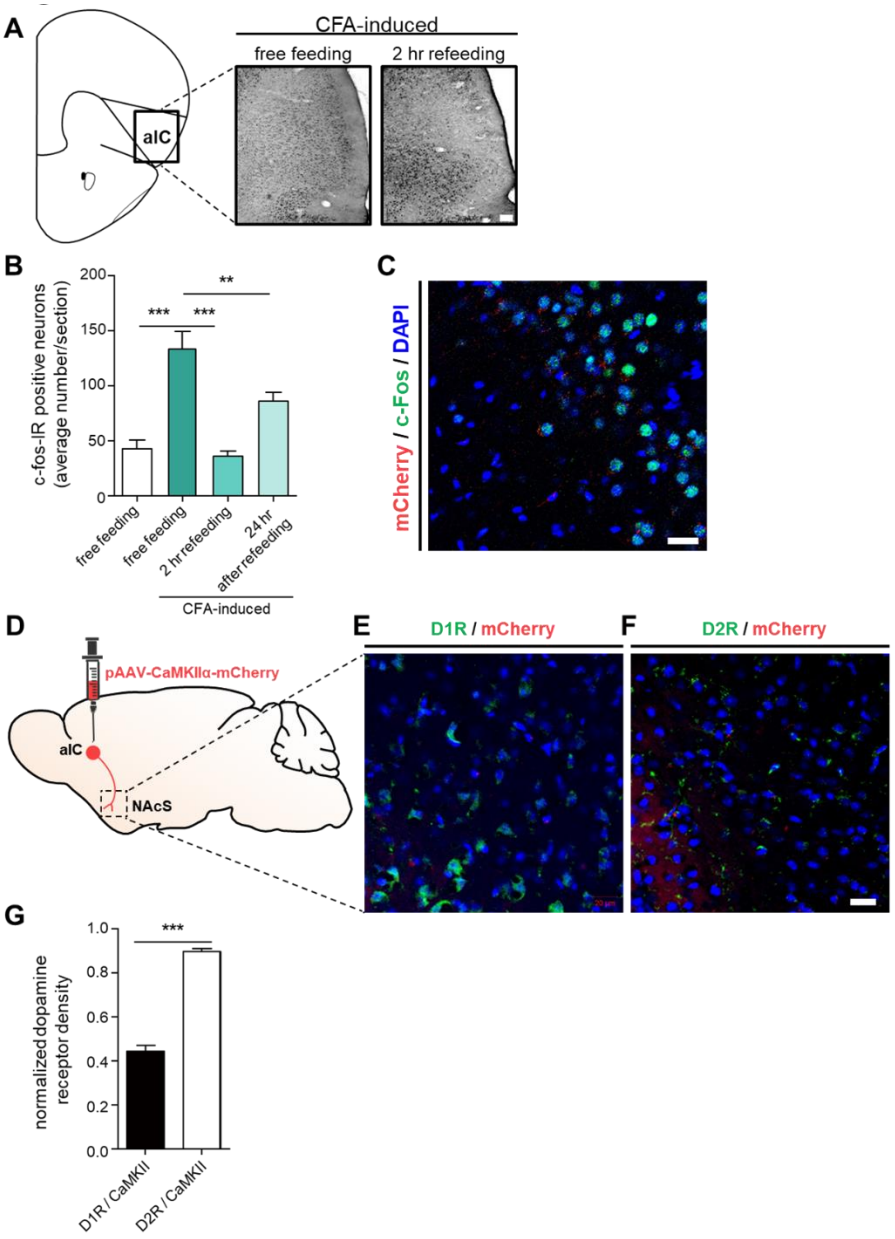

A
CFA-induced
free feeding
2 hr refeeding
aIC
B
c-fos-IR positive neurons
(average number/section)
200
150
100
50
0
**
***
***
free feeding
free feeding
2 hr refeeding
24 hr
after refeeding
CFA-induced
C
mCherry / c-Fos / DAPI
D
pAAV-CaMKIIα-mCherry
aIC
NAcS
E
D1R / mCherry
F
D2R / mCherry
G
normalized dopamine
receptor density
1.0
0.8
0.6
0.4
0.2
0.0
***
D1R / CaMKII
D2R / CaMKII

Figure 13. Involvement of aIC in refeeding-induced analgesia under chronic inflammatory pain

(A) Regions of interest in coronal sections based on the mouse brain atlas by Paxinos, George, and Keith B.J. Franklin. Representative c-Fos photomicrographs observed in the aIC. Scale bar represents 100 μm; magnification 10X. (B) Bar graphs show mean c-Fos positive cell numbers. (C) Representative images showing c-Fos (green) neurons co-labeled with CaMKII (red) in the aIC. Scale bar represents 20 μm; magnification 200X (1.5). (D) Sagittal view of virus injection into the aIC. (E) Representative images showing hM3D (red) neurons co-labeled with D1R (green) in the NAcS. (F) Representative images showing hM3D (red) neurons co-labeled with D2R (green) in the NAcS. Scale bar represents 20 μm; magnification 100X (1.5). (G) Normalized D1R or D2R expressing neurons co-labeled with CaMKII. Unpaired t-test. Data are present as mean SEM. **p<0.001, ***P < 0.0001.

Figure 14

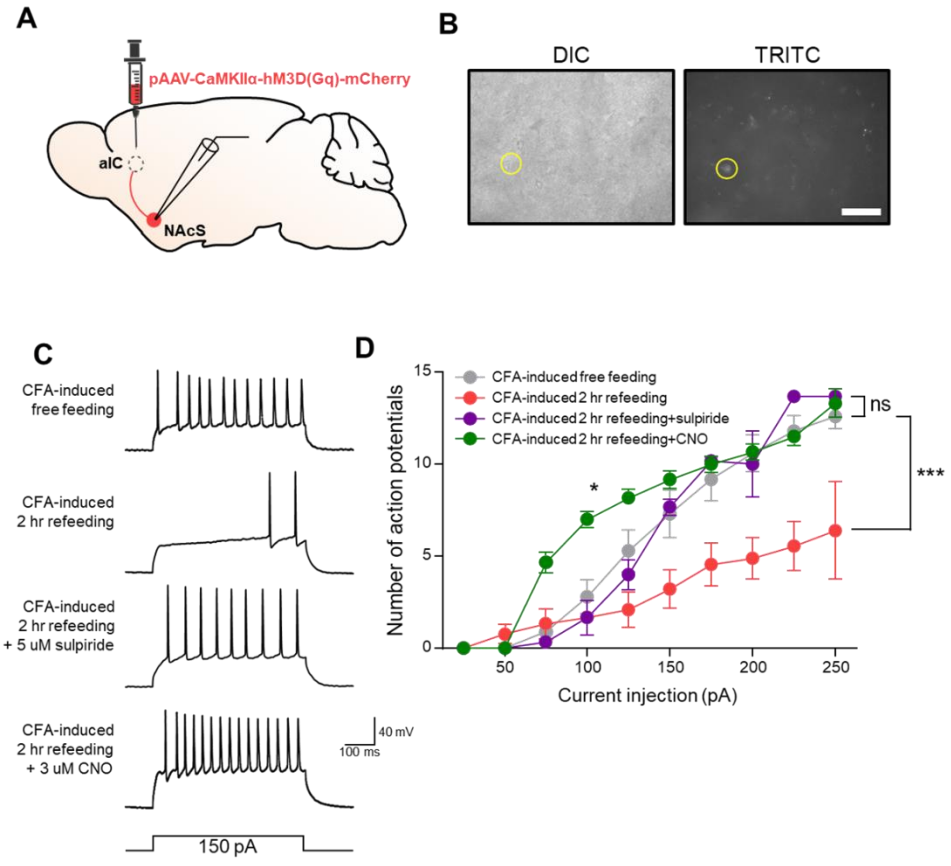

A
pAAV-CaMKIIα-hM3D(Gq)-mCherry
aIC
NAcS
B
DIC
TRITC
C
CFA-induced free feeding
CFA-induced 2 hr refeeding
CFA-induced 2 hr refeeding + 5 uM sulpiride
CFA-induced 2 hr refeeding + 3 uM CNO
40 mV
100 ms
150 pA
D
CFA-induced free feeding
CFA-induced 2 hr refeeding
CFA-induced 2 hr refeeding+sulpiride
CFA-induced 2 hr refeeding+CNO
Number of action potentials
Current injection (pA)
ns
***
*

Figure 14. Effect of refeeding on the excitability of D2R-expressing neurons in the NAcS in inflammatory chronic pain

(A) Sagittal view of virus injection into aIC and whole-cell patch recording in NAcS. (B) Fluorescent (left) and infrared (right) image of patch-clamp recording from hM3Dq neuron. Yellow circle indicates recorded neuron. Scale bar represents 10 μm; magnification 40X. (C) Example of current-clamp recording using 150-pA current step in chronic pain model. (D) Mean number of spikes evoked by current steps (from 0 to +250 pA in 25 pA increments) (n=8 and 7 for free fed and refed, respectively). Two-way ANOVA followed by Bonferroni's post hoc test. Data are represented as mean ± SEM, $^{**}p < 0.001$, $^{***}p < 0.0001$.

Figure 15

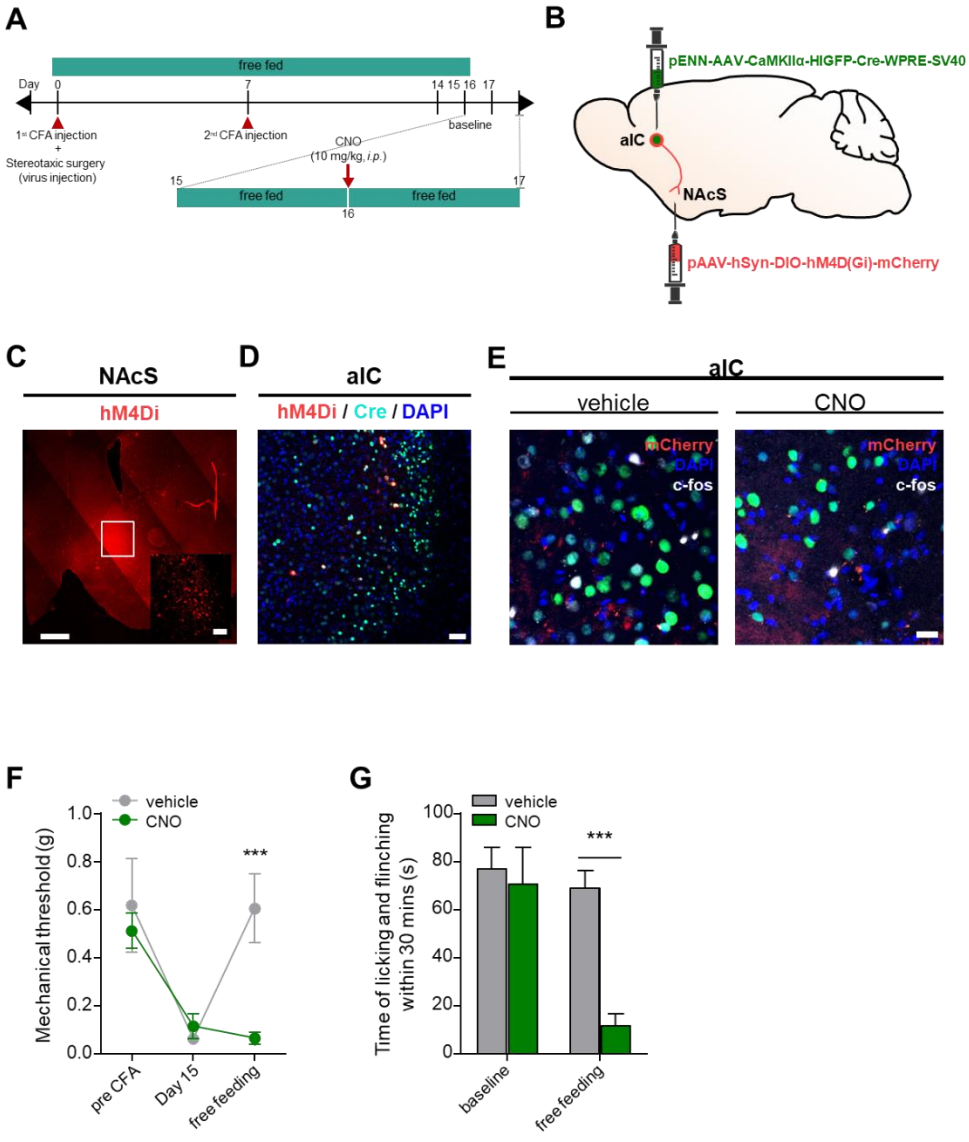

A
free fed
Day 0 7 14 15 16 17
1st CFA injection + Stereotaxic surgery (virus injection)
2nd CFA injection
CNO (10 mg/kg, i.p.)
baseline
15 16 17
free fed
free fed
B
pENN-AAV-CaMKIIα-HIGFP-Cre-WPRE-SV40
aIC
NAcS
pAAV-hSyn-DIO-hM4D(Gi)-mCherry
C
NAcS
hM4Di
D
aIC
hM4Di / Cre / DAPI
E
aIC
vehicle
CNO
mCherry
DAPI
c-fos
F
vehicle
CNO
Mechanical threshold (g)
1.0
0.8
0.6
0.4
0.2
0.0
***
pre CFA
Day 15
free feeding
G
vehicle
CNO
Time of licking and flinching within 30 mins (s)
100
80
60
40
20
0
***
baseline
free feeding

Figure 15. Effect of inhibition of aIC^{CaMKII}-NAcS circuit on chronic inflammatory pain behavior

(A) Schematic diagram of experiment schedule. (B) Sagittal view of virus injection into the aIC and the NAcS. (C) Representative fluorescence image illustrating expression of hM4Di in the NAcS. Scale bar represents 500 μm, 20 μm in the box; maginification 100X tiled scan and 400X. (D) Representative fluorescence image illustrating expression of hM4Di, Cre, and DAPI in the aIC. Scale bar represents 50 μm. (E) Representative images of c-Fos (grey), Cre (green) and hM3Di (red) expression in the aIC that were administered vehicle or CNO. Scale bar represents 10 μm; magnification 200X (1.5) (F) Change in mechanical threshold in the CFA group after vehicle or CNO injection (n=9 and 7 for CNO and vehicle, respectively). (G) Change in licking and flinching behavior of CFA group after vehicle or CNO injection (n=8 and 7 for sulpiride and vehicle, respectively). Two-way ANOVA followed by Bonferroni's post hoc test. Data are represented as mean ± SEM, $^{**}p < 0.01$, $^{****}p < 0.0001$.

Figure 16

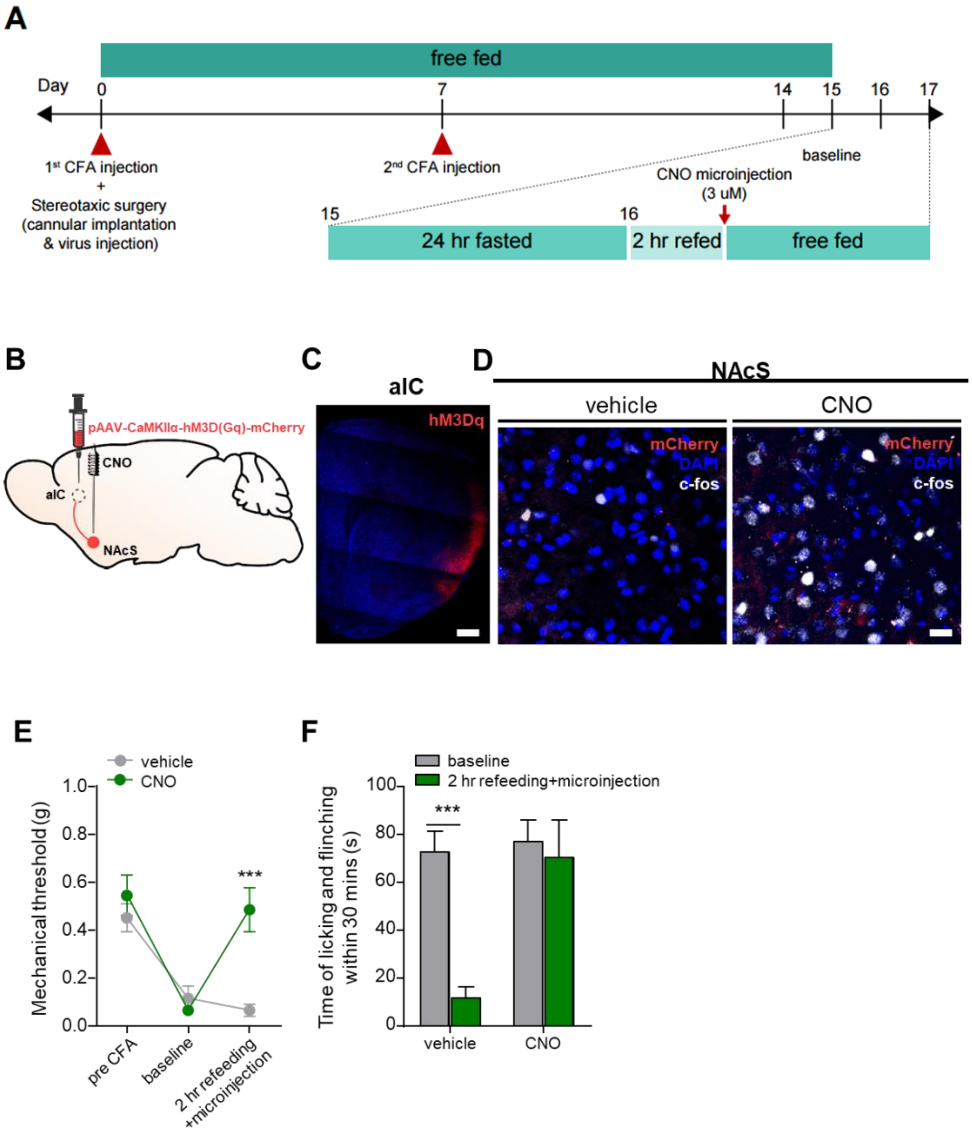

A
free fed
Day
0
7
14
15
16
17
1st CFA injection
+
Stereotaxic surgery
(cannular implantation
& virus injection)
2nd CFA injection
baseline
CNO microinjection
(3 uM)
15
16
24 hr fasted
2 hr refed
free fed
B
pAAV-CaMKIIα-hM3D(Gq)-mCherry
CNO
aIC
NAcS
C
aIC
hM3Dq
D
NAcS
vehicle
CNO
mCherry
DAPI
c-fos
E
vehicle
CNO
Mechanical threshold (g)
1.0
0.8
0.6
0.4
0.2
0.0
***
pre CFA
baseline
2 hr refeeding
+microinjection
F
baseline
2 hr refeeding+microinjection
Time of licking and flinching
within 30 mins (s)
100
80
60
40
20
0
***
vehicle
CNO

Figure 16. Effect of chemogenetic activation of aIC^{CaMKII}-NAcS circuit on refeeding- induced analgesia

(A) Schematic diagram of experiment schedule. (B) Sagittal view of virus injection into the aIC and cannula implantation above NAcS. (C) Representative fluorescence image illustrating expression of hM3Dq at the injection site. Scale bar represents 500 μm. (D) Representative images of c-Fos (grey) and hM3Dq (red) expression in the NAcS that were administered vehicle or CNO. Scale bar represents 20 μm; magnification 200X (1.5). (E) Change in mechanical threshold with CNO or vehicle infusion in the CFA group (n=9 and 6 for sulpiride and vehicle, respectively). (F) Change in time of licking and flinching with CNO or vehicle infusion in chronic inflammatory pain group (n=10 and 6 for sulpiride and vehicle, respectively). Two-way ANOVA followed by Bonferroni. Data are represented as mean ± SEM, $^{**}p < 0.01$, $^{****}p < 0.0001$.

Figure 17

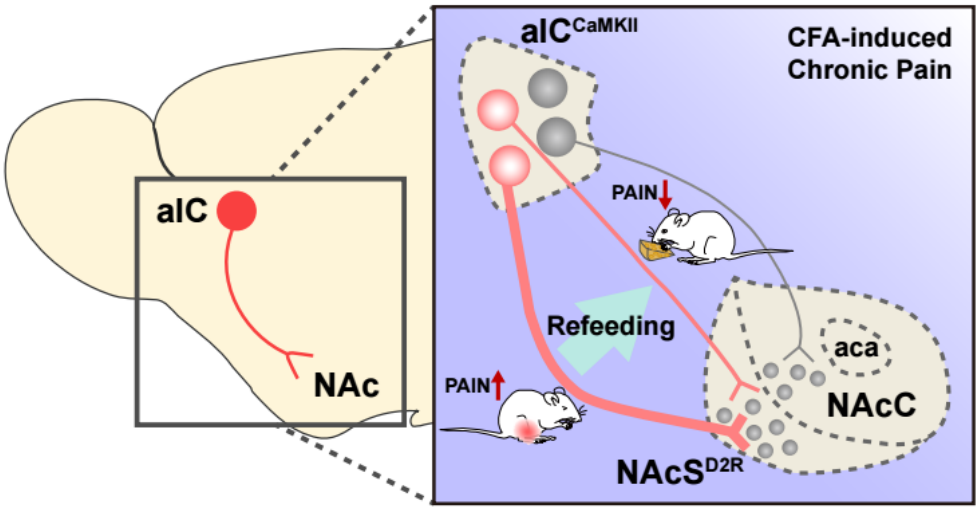

aIC
NAc
aIC CaMKII
CFA-induced
Chronic Pain
PAIN
Refeeding
aca
NAcC
PAIN
NAcS D2R

Figure 17. Schematic summary showing that activity of aIC^{CaMKII}-NAcS circuit plays a key role in refeeding-induced analgesia

Discussion

We demonstrate in this study that glutamatergic neurons in the aIC projecting to the $NAcS^{D2R}$ mediate refeeding-induced analgesia. Our results indicate that refeeding produces analgesic effect by suppressing neuronal activities of aIC^{CaMKII}-$NAcS^{D2R}$ circuit which are enhanced under chronic pain condition. Our work thus delineates important neural circuit responsible for refeeding-induced analgesia.

Chronic pain entails structural and functional changes in the mesolimbic system including the NAc. In the previous study, inhibition of the NAcS with lidocaine ameliorated chronic pain behavior but not acute pain behavior. By contrast, inhibition of the NAcC exacerbated acute pain behavior and optogenetic activation of the NAcC ameliorated chronic pain behavior. These suggest that activation of the NAcS mediates pain perception under chronic pain condition while activation of the NAcC alleviates pain in both acute and chronic pain condition. In line with these findings, we showed activated NAcS with pain chronification which was suppressed by refeeding. These implicate that the NAcS is involved in the refeeding-induced analgesia, rather than the NAcC. In the present study, the NAcS was activated under chronic pain condition and suppressed by 2 hr refeeding, but the NAcC was not affected by refeeding (Fig 2). These results suggest that function of the NAcS in regard with pain behavior may alter with pain chronification and may restored by refeeding.

Dopamine release and the level of c-Fos expression increases within the NAcC in response to reward stimuli such as addictive drug

administration or refeeding. However, reward responsiveness is reduced in chronic pain condition. Dopamine release induced by sucrose solution intake or morphine as a reward is suppressed and dopamine signaling is reduced in the NAcC under chronic pain condition. Similarly, we found that refeeding, a reward stimulus, did not increase the level of c-Fos expression within the NAcC under chronic pain condition (Fig. 3B) whereas c-Fos expression increased in response to refeeding in the naïve group (Fig. S2). These findings indicate deficits in reward-related function of the NAcC under chronic pain condition which may result from hypodopaminergic state. However, dopamine concentration in the NAcC needs to be measured in the CFA-induced free fed and refed group to confirm dysfunction of the NAcC in chronic inflammatory pain. Together, we assume that the reward effect from refeeding may not be involved in the refeeding-induced analgesia.

D1R and D2R expressing neurons in the NAcS play apposite roles in modulating behavior. Some evidences indicate that D1R is important for reward learning while D2R elicits behavioral aversion. Pain states have been associated with D2R in both preclinical animal models and human imaging studies. A previous study reported that contribution of D2R to anti-hyperalgesic was greater than that of D1R in the chronic pain. In other study, chronic pain only elevated D2R protein level, but not D1R protein level. Moreover, a previous study suggest that intrinsic excitability of the GABAergic $NAcS^{D2R}$ whose activity suppresses by dopamine binding was substantially elevated in chronic neuropathic pain model due to lack of dopamine. Similarly, we showed elevated D2R expression in

chronic pain condition (Figs. 2D, 2E) and enhanced excitability of D2R expressing neurons within the NAcS (Fig. 5D). These findings suggest that the activation of D2R expressing neurons within the NAcS may mediate pain behavior in the chronic inflammatory pain and refeeding may induce analgesia by decreasing its activity. However, further study is needed to clarify the circuits downstream of the NAcS.

Plastic changes such as long-term potentiation (LTP) and long-term depression (LTD) in the brain synapses have been observed in chronic pain. The brain interprets sensory inputs to affective responses or pain perception and enhances, amplifies, or exaggerates such sensory experience. Previous studies showed plasticity changes in the ACC and IC in chronic pain conditions via enhanced NMDA receptors (NMDAR) and AMPA receptors (AMPAR). By contrast, within the $NAcS^{D2R}$, AMPAR/NMDAR ratio was decreased with prolonged decay time which indicates an increase in the GluN2B subunit of NMDAR. Interestingly, infralimbic-evoked $NAcS^{D2R}$ synapse strength was weakened, whereas hippocampus-evoked $NAcS^{D2R}$ synapse strength was increased without change in decay time in chronic pain. The present study showed increased activity of aIC^{CaMKII}-NAc^{D2R} in chronic pain. Hence, I assume that synapse strength is increased, similar to the hippocampus-$NAcS^{D2R}$. However, further study is needed to confirm synaptic changes in the aIC^{CaMKII}-NAc^{D2R} in free-fed and refed chronic pain group.

Although anatomical connectivity between the aIC and NAcS has been reported, the functional connectivity of aIC^{CaMKII}-$NAcS^{D2R}$ is yet to be identified. Consistent with this study, we confirmed glutamatergic

projection from the aIC to the $NAcS^{D2R}$ neurons (Figs. 4E, 4F) and further revealed functional connectivity through slice patch clamp recordings. pAAV-CaMKIIa-hM3D(Gq)-mCherry was injected into the aIC to record neural spikes in the mCherry expressing $NAcS^{D2R}$ neurons. The excitability of the $NAcS^{D2R}$ neurons receiving aIC^{CaMKII} projection was increased under chronic pain condition, but suppressed by refeeding (Figs. 5C, 5D). Moreover, we confirmed role of aIC^{CaMKII}-$NAcS^{D2R}$ circuit in chronic inflammatory pain with chemogenetics. Inhibition of aIC^{CaMKII}-$NAcS^{D2R}$ circuit alleviated chronic inflammatory pain behavior (Figs. 6F, 6G) while activation reversed refeeding-induced analgesia (Figs. 7E, 7F). These results imply that activation of aIC^{CaMKII}-$NAcS^{D2R}$ circuit may mediate chronic pain behavior. Unlike our results, a previous human fMRI study reported that the functional connectivity of the aIC-NAc is decreased in chronic pain. This discrepancy might be due to either differences in subregions of the NAc or differences in the types of pain.

Both chronic pain and feeding behavior involves complex brain circuits that include sensory, reward and homeostasis. In this study, we focused on the aIC and NAcS which are known to associate with pain and feeding behavior. However, we do not exclude the possibility that other brain regions may also be participated in the refeeding-induced analgesia, as numerous brain regions interact to process pain and feeding behavior. Indeed, the whole brain c-Fos analysis show possible involvement of other brain regions such as basolateral amygdala, medial prefrontal cortex, lateral parabrachial nucleus and ventromedial hypothalamus (submitted). Future study of different brain regions will further help us to understand

cellular mechanism and brain circuits for refeeding-induced analgesia.

Refeeding engages multiple features including eating behavior, calorie recovery and stomach expansion. It is well elucidated that mastication influences on the dopamine release at the pre-synapse in the striatum and glucose sensing neurons are located in the reward system in the brain. Moreover, the satiety signal that derives from gastric distention and intestinal nutrients that travels to the GLP-1 producing neurons in the NTS which directly activates the dopaminergic pathways of the mesolimbic system. These findings support our hypothesis that dopamine system may play an important role in the refeeding-induced analgesia. However, refeeding also engages multiple factors including systemic hormonal and immune system changes. Imaging studies in human report that gut hormones such as ghrelin and CCK affect activation of the aIC and NAcS to regulate appetite. Also, it is known that short-term fasting depletes naïve B cells by promoting apoptosis, and the number of naïve B cells are restored by refeeding . Thus, it is highly likely that both endocrine and immune system are involved in in the refeeding-induced analgesia.

From this study, we demonstrate for the first-time functional significance of aIC-NAcS circuit in the modulation of pain behavior. Importantly, this study suggests modification of feeding behavior can restore altered brain function and induce analgesic effect in chronic inflammatory pain condition even without medical treatment. Through verifying a mechanistic understanding for the suppression of chronic inflammatory pain behavior by feeding behavior, our work provides novel

therapeutic targets for the development of chronic pain management.

General discussion

Every day, more than 128 people in the United States die from misuse and addiction of opioids including prescription pain relievers, heroin, and synthetic opioids such as fentanyl. Recently, to confront opioid crisis, research on pain has been increased. These areas included understanding unique properties of acute and chronic pain and mechanism of the pain chronification. Also, identifying new, potent non-opioid analgesics and other pain-treatment strategies and biomarkers of pain has become emerging trends in neuroscience field. Similarly, I attempted to explore effective alternative treatments for chronic pain to reduced use of opioid to prevent opioid crisis in this thesis.

As escaping from aversive state is considered to be a reward, pain relief can be considered as a reward. Similarly, refeeding after fasting is a reward, because fasting is an aversive state that we want to avoid. Hence, my initial hypothesis was that the reward system may involve in the refeeding-induced analgesia. However, the results showed that the refeeding induces analgesia via suppressing facilitated NAcS in chronic pain conditions which is completely different from my hypothesis. The involvement of the NAc in pain chronification has been studied, but the functions of subregions in pain perception have not yet been well elucidated. This study provides evidence that the activation of the NAcS may aggravates pain perception, however further study is needed to confirm.

Naloxone, a non-specific opioid antagonist, is a medicine that

reverses effects of opioid overdose. Interestingly, I demonstrated paradoxical analgesic effect of naloxone in chronic pain condition via suppressing facilitated kappa opioid system. I hypothesized that prodynorphin (kappa opioid receptor ligand) is increased in the brain areas related to affective pain behavior in chronic inflammatory pain conditions. Indeed, the results showed that the density of prodynorphin and c-Fos expression (submitted) were increased in the NAc and CeA. By contrast, density of prodynorphin was suppressed and the level of c-Fos expression was enhanced in the anterior cingulate cortex (ACC) in chronic inflammatory pain conditions. Interestingly, recent studies have shown that the location of KOR is important in behavioral effects. For example, kappa stimulation in the rostral part of the nucleus accumbens shell (NAcS) enhances reward behavior, whereas kappa stimulation of the caudal part induces aversive behavior. Similar to the ACC, lack of KOR and elevated c-Fos level in basolateral amygdala (BLA) is associated with negative effect such as stress and anxiolytic behavior. These results suggest that both facilitation and suppression of kappa opioid system may mediate pain behavior depend on the neuroanatomical location. Thus, I assume that suppression of kappa opioid system in the ACC may mediate affective pain behavior. However, further studies are needed to confirm whether this hypothesis is correct.

The current thesis aimed to reveal pharmacological and non-pharmacological treatments for chronic inflammatory pain to minimize the use of opioids. It is hoped that the results of the current thesis will

lead to a deeper understanding of chronic pain, ultimately contributing to better treatment of chronic pain.

References

Al-Hasani, R., and M. R. Bruchas. "Molecular Mechanisms of Opioid Receptor-Dependent Signaling and Behavior." *Anesthesiology* 115, no. 6 (Dec 2011): 1363-81.

Alhadeff, A. L., Z. Su, E. Hernandez, M. L. Klima, S. Z. Phillips, R. A. Holland, C. Guo*, et al.* "A Neural Circuit for the Suppression of Pain by a Competing Need State." *Cell* 173, no. 1 (Mar 22 2018): 140-52 e15.

Anderson, R. I., M. F. Lopez, W. C. Griffin, H. L. Haun, D. W. Bloodgood, D. Pati, K. M. Boyt, T. L. Kash, and H. C. Becker. "Dynorphin-Kappa Opioid Receptor Activity in the Central Amygdala Modulates Binge-Like Alcohol Drinking in Mice." *Neuropsychopharmacology* 44, no. 6 (May 2019): 1084-92.

Anderson, S. M., H. D. Schmidt, and R. C. Pierce. "Administration of the D2 Dopamine Receptor Antagonist Sulpiride into the Shell, but Not the Core, of the Nucleus Accumbens Attenuates Cocaine Priming-Induced Reinstatement of Drug Seeking." *Neuropsychopharmacology* 31, no. 7 (Jul 2006): 1452-61.

Bai, Y., L. T. Ma, Y. B. Chen, D. Ren, Y. B. Chen, Y. Q. Li, H. K. Sun*, et al.* "Anterior Insular Cortex Mediates Hyperalgesia Induced by Chronic Pancreatitis in Rats." *Mol Brain* 12, no. 1 (Sep 4 2019): 76.

Baliki, M. N., P. Y. Geha, H. L. Fields, and A. V. Apkarian. "Predicting Value of Pain and Analgesia: Nucleus Accumbens Response to Noxious Stimuli Changes in the Presence of Chronic Pain." *Neuron* 66, no. 1 (Apr 15 2010): 149-60.

Bassareo, V., and G. Di Chiara. "Modulation of Feeding-Induced Activation of Mesolimbic Dopamine Transmission by Appetitive Stimuli and Its Relation to Motivational State." *Eur J Neurosci* 11, no. 12 (Dec 1999): 4389-97.

Becerra, L., and D. Borsook. "Signal Valence in the Nucleus Accumbens to Pain Onset and Offset." *Eur J Pain* 12, no. 7 (Oct 2008): 866-9.

Becker, S., W. Gandhi, and P. Schweinhardt. "Cerebral Interactions of Pain and Reward and Their Relevance for Chronic Pain." *Neurosci Lett* 520, no. 2 (Jun 29 2012): 182-7.

Benarroch, E. E. "Endogenous Opioid Systems: Current Concepts and Clinical Correlations." *Neurology* 79, no. 8 (Aug 21 2012): 807-14. "Involvement of the Nucleus Accumbens and Dopamine System in Chronic Pain." *Neurology* 87, no. 16 (Oct 18 2016): 1720-26.

Blevins, J. E., B. G. Stanley, and R. D. Reidelberger. "Brain Regions Where Cholecystokinin Suppresses Feeding in Rats." *Brain Res* 860, no. 1-2 (Mar 31 2000): 1-10.

Bodnar, R., D. Paul, and G. W. Pasternak. "Synergistic Analgesic Interactions between the Periaqueductal Gray and the Locus Coeruleus." *Brain Res* 558, no. 2 (Sep 6 1991): 224-30.

Boyd, A., and I. B. Holland. "Intermediate Location in the Assembly of the Matrix Protein or Porin into the Outer Membrane of Escherichia Coli." *J Bacteriol* 143, no. 3 (Sep 1980): 1538-41.

Bushnell, M. C., M. Ceko, and L. A. Low. "Cognitive and Emotional Control of Pain and Its Disruption in Chronic Pain." *Nat Rev Neurosci* 14, no. 7 (Jul 2013): 502-11.

Cahill, C. M., A. M. Taylor, C. Cook, E. Ong, J. A. Moron, and C. J. Evans. "Does the Kappa Opioid Receptor System Contribute to Pain Aversion?". *Front Pharmacol* 5 (2014): 253.

Carla E. Zelaya, Ph.D., James M. Dahlhamer, Ph.D., Jacqueline W. Lucas, M.P.H., and Eric M. Connor, B.S. "Chronic Pain and High-Impact Chronic Pain among U.S. Adults, 2019." *NCHS Data Brief* (11, 2020).

Castro, D. C., and K. C. Berridge. "Opioid Hedonic Hotspot in Nucleus Accumbens Shell: Mu, Delta, and Kappa Maps for Enhancement of Sweetness "Liking" and "Wanting"." *J Neurosci* 34, no. 12 (Mar 19 2014): 4239-50.

CDC. "Opioid Overdose." (2021).

Chang, P. C., S. L. Pollema-Mays, M. V. Centeno, D. Procissi, M. Contini, A. T. Baria, M. Martina, and A. V. Apkarian. "Role of Nucleus Accumbens in Neuropathic Pain: Linked Multi-Scale Evidence in the Rat Transitioning to Neuropathic Pain." *Pain* 155, no. 6 (Jun 2014): 1128-39.

Chaparro, L. E., A. D. Furlan, A. Deshpande, A. Mailis-Gagnon, S. Atlas, and D. C. Turk. "Opioids Compared with Placebo or Other Treatments for Chronic Low Back Pain: An Update of the Cochrane Review." *Spine (Phila Pa 1976)* 39, no. 7 (Apr 1 2014): 556-63.

Chartoff, E. H., and M. Mavrikaki. "Sex Differences in Kappa Opioid Receptor Function and Their Potential Impact on Addiction." *Front Neurosci* 9 (2015): 466.

Chavkin, C. "The Therapeutic Potential of Kappa-Opioids for Treatment of Pain and Addiction." *Neuropsychopharmacology* 36, no. 1 (Jan 2011): 369-70.

Chen, K. Y., L. Chen, and J. Mao. "Buprenorphine-Naloxone Therapy in Pain Management." *Anesthesiology* 120, no. 5 (May 2014): 1262-74.

Chen, L., T. Vo, L. Seefeld, C. Malarick, M. Houghton, S. Ahmed, Y. Zhang, *et al.* "Lack of Correlation between Opioid Dose Adjustment and Pain Score Change in a Group of Chronic Pain Patients." *J Pain* 14, no. 4 (Apr 2013): 384-92.

Choi, S. S., K. J. Han, H. K. Lee, E. J. Han, and H. W. Suh. "Possible Antinociceptive Mechanisms of Opioid Receptor Antagonists in the Mouse Formalin Test." *Pharmacol Biochem Behav* 75, no. 2 (May 2003): 447-57.

Chou, R., J. A. Turner, E. B. Devine, R. N. Hansen, S. D. Sullivan, I. Blazina, T. Dana, C. Bougatsos, and R. A. Deyo. "The Effectiveness and Risks of Long-Term Opioid Therapy for Chronic Pain: A Systematic Review for a National Institutes of Health Pathways to Prevention Workshop." *Ann Intern Med* 162, no. 4 (Feb 17 2015): 276-86.

Clifton, P. G., I. N. Rusk, and S. J. Cooper. "Effects of Dopamine D1 and Dopamine D2 Antagonists on the Free Feeding and Drinking Patterns of Rats." *Behav Neurosci* 105, no. 2 (Apr 1991): 272-81.

Cole, S. L., M. J. F. Robinson, and K. C. Berridge. "Optogenetic Self-Stimulation in the Nucleus Accumbens: D1 Reward Versus D2 Ambivalence." *PLoS One* 13, no. 11 (2018): e0207694.

Conway, S. M., D. Puttick, S. Russell, D. Potter, M. F. Roitman, and E. H. Chartoff. "Females Are Less Sensitive Than Males to the Motivational- and Dopamine-Suppressing Effects of Kappa Opioid Receptor Activation." *Neuropharmacology* 146 (Mar 1 2019): 231-41.

Cox, B., T. Kislinger, and A. Emili. "Integrating Gene and Protein Expression Data: Pattern Analysis and Profile Mining." *Methods* 35, no. 3 (Mar 2005): 303-14.

David, V., T. P. Durkin, and P. Cazala. "Differential Effects of the Dopamine D2/D3 Receptor Antagonist Sulpiride on Self-Administration of Morphine into the Ventral Tegmental Area or the Nucleus Accumbens." *Psychopharmacology (Berl)* 160, no. 3 (Mar 2002): 307-17.

Deyo, R. A., M. Von Korff, and D. Duhrkoop. "Opioids for Low Back Pain." *BMJ* 350 (Jan 5 2015): g6380.

DosSantos, M. F., B. S. Moura, and A. F. DaSilva. "Reward Circuitry Plasticity in Pain Perception and Modulation." *Front Pharmacol* 8 (2017):

790.
Dragan, S., M. C. Serban, G. Damian, F. Buleu, M. Valcovici, and R. Christodorescu. "Dietary Patterns and Interventions to Alleviate Chronic Pain." *Nutrients* 12, no. 9 (Aug 19 2020).
Dworkin, R. H. "An Overview of Neuropathic Pain: Syndromes, Symptoms, Signs, and Several Mechanisms." *Clin J Pain* 18, no. 6 (Nov-Dec 2002): 343-9.
Finnerup, N. B., S. H. Sindrup, and T. S. Jensen. "The Evidence for Pharmacological Treatment of Neuropathic Pain." *Pain* 150, no. 3 (Sep 2010): 573-81.
Frank, S., S. Kullmann, and R. Veit. "Food Related Processes in the Insular Cortex." *Front Hum Neurosci* 7 (2013): 499.
Gehrlach, D. A., C. Weiand, T. N. Gaitanos, E. Cho, A. S. Klein, A. A. Hennrich, K. K. Conzelmann, and N. Gogolla. "A Whole-Brain Connectivity Map of Mouse Insular Cortex." *Elife* 9 (Sep 17 2020).
Grenald, S. A., M. A. Young, Y. Wang, M. H. Ossipov, M. M. Ibrahim, T. M. Largent-Milnes, and T. W. Vanderah. "Synergistic Attenuation of Chronic Pain Using Mu Opioid and Cannabinoid Receptor 2 Agonists." *Neuropharmacology* 116 (Apr 2017): 59-70.
Guindon, J., and A. G. Hohmann. "The Endocannabinoid System and Pain." *CNS Neurol Disord Drug Targets* 8, no. 6 (Dec 2009): 403-21.
Hack, C. J. "Integrated Transcriptome and Proteome Data: The Challenges Ahead." *Brief Funct Genomic Proteomic* 3, no. 3 (Nov 2004): 212-9.
Hamlin, A. S., G. P. McNally, R. F. Westbrook, and P. B. Osborne. "Induction of Fos Proteins in Regions of the Nucleus Accumbens and Ventrolateral Striatum Correlates with Catalepsy and Stereotypic Behaviours Induced by Morphine." *Neuropharmacology* 56, no. 4 (Mar 2009): 798-807.
Hargraves, W. A., and I. D. Hentall. "Analgesic Effects of Dietary Caloric Restriction in Adult Mice." *Pain* 114, no. 3 (Apr 2005): 455-61.
Harris, H. N., and Y. B. Peng. "Evidence and Explanation for the Involvement of the Nucleus Accumbens in Pain Processing." *Neural Regen Res* 15, no. 4 (Apr 2020): 597-605.
Heffner, T. G., J. A. Hartman, and L. S. Seiden. "Feeding Increases Dopamine Metabolism in the Rat Brain." *Science* 208, no. 4448 (Jun 6 1980): 1168-70.
Heiman, M., A. Schaefer, S. Gong, J. D. Peterson, M. Day, K. E. Ramsey, M. Suarez-Farinas*, et al.* "A Translational Profiling Approach for the

Molecular Characterization of Cns Cell Types." *Cell* 135, no. 4 (Nov 14 2008): 738-48.
Heimer, L., G. F. Alheid, J. S. de Olmos, H. J. Groenewegen, S. N. Haber, R. E. Harlan, and D. S. Zahm. "The Accumbens: Beyond the Core-Shell Dichotomy." *J Neuropsychiatry Clin Neurosci* 9, no. 3 (Summer 1997): 354-81.
Henry, D. E., A. E. Chiodo, and W. Yang. "Central Nervous System Reorganization in a Variety of Chronic Pain States: A Review." *PM R* 3, no. 12 (Dec 2011): 1116-25.
Hernandez, L., and B. G. Hoebel. "Feeding and Hypothalamic Stimulation Increase Dopamine Turnover in the Accumbens." *Physiol Behav* 44, no. 4-5 (1988): 599-606.
Hikida, T., K. Kimura, N. Wada, K. Funabiki, and S. Nakanishi. "Distinct Roles of Synaptic Transmission in Direct and Indirect Striatal Pathways to Reward and Aversive Behavior." *Neuron* 66, no. 6 (Jun 24 2010): 896-907.
Iannetti, G. D., and A. Mouraux. "From the Neuromatrix to the Pain Matrix (and Back)." *Exp Brain Res* 205, no. 1 (Aug 2010): 1-12.
Ikeda, E., T. Li, H. Kobinata, S. Zhang, and J. Kurata. "Anterior Insular Volume Decrease Is Associated with Dysfunction of the Reward System in Patients with Chronic Pain." *Eur J Pain* 22, no. 6 (Jul 2018): 1170-79.
Jang, S. P., S. H. Park, J. S. Jung, H. J. Lee, J. W. Hong, J. Y. Lee, and H. W. Suh. "Characterization of Changes of Pain Behavior and Signal Transduction System in Food-Deprived Mice." *Anim Cells Syst (Seoul)* 22, no. 4 (2018): 227-33.
Johansen, J. P., and H. L. Fields. "Glutamatergic Activation of Anterior Cingulate Cortex Produces an Aversive Teaching Signal." *Nat Neurosci* 7, no. 4 (Apr 2004): 398-403.
Johansen, J. P., H. L. Fields, and B. H. Manning. "The Affective Component of Pain in Rodents: Direct Evidence for a Contribution of the Anterior Cingulate Cortex." *Proc Natl Acad Sci U S A* 98, no. 14 (Jul 3 2001): 8077-82.
Juarez-Salinas, D. L., J. M. Braz, A. Etlin, S. Gee, V. Sohal, and A. I. Basbaum. "Gabaergic Cell Transplants in the Anterior Cingulate Cortex Reduce Neuropathic Pain Aversiveness." *Brain* 142, no. 9 (Sep 1 2019): 2655-69.
Kato, T., S. Ide, and M. Minami. "Pain Relief Induces Dopamine Release in

the Rat Nucleus Accumbens During the Early but Not Late Phase of Neuropathic Pain." *Neurosci Lett* 629 (Aug 26 2016): 73-78.

Kc, E., H. C. Moon, S. Kim, H. K. Kim, S. Y. Won, S. H. Hyun, and Y. S. Park. "Optical Modulation on the Nucleus Accumbens Core in the Alleviation of Neuropathic Pain in Chronic Dorsal Root Ganglion Compression Rat Model." *Neuromodulation* 23, no. 2 (Feb 2020): 167-76.

Kim, J., B. H. Park, J. H. Lee, S. K. Park, and J. H. Kim. "Cell Type-Specific Alterations in the Nucleus Accumbens by Repeated Exposures to Cocaine." *Biol Psychiatry* 69, no. 11 (Jun 1 2011): 1026-34.

Kirkham, T. C. "Endocannabinoids and the Non-Homeostatic Control of Appetite." *Curr Top Behav Neurosci* 1 (2009): 231-53.

Klawonn, A. M., and R. C. Malenka. "Nucleus Accumbens Modulation in Reward and Aversion." *Cold Spring Harb Symp Quant Biol* 83 (2018): 119-29.

Knoll, A. T., E. G. Meloni, J. B. Thomas, F. I. Carroll, and W. A. Carlezon, Jr. "Anxiolytic-Like Effects of Kappa-Opioid Receptor Antagonists in Models of Unlearned and Learned Fear in Rats." *J Pharmacol Exp Ther* 323, no. 3 (Dec 2007): 838-45.

Koekkoek, L. L., J. D. Mul, and S. E. la Fleur. "Glucose-Sensing in the Reward System." *Front Neurosci* 11 (2017): 716.

Kolesnikov, Y., S. Jain, R. Wilson, and G. W. Pasternak. "Peripheral Kappa 1-Opioid Receptor-Mediated Analgesia in Mice." *Eur J Pharmacol* 310, no. 2-3 (Aug 29 1996): 141-3.

Kowaltowski, Fernanda M. CerqueiraBruno ChausseAlicia J. *Intermittent Fasting Effects on the Central Nervous System: How Hunger Modulates Brain Function*. Handbook of Famine, Starvation, and Nutrient Deprivation. Springer, Cham, 2019. doi:https://doi.org/10.1007/978-3-319-55387-0_29.

Kravitz, A. V., L. D. Tye, and A. C. Kreitzer. "Distinct Roles for Direct and Indirect Pathway Striatal Neurons in Reinforcement." *Nat Neurosci* 15, no. 6 (Jun 2012): 816-8.

Krebs, E. E., A. Gravely, S. Nugent, A. C. Jensen, B. DeRonne, E. S. Goldsmith, K. Kroenke, M. J. Bair, and S. Noorbaloochi. "Effect of Opioid Vs Nonopioid Medications on Pain-Related Function in Patients with Chronic Back Pain or Hip or Knee Osteoarthritis Pain: The Space Randomized Clinical Trial." *JAMA* 319, no. 9 (Mar 6 2018): 872-82.

Krugel, U., T. Schraft, H. Kittner, W. Kiess, and P. Illes. "Basal and Feeding-

Evoked Dopamine Release in the Rat Nucleus Accumbens Is Depressed by Leptin." *Eur J Pharmacol* 482, no. 1-3 (Dec 15 2003): 185-7.

Kuhla, A., S. Lange, C. Holzmann, F. Maass, J. Petersen, B. Vollmar, and A. Wree. "Lifelong Caloric Restriction Increases Working Memory in Mice." *PLoS One* 8, no. 7 (2013): e68778.

Lee, G. J., S. A. Kim, Y. J. Kim, and S. B. Oh. "Naloxone-Induced Analgesia Mediated by Central Kappa Opioid System in Chronic Inflammatory Pain." *Brain Res* 1762 (Jul 1 2021): 147445.

Lee, J. Y., G. J. Lee, P. R. Lee, C. H. Won, D. Kim, Y. Kang, and S. B. Oh. "The Analgesic Effect of Refeeding on Acute and Chronic Inflammatory Pain." *Sci Rep* 9, no. 1 (Nov 14 2019): 16873.

Lee, P. R., S. Y. Yoon, H. W. Kim, J. H. Yeo, Y. H. Kim, and S. B. Oh. "Peripheral Gabaa Receptor-Mediated Signaling Facilitates Persistent Inflammatory Hypersensitivity." *Neuropharmacology* 135 (Jun 2018): 572-80.

Li, L., Z. Wang, and Z. Zuo. "Chronic Intermittent Fasting Improves Cognitive Functions and Brain Structures in Mice." *PLoS One* 8, no. 6 (2013): e66069.

Li, Z., Z. Chen, G. Fan, A. Li, J. Yuan, and T. Xu. "Cell-Type-Specific Afferent Innervation of the Nucleus Accumbens Core and Shell." *Front Neuroanat* 12 (2018): 84.

Liu, C. M., and S. E. Kanoski. "Homeostatic and Non-Homeostatic Controls of Feeding Behavior: Distinct Vs. Common Neural Systems." *Physiol Behav* 193, no. Pt B (Sep 1 2018): 223-31.

Liu, S. S., S. Pickens, N. E. Burma, I. Ibarra-Lecue, H. Yang, L. Xue, C. Cook*, et al.* "Kappa Opioid Receptors Drive a Tonic Aversive Component of Chronic Pain." *J Neurosci* 39, no. 21 (May 22 2019): 4162-78.

Liu, Y., Y. Ni, W. Zhang, Y. E. Sun, Z. Ma, and X. Gu. "Antinociceptive Effects of Caloric Restriction on Post-Incisional Pain in Nonobese Rats." *Sci Rep* 7, no. 1 (May 11 2017): 1805.

Lobo, M. K., H. E. Covington, 3rd, D. Chaudhury, A. K. Friedman, H. Sun, D. Damez-Werno, D. M. Dietz*, et al.* "Cell Type-Specific Loss of Bdnf Signaling Mimics Optogenetic Control of Cocaine Reward." *Science* 330, no. 6002 (Oct 15 2010): 385-90.

Lu, C., T. Yang, H. Zhao, M. Zhang, F. Meng, H. Fu, Y. Xie, and H. Xu. "Insular Cortex Is Critical for the Perception, Modulation, and Chronification of Pain." *Neurosci Bull* 32, no. 2 (Apr 2016): 191-201.

Lutz, A., D. R. McFarlin, D. M. Perlman, T. V. Salomons, and R. J. Davidson. "Altered Anterior Insula Activation During Anticipation and Experience of Painful Stimuli in Expert Meditators." *Neuroimage* 64 (Jan 1 2013): 538-46.

Magnusson, J. E., and R. V. Martin. "Additional Evidence for the Involvement of the Basal Ganglia in Formalin-Induced Nociception: The Role of the Nucleus Accumbens." *Brain Res* 942, no. 1-2 (Jun 28 2002): 128-32.

Martikainen, I. K., E. B. Nuechterlein, M. Pecina, T. M. Love, C. M. Cummiford, C. R. Green, C. S. Stohler, and J. K. Zubieta. "Chronic Back Pain Is Associated with Alterations in Dopamine Neurotransmission in the Ventral Striatum." *J Neurosci* 35, no. 27 (Jul 8 2015): 9957-65.

Massaly, N., B. A. Copits, A. R. Wilson-Poe, L. Hipolito, T. Markovic, H. J. Yoon, S. Liu*, et al.* "Pain-Induced Negative Affect Is Mediated Via Recruitment of the Nucleus Accumbens Kappa Opioid System." *Neuron* 102, no. 3 (May 8 2019): 564-73 e6.

Mateo, Y., C. M. Lack, D. Morgan, D. C. Roberts, and S. R. Jones. "Reduced Dopamine Terminal Function and Insensitivity to Cocaine Following Cocaine Binge Self-Administration and Deprivation." *Neuropsychopharmacology* 30, no. 8 (Aug 2005): 1455-63.

McGivern, R. F., and G. G. Berntson. "Mediation of Diurnal Fluctuations in Pain Sensitivity in the Rat by Food Intake Patterns: Reversal by Naloxone." *Science* 210, no. 4466 (Oct 10 1980): 210-1.

Mitsi, V., and V. Zachariou. "Modulation of Pain, Nociception, and Analgesia by the Brain Reward Center." *Neuroscience* 338 (Dec 3 2016): 81-92.

Mogil, J. S. "The Etiology and Symptomatology of Spontaneous Pain." *J Pain* 13, no. 10 (Oct 2012): 932-3; discussion 34-5.

Moradi, M., M. Yazdanian, and A. Haghparast. "Role of Dopamine D2-Like Receptors within the Ventral Tegmental Area and Nucleus Accumbens in Antinociception Induced by Lateral Hypothalamus Stimulation." *Behav Brain Res* 292 (Oct 1 2015): 508-14.

Morgan, M. J., and K. B. Franklin. "Dopamine Receptor Subtypes and Formalin Test Analgesia." *Pharmacol Biochem Behav* 40, no. 2 (Oct 1991): 317-22.

Nagai, M., R. Noguchi, D. Takahashi, T. Morikawa, K. Koshida, S. Komiyama, N. Ishihara*, et al.* "Fasting-Refeeding Impacts Immune Cell

Dynamics and Mucosal Immune Responses." *Cell* 178, no. 5 (Aug 22 2019): 1072-87 e14.

Narita, M., Y. Kishimoto, Y. Ise, Y. Yajima, K. Misawa, and T. Suzuki. "Direct Evidence for the Involvement of the Mesolimbic Kappa-Opioid System in the Morphine-Induced Rewarding Effect under an Inflammatory Pain-Like State." *Neuropsychopharmacology* 30, no. 1 (Jan 2005): 111-8.

Navratilova, E., C. W. Atcherley, and F. Porreca. "Brain Circuits Encoding Reward from Pain Relief." *Trends Neurosci* 38, no. 11 (Nov 2015): 741-50.

Navratilova, E., G. Ji, C. Phelps, C. Qu, M. Hein, V. Yakhnitsa, V. Neugebauer, and F. Porreca. "Kappa Opioid Signaling in the Central Nucleus of the Amygdala Promotes Disinhibition and Aversiveness of Chronic Neuropathic Pain." *Pain* 160, no. 4 (Apr 2019): 824-32.

Navratilova, E., J. Y. Xie, A. Okun, C. Qu, N. Eyde, S. Ci, M. H. Ossipov*, et al.* "Pain Relief Produces Negative Reinforcement through Activation of Mesolimbic Reward-Valuation Circuitry." *Proc Natl Acad Sci U S A* 109, no. 50 (Dec 11 2012): 20709-13.

Neugebauer, V., V. Galhardo, S. Maione, and S. C. Mackey. "Forebrain Pain Mechanisms." *Brain Res Rev* 60, no. 1 (Apr 2009): 226-42.

Neugebauer, V., W. Li, G. C. Bird, and J. S. Han. "The Amygdala and Persistent Pain." *Neuroscientist* 10, no. 3 (Jun 2004): 221-34.

Niikura, K., M. Narita, E. R. Butelman, M. J. Kreek, and T. Suzuki. "Neuropathic and Chronic Pain Stimuli Downregulate Central Mu-Opioid and Dopaminergic Transmission." *Trends Pharmacol Sci* 31, no. 7 (Jul 2010): 299-305.

Nijs, J., E. D'Hondt, P. Clarys, T. Deliens, A. Polli, A. Malfliet, I. Coppieters*, et al.* "Lifestyle and Chronic Pain across the Lifespan: An Inconvenient Truth?". *PM R* 12, no. 4 (Apr 2020): 410-19.

Ozaki, S., M. Narita, M. Narita, M. Iino, J. Sugita, Y. Matsumura, and T. Suzuki. "Suppression of the Morphine-Induced Rewarding Effect in the Rat with Neuropathic Pain: Implication of the Reduction in Mu-Opioid Receptor Functions in the Ventral Tegmental Area." *J Neurochem* 82, no. 5 (Sep 2002): 1192-8.

Palmisano, M., F. F. Caputi, D. Mercatelli, P. Romualdi, and S. Candeletti. "Dynorphinergic System Alterations in the Corticostriatal Circuitry of Neuropathic Mice Support Its Role in the Negative Affective Component of Pain." *Genes Brain Behav* 18, no. 6 (Jul

2019): e12467.
Patterson, R. E., G. A. Laughlin, A. Z. LaCroix, S. J. Hartman, L. Natarajan, C. M. Senger, M. E. Martinez*, et al.* "Intermittent Fasting and Human Metabolic Health." *J Acad Nutr Diet* 115, no. 8 (Aug 2015): 1203-12.
Phillips, M. C. L. "Fasting as a Therapy in Neurological Disease." *Nutrients* 11, no. 10 (Oct 17 2019).
Pollack, A. "Coactivation of D1 and D2 Dopamine Receptors: In Marriage, a Case of His, Hers, and Theirs." *Sci STKE* 2004, no. 255 (Oct 19 2004): pe50.
Racine, M. "Chronic Pain and Suicide Risk: A Comprehensive Review." *Prog Neuropsychopharmacol Biol Psychiatry* 87, no. Pt B (Dec 20 2018): 269-80.
Rainville, P., G. H. Duncan, D. D. Price, B. Carrier, and M. C. Bushnell. "Pain Affect Encoded in Human Anterior Cingulate but Not Somatosensory Cortex." *Science* 277, no. 5328 (Aug 15 1997): 968-71.
Rasakham, K., and L. Y. Liu-Chen. "Sex Differences in Kappa Opioid Pharmacology." *Life Sci* 88, no. 1-2 (Jan 3 2011): 2-16.
Reddan, M. C., and T. D. Wager. "Brain Systems at the Intersection of Chronic Pain and Self-Regulation." *Neurosci Lett* 702 (May 29 2019): 24-33.
Ren, W., M. V. Centeno, S. Berger, Y. Wu, X. Na, X. Liu, J. Kondapalli*, et al.* "The Indirect Pathway of the Nucleus Accumbens Shell Amplifies Neuropathic Pain." *Nat Neurosci* 19, no. 2 (Feb 2016): 220-2.
Sah, D. W., M. H. Ossipo, and F. Porreca. "Neurotrophic Factors as Novel Therapeutics for Neuropathic Pain." *Nat Rev Drug Discov* 2, no. 6 (Jun 2003): 460-72.
Sang, C. N., R. H. Gracely, M. B. Max, and G. J. Bennett. "Capsaicin-Evoked Mechanical Allodynia and Hyperalgesia Cross Nerve Territories. Evidence for a Central Mechanism." *Anesthesiology* 85, no. 3 (Sep 1996): 491-6.
Saper, C. B., T. C. Chou, and J. K. Elmquist. "The Need to Feed: Homeostatic and Hedonic Control of Eating." *Neuron* 36, no. 2 (Oct 10 2002): 199-211.
Schultz, W. "Behavioral Theories and the Neurophysiology of Reward." *Annu Rev Psychol* 57 (2006): 87-115.
Schutter, U., S. Grunert, C. Meyer, T. Schmidt, and T. Nolte. "Innovative Pain Therapy with a Fixed Combination of Prolonged-Release

Oxycodone/Naloxone: A Large Observational Study under Conditions of Daily Practice." *Curr Med Res Opin* 26, no. 6 (Jun 2010): 1377-87.

Schwartz, N., P. Temkin, S. Jurado, B. K. Lim, B. D. Heifets, J. S. Polepalli, and R. C. Malenka. "Chronic Pain. Decreased Motivation During Chronic Pain Requires Long-Term Depression in the Nucleus Accumbens." *Science* 345, no. 6196 (Aug 1 2014): 535-42.

Sehgal, N., H. S. Smith, and L. Manchikanti. "Peripherally Acting Opioids and Clinical Implications for Pain Control." *Pain Physician* 14, no. 3 (May-Jun 2011): 249-58.

Selley, D. E., M. F. Lazenka, L. J. Sim-Selley, J. R. Secor McVoy, D. N. Potter, E. H. Chartoff, W. A. Carlezon, Jr., and S. S. Negus. "Attenuated Dopamine Receptor Signaling in Nucleus Accumbens Core in a Rat Model of Chemically-Induced Neuropathy." *Neuropharmacology* 166 (Apr 2020): 107935.

Seminowicz, D. A., B. Remeniuk, S. R. Krimmel, M. T. Smith, F. S. Barrett, A. B. Wulff, A. J. Furman*, et al.* "Pain-Related Nucleus Accumbens Function: Modulation by Reward and Sleep Disruption." *Pain* 160, no. 5 (May 2019): 1196-207.

Senba, E., and K. Kami. "A New Aspect of Chronic Pain as a Lifestyle-Related Disease." *Neurobiol Pain* 1 (Jan-Jul 2017): 6-15.

Seymour, B., and F. Mancini. "Hierarchical Models of Pain: Inference, Information-Seeking, and Adaptive Control." *Neuroimage* 222 (Nov 15 2020): 117212.

Shyu, B. C., W. F. Chen, and H. C. Shih. "Electrically and Mechanically Evoked Nociceptive Neuronal Responses in the Rat Anterior Cingulate Cortex." *Acta Neurochir Suppl* 101 (2008): 23-5.

Siahposht-Khachaki, A., P. Pourreza, S. Ezzatpanah, and A. Haghparast. "Nucleus Accumbens Dopamine Receptors Mediate Hypothalamus-Induced Antinociception in the Rat Formalin Test." *Eur J Pain* 21, no. 7 (Aug 2017): 1285-94.

Simonin, F., O. Valverde, C. Smadja, S. Slowe, I. Kitchen, A. Dierich, M. Le Meur*, et al.* "Disruption of the Kappa-Opioid Receptor Gene in Mice Enhances Sensitivity to Chemical Visceral Pain, Impairs Pharmacological Actions of the Selective Kappa-Agonist U-50,488h and Attenuates Morphine Withdrawal." *EMBO J* 17, no. 4 (Feb 16 1998): 886-97.

Song, S., Y. Zhang, J. Qiu, X. Li, K. Ma, S. Chen, and H. Chen. "Brain Structures

Associated with Eating Behaviors in Normal-Weight Young Females." *Neuropsychologia* 133 (Oct 2019): 107171.

Starr, C. J., L. Sawaki, G. F. Wittenberg, J. H. Burdette, Y. Oshiro, A. S. Quevedo, and R. C. Coghill. "Roles of the Insular Cortex in the Modulation of Pain: Insights from Brain Lesions." *J Neurosci* 29, no. 9 (Mar 4 2009): 2684-94.

Takahashi H., Funaki Y., Tsuboi A., Watanabe M., Yamaguchi S. "The Influence of Mastication on the Dopaminergic System in the Rat Brain." *Interface Oral Health Science 2011* (2012): 156-58.

Talbot, K., V. J. Madden, S. L. Jones, and G. L. Moseley. "The Sensory and Affective Components of Pain: Are They Differentially Modifiable Dimensions or Inseparable Aspects of a Unitary Experience? A Systematic Review." *Br J Anaesth* 123, no. 2 (Aug 2019): e263-e72.

Taylor, A. M. W., S. Becker, P. Schweinhardt, and C. Cahill. "Mesolimbic Dopamine Signaling in Acute and Chronic Pain: Implications for Motivation, Analgesia, and Addiction." *Pain* 157, no. 6 (Jun 2016): 1194-98.

Tuulari, J. J., L. Tuominen, F. E. de Boer, J. Hirvonen, S. Helin, P. Nuutila, and L. Nummenmaa. "Feeding Releases Endogenous Opioids in Humans." *J Neurosci* 37, no. 34 (Aug 23 2017): 8284-91.

Valjent, E., J. Bertran-Gonzalez, D. Herve, G. Fisone, and J. A. Girault. "Looking Bac at Striatal Signaling: Cell-Specific Analysis in New Transgenic Mice." *Trends Neurosci* 32, no. 10 (Oct 2009): 538-47.

Wang, X., S. Ma, H. Wu, X. Shen, S. Xu, X. Guo, M. L. Bolick, S. Wu, and F. Wang. "Macrophage Migration Inhibitory Factor Mediates Peripheral Nerve Injury-Induced Hypersensitivity by Curbing Dopaminergic Descending Inhibition." *Exp Mol Med* 50, no. 2 (Feb 16 2018): e445.

Wawrzczak-Bargiela, A., B. Ziolkowska, A. Piotrowska, J. Starnowska-Sokol, E. Rojewska, J. Mika, B. Przewlocka, and R. Przewlocki. "Neuropathic Pain Dysregulates Gene Expression of the Forebrain Opioid and Dopamine Systems." *Neurotox Res* 37, no. 4 (Apr 2020): 800-14.

Wood, P. B. "Role of Central Dopamine in Pain and Analgesia." *Expert Rev Neurother* 8, no. 5 (May 2008): 781-97.

Wu, Q., M. B. Lemus, R. Stark, J. A. Bayliss, A. Reichenbach, S. H. Lockie, and Z. B. Andrews. "The Temporal Pattern of Cfos Activation in Hypothalamic, Cortical, and Brainstem Nuclei in Response to

Fasting and Refeeding in Male Mice." *Endocrinology* 155, no. 3 (Mar 2014): 840-53.

Wu, Y., C. Chen, M. Chen, K. Qian, X. Lv, H. Wang, L. Jiang, *et al.* "The Anterior Insular Cortex Unilaterally Controls Feeding in Response to Aversive Visceral Stimuli in Mice." *Nat Commun* 11, no. 1 (Jan 31 2020): 640.

Yang, S., and M. C. Chang. "Chronic Pain: Structural and Functional Changes in Brain Structures and Associated Negative Affective States." *Int J Mol Sci* 20, no. 13 (Jun 26 2019).

Zanchi, D., A. Depoorter, L. Egloff, S. Haller, L. Mahlmann, U. E. Lang, J. Drewe, *et al.* "The Impact of Gut Hormones on the Neural Circuit of Appetite and Satiety: A Systematic Review." *Neurosci Biobehav Rev* 80 (Sep 2017): 457-75.

Zhang, W. H., W. Z. Liu, Y. He, W. J. You, J. Y. Zhang, H. Xu, X. L. Tian, *et al.* "Chronic Stress Causes Projection-Specific Adaptation of Amygdala Neurons Via Small-Conductance Calcium-Activated Potassium Channel Downregulation." *Biol Psychiatry* 85, no. 10 (May 15 2019): 812-28.

Zhuo, M. "Long-Term Cortical Synaptic Changes Contribute to Chronic Pain and Emotional Disorders." *Neurosci Lett* 702 (May 29 2019): 66-70.

Zubieta, J. K., Y. R. Smith, J. A. Bueller, Y. Xu, M. R. Kilbourn, D. M. Jewett, C. R. Meyer, R. A. Koeppe, and C. S. Stohler. "Regional Mu Opioid Receptor Regulation of Sensory and Affective Dimensions of Pain." *Science* 293, no. 5528 (Jul 13 2001): 311-5.

국문초록

마약성 진통제 사용의 대체 치료법:

나록손과 섭식조절을 이용한 진통효과 검증

만성통증은 전 세계적으로 빈번하게 발병하는 질병 중 하나이다. 중증도 이상의 만성통증을 치료하기 위해서는 마약성 진통제가 주로 사용된다. 하지만 마약성 진통제는 외상으로부터 기인하는 통증에는 효과적이나 그 외의 만성통증을 치료하는 데에는 한계가 있는 것으로 알려져 있다. 마약성 진통제의 오남용이나 중독은 심각한 사회문제로 급부상하고 있으며 큰 경제적 손실을 야기한다. 특히 미국에서는 2019 년에 마약성 진통제 과다 복용으로 인한 사망자 수가 5 만여명에 달했을 정도로 심각한 사회문제로 자리잡고 있다. 따라서 마약성 진통제를 대체할 치료법 개발이 필수적이다. 이에 본 논문에서는 마약성 진통제를 대체할 만성통증 치료 방법을 살펴보았다.

첫 번째 장에서는 약물학적 치료에 중점을 두고 연구하였다. 만성통증은 뇌의 오피오이드 시스템(opioid system)을 변화시키고 이로 인해 통증이 악화되는 것으로 알려져 있다 본 연구에서는 complete Freund adjuvant (CFA)라는 약물을 주사하여 염증성 만성통증을 유발한 쥐의 측좌핵(側坐核)(nucleus accumbens)과 편도체 (扁桃體) (central nucleus of amygdala)에서 카파

오피오이드 시스템(kappa opioid system)이 강화되고 뮤 오피오이드 시스템(mu-opioid system)이 억제됨을 추가로 보여주었다. 이어서 불특정 오피오이드 수용체의 길항제(antagonist)인 나록손 (naloxone) (10 mg/kg)을 주사했을 때 자발통이 감소되는 것을 확인하였다. 카파 오피오이드 수용체의 길항제인 nor-BNI 를 주사하였을 때에도 자발통이 감소하였다. 마지막으로 나록손과 nor-BNI 를 뇌의 측좌핵과 편도체에 직접 주사했을 때 역시 자발통이 줄어드는 것을 확인하였다. 이를 통해 오피오이드의 길항제가 만성통증을 치료하는데 효과적임을 확인하였다.

만성통증은 심리적, 인지적 요소가 관련 되어있어 약물만으로는 치료가 어렵다. 따라서 두 번째 장에서는 비약물학적 만성통증 치료방법에 대하여 연구하였다. 운동부족, 스트레스, 수면부족, 불규칙한 섭식행동과 같은 비건전한 생활습관은 만성통증과 밀접한 관련이 있다고 알려져 있다. 그 중에서도 섭식습관 개선은 모두가 쉽게 접근할 수 있는 방법으로 이번 장에서는 섭식습관 개선을 통한 만성통증 치료에 초점을 두고 연구하였다. 현재까지 많은 연구들이 24 시간 단식과, 24 시간 단식 후 2 시간 재섭취가 진통효과를 나타낼 수 있음을 보여주었다. 24 시간 단식으로 인한 진통효과의 기전은 밝혀져 있지만 24 시간 단식 후 2 시간 재섭취의 진통효과의 기전은 아직 밝혀지지 않고 있어 본 연구에서는 그 기전을 찾는 것을 목표로

하였다. 첫째로, 염증성 만성통증 상황에 측좌핵(側坐核)(nucleus accumbens)의 외피(外皮)(shell)와 뇌섬엽 (腦島葉) (insular cortex)의 앞쪽부분에서 신경활동이 증가했음을 확인하였다. 만성통증 상황에 뇌섬엽 앞부분에서 측좌핵 껍질로 신호를 전달하는 신경세포의 활성이 증가되고 재섭취 후에는 그 활성이 다시 감소되는 것을 확인하였다. 나아가 화학유전학 기법을 이용하여 이 회로를 억제하였을 때 만성통증이 감소하고, 이 회로를 활성화하였을 때 재섭취로 인하여 생겼던 진통 반응이 사라지는 것을 추가로 확인함으로써 24 시간 단식 후 2 시간 재섭취로 인한 진통 기전에는 뇌섬엽 앞부분과 측좌핵의 외피간의 신경회로가 관여하고 있다는 것을 알 수 있었다.

이 논문은 마약성 진통제 사용을 줄이며 부작용 없이 만성통증을 치료하는 약물학적, 비약물학적 방법에 대하여 소개한다.

주요어: 만성통증; 변연계; 나록손; 시간 제한 섭식; 도파민; 뇌섬엽;

학생번호: 2017-21571