Glia-mediated cerebellar pain modulation

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Abstract

Clinical studies have revealed that the cerebellum is activated by noxious stimuli or pathological pain, and its removal results in somatosensory dysfunction. However, the neural circuit, cellular and molecular mechanisms underlying pain transmission and modulation in the cerebellum remain unknown. Here, we show that Bergmann glia (BG) relay noradrenaline (NA) signals ascending from the locus coeruleus (LC) to Purkinje cells (PCs), contributing to pain behavior. Using two-photon microscopy and optogenetics in mice, we found that the LC releases NA in the cerebellar cortex during peripheral noxious electrical stimuli, which elevates intracellular calcium in most BG. This global calcium activation of BG, called "flare", was also elicited during capsaicin-induced pain, and chemogenetic inactivation of BG or LC terminals inhibited not only BG flares but also pain behavior. Pharmacological blockade or genetic knockdown of BG alpha-1 adrenergic receptors (α1-ARs) also suppressed capsaicin-induced BG flares and pain behavior. Electrophysiological recordings indicated that capsaicin-induced pain accompanies the reduction of PC activity, which was mimicked by chemogenetic activation of BG and prevented when flares were blocked by prazosin, an α1-AR blocker. Taken together, our study identified a glia-mediated mechanism of cerebellar pain processing, providing new insights into the cerebellum as an active pain modulator.

One-Sentence Summary: Identification of glia-mediated mechanism of pain information processing in the cerebellum.

Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage [1]. Thus, pain information processing should involve multiple brain regions [2, 3], among which the cerebellum has been relatively neglected in the field of pain medicine. The cerebellum plays an important role in coordinating movements while receiving diverse sensory inputs [4]. Interestingly, human brain imaging studies have consistently reported that the cerebellum is activated during physiological and pathological pain states [5-9], whereas research on cerebellar stimulation of pain has shown contradictory results (i.e., analgesia or hyperalgesia) [6]. To our knowledge, no studies have revealed how peripheral pain signals are transmitted and modulated within the cerebellar cortex.

The noradrenergic system transmits, processes, and modulates pain information throughout the entire brain [10]. The locus coeruleus (LC), a major source of noradrenaline (NA), is activated by noxious peripheral stimuli [10] and has ascending projections to the prefrontal cortex and limbic system, which are known pain-related higher centers, and the cerebellum [11]. Activated LC neurons release NA from their nerve terminals in the form of volume transmission, affecting innervating brain regions [12-15]. Previous studies have shown that the LC-NA system innervating the cerebellum is actively involved in motor control and cognitive behaviors [16-18]. We postulated that the cerebellum receives pain signals via the LC-NA system.

Bergmann glia (BG) are tightly associated with Purkinje cells (PCs) and can modulate their activity [19-21]. Since BG strongly responds to NA inputs by elevating intracellular calcium [17, 22, 23], LC-NA-activated BG is a strong candidate that mediates cerebellar pain processing. In this study, we revealed that peripheral noxious stimuli induced NA release from LC terminals in the cerebellar cortex, triggering BG calcium activation. We also show that alpha-1 adrenergic receptors (α1-ARs) of BG are essential for LC-BG activation and pain behavior. Finally, we demonstrated that the α1-AR-mediated BG activation changes the PC outputs during pain. Our findings provide a critical mechanism for cerebellar pain modulation, which may represent a novel therapeutic target for pain medicine.

Result

To test whether LC neuronal axons project to the cerebellar cortex, we performed dual injection of AAV-TH-cre and AAV-DIO-mCherry in the LC, achieving mCherry expression in LC neurons that were co-labeled with dopamine beta-hydroxylase (DBH), an LC neuronal marker. Co-labeled signals were observed in the cerebellar cortex vermis lobule 4/5 (Fig. 1a), which is part of the spinocerebellum and receives somatosensory input [24]. Next, to examine whether pain information is transmitted from the LC to the cerebellar cortex, we co-expressed the genetically encoded calcium indicator (GECI) GCaMP6s and inhibitory DREADD hM4Di in LC neurons (Fig. 1b). *In vivo* two-photon imaging assisted by CNMF_E region-of-interest detection showed that the calcium level of LC terminals in the molecular layer of the cerebellar cortex increased in response to noxious electrical stimuli (10 mA) applied to the hind paw of anesthetized mice. The noxious stimuli-induced calcium signals of LC terminals were diminished when compound 21 (C21), an hM4Di agonist, was injected into the cerebellum (Fig. 1c-e). These results indicate that LC conveys noxious information to the cerebellum.

To test whether LC terminals carrying noxious information activate BG through NA release, we performed simultaneous calcium imaging of LC terminals and BG in the molecular layer of the cerebellar cortex during noxious electrical stimuli in the hind paw (Fig. 2a). The calcium signals in the LC terminals immediately increased as the stimulus was given, whereas the BG calcium signal gradually increased with a delay of several seconds (Fig. 2b). Quantitative analysis showed that both calcium signals from the LC terminals and BG were significantly increased after noxious stimulation (Fig. 2c). Based on the order of calcium activation timing between the LC and BG, we reasoned that LC terminals activated by noxious stimuli release NA, which in turn activates BG. We used a red-shift opsin (ChrimsonR) that can forcibly activate LC terminals in the cerebellar cortex (Fig. 2d, Fig. S1). Using NA-sensor (Grab-NE) [25], the measurement of the levels of NA acting on BG during the optogenetic activation of the LC revealed that NA levels were elevated from the onset of laser stimulation (593.5 nm), peaked near the last laser stimulus (peak latency: 3.82 ± 0.05 s), and rapidly returned to the baseline when the laser was turned off (Fig. 2e-g). Interestingly, upon optogenetic activation of LC terminals, the BG calcium levels increased after a short delay from the laser onset and continued to elevate even after the cessation of LC activation (peak latency: 6.82 ± 0.23 s), and slowly diminished thereafter (Fig. 2h-k). These results suggest that noxious stimuli activate cerebellar LC terminals to release NA, which in turn activates BG.

To determine the role of the LC-NA-activated BG circuit in pain behavior, we used a capsaicin-induced pain model [26]. When capsaicin was injected into the hind paw, licking behavior was prominent in response to pain and diminished within 10 min (Fig. 3a). Upon capsaicin injection, BGs showed a distinct pattern of global calcium elevation similar to that observed with noxious electrical stimulation (Fig. 3b). This so-called “flare” is characterized by synchronized and simultaneous activation of the entire BG [27]. In our 5-min recordings after capsaicin injection, BG displayed 2-5 flare activity (Fig. 3c). To test whether the BG flare plays a role in pain behavior, we blocked the capsaicin-induced BG calcium response using BG-specific hM4di activation (Fig. 3d-f, Fig. S2). Upon clozapine N-oxide (CNO) administration, capsaicin-induced licking behavior was significantly reduced (Fig. 3d), and BG flares were also blocked (Fig. 3e-f). In addition, we investigated the role of LC terminals in the cerebellar cortex in this behavioral change and BG flares. The total duration of capsaicin-induced licking behavior was reduced when C21 was locally applied through the cannula implanted in cerebellar vermis lobule 4/5 of the LC-specific hM4di-expressing mice (Fig. 3g). Capsaicin-induced BG flares

were completely blocked (Fig. 3h-i). Taken together, these results demonstrate that capsaicin-induced acute pain is expressed as waves of BG flares in the cerebellum through LC activation, and blocking these flares can inhibit pain behavior.

Next, to investigate which NA receptor mediates capsaicin-induced BG flares and pain behavior, we performed *in vivo* calcium imaging of BG as well as behavioral analysis with the local administration of NA receptor blockers (prazosin, PRZ, α1-AR blocker; yohimbine, YOH, α2-AR blocker; propranolol, PROP, β-AR blocker) through a cannula targeting the imaging area (Fig. 4a). Among the drugs tested, only prazosin completely blocked capsaicin-induced BG flares (Fig. 4b). The capsaicin-induced licking behavior was also reduced in the prazosin-infused group (Fig. 4c). In the slice recording, we further confirmed that BG calcium elevation during optogenetic activation of the LC terminal was completely blocked by bath application of prazosin (Fig. 4d-f). Prazosin application did not affect the activity of PCs, the sole output neurons in the cerebellum (Fig. S3). To further confirm the cell-type-specific genetic manipulation, BG-specific α1-AR knockdown (KD) was achieved by injecting Cre-dependent α1-AR shRNA virus into Glast-cre/ert2 mice, a transgenic strain specifically expressing Cre in BG (Fig. 4g, i). The calcium response of α1-AR-KD glial cells was completely abolished in response to the α1-AR agonist but not to the protease-activated receptor 1 (PAR-1) agonist (Fig. S4a-c). α1-AR protein expression was reduced by approximately 70% in the cerebellar cortex of the α1-AR KD group (Fig. S4d-f). α1-AR KD completely blocked capsaicin-induced BG flares and significantly reduced capsaicin-induced licking behavior compared to the sh-scramble-injected group (Fig. 4h-i). The results indicate that α1-AR on BG mediates pain information processing in the LC-cerebellum circuitry.

Finally, we examined whether capsaicin-induced BG activation affects cerebellar cortical output, PC activity. *In vivo* electrophysiology recordings of cerebellar PCs indicate that capsaicin injection into the hind paw results in a reduction in firing frequency. Importantly, this reduction was blocked by microinjection of prazosin, an α1-AR blocker, into the cerebellar cortex (Fig. 5a-c). To determine whether BG activation is responsible for the reduction in PC firing, we performed cell-attached *ex vivo* electrophysiology recordings with BG-specific chemogenetic manipulation (Fig. 5d). The spontaneous firing rate of PCs was reduced when BG was activated using the CNO application under hM3Dq expression. Inhibiting BG with hM4Di expression by CNO did not affect the spontaneous firing rate of PCs (Fig. 5e-f). As prazosin treatment significantly prevented BG activation and capsaicin-induced reduction of PC firing, we speculated that the reduced PC output to the deep cerebellar nucleus (DCN) during pain could be restored in the absence of BG involvement. Thus, the restored PC output may yield stronger inhibition of the DCN compared to the normal pain state. To confirm DCN activity during prazosin application, we examined the expression of the neuronal activity marker c-Fos. Consequently, c-Fos expression in DCN was increased by capsaicin-induced pain, and the prazosin group had distinctly lower c-Fos expression than the saline control group after capsaicin injection (Fig. 5g-h). Furthermore, to investigate the functional role of the DCN in pain processing, we applied muscimol, a GABA-A receptor agonist, to the DCN through the cannula and assessed the changes in capsaicin-induced nociceptive behavior (Fig. 5i). The results suggest that pain information is transmitted from the LC to the cerebellum via activation of α1-AR on BG, suppression of PCs, and subsequent disinhibition of the DCN, furthermore, manipulating this circuitry can reduce nociceptive behavior in the pain state.

Discussion

Previous studies have consistently reported that the cerebellum is responsive to painful stimuli [5-9], indicating that the cerebellum might play a critical role in pain processing. However, its contribution to pain processing has not been fully elucidated and in-depth studies are lacking. Here, we found evidence that demonstrated a significant role of the cerebellum in nociceptive processing. First, at the circuit level, nociceptive signals entered the cerebellum through the LC, and the NA released from the LC terminal elevated the BG calcium. Second, at the cellular level, the firing of PCs, which is the sole output of the cerebellum, is modulated by BG calcium activity. Third, at the molecular level, the increased BG calcium activity and PC activity regulation in pain states were dependent on the α1-AR of BG. Finally, when an intervention was applied to the cerebellum, the expected nociceptive behavior was blocked. In summary, we demonstrated that NA delivered from LC in a pain state increases α1-AR dependent BG calcium activity, thereby regulating PC output and contributing to pain information processing. Through this step-by-step confirmation, we have clearly elucidated the pain modulation mechanism of the cerebellum and suggest that the cerebellum is actively involved in pain information processing.

The LC provides noradrenergic projections to the entire brain and strongly influences pain processing through the release of NA, one of the neuromodulators [28]. NA is involved in both pain inhibition and facilitation [28, 29], maintenance of chronic pain [28, 30], and modulation of pain perception in the brain [28, 31]. NA release into the cerebellum, followed by BG calcium activation, is rapidly induced by electrical stimulation. In addition, only blocking α1-AR in BG significantly reduced pain behavior. It was clearly verified that pain information was processed through the NA released to the cerebellum in the pain state, and the intervention of this pathway attenuated pain behavior without motor dysfunction, indicating the role of the cerebellum in pain perception.

In this study, we suggested that NA reduces PC firing via BG activation. We found that the reduction in PC firing did not occur when α1-AR was blocked by prazosin (Fig. S3), and BG-specific hM3Dq activation induced decreased PC firing (Fig. 5d-f). Our results revealed that α1-AR in BG is sufficient to modulate cerebellar output. Several previous studies have proposed that BG could regulate PC activity [19-21, 32, 33]. Among them, first, interneurons can be activated by gliotransmission and can inhibit PC activity [33]. Second, BG directly releases GABA to PCs through the Bestrophin-1 channel [32] and tonic inhibitory currents can hyperpolarize PCs. These two mechanisms may underlie the BG-dependent reduction of PC activity in the pain state.

The manner in which pain information is processed in cerebellar microcircuits has not yet been elucidated. Strikingly, our study demonstrated that pain information through the LC-BG pathway contributes to the manifestation of pain behavior by reducing PC firing, thereby disinhibiting DCN activity [34, 35]. Cerebellar infusion of prazosin prevented the pain-induced reduction of PC firing, resulting in attenuation of pain behavior. Remarkably, muscimol-induced DCN inhibition (blocking disinhibition) also significantly attenuated pain behavior (Fig. 5) without affecting motor coordination (Fig. S5h), validating the functional role of the PC-DCN circuit in pain processing. Previous studies used muscimol (10 mM) for DCN inactivation, which results in impaired motor learning via the eyeblink and rotarod tests [36-39]. However, we tested several doses of muscimol to exclude the possibility of nociceptive behavioral changes due to motor dysfunction, we used 100 times lower concentrations (100 μM) with no motor impairment. Consequently, these data show that pain information is processed in the cerebellar

cortex by reducing PC firing to enhance DCN activity, which leads to the manifestation of pain behavior.

Clinically, NA agonists or inhibitors have been used to treat hypertension for a long time. Although there are not many examples of the α1-AR blockade, partial pain relief has been confirmed in patients with complex regional pain syndrome [40, 41]. However, it is difficult to explain the exact mechanism of these effects owing to the limitations of systemic application and side effects. Our study, which blocks α1-AR specifically in the cerebellum, showed a significant analgesic effect, implicating an exquisite approach to the cerebellum with the α1-AR blockade. Notably, capsaicin-induced pain behavior was reduced by local manipulation of the cerebellum, without affecting motor function (Fig. S5). Our study clearly showed that pain could be modulated by blocking only BG in the cerebellar vermis lobule 4/5 without the risk of side effects or systemic effects. Eventually, our research will form the basis for a new approach to controlling pain by applying optogenetics, chemogenetics, and electroceutical tools to specific cells in specific areas at the clinical level.

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Author contributions

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Competing interests

SHK, SKK, SJK hold patent applications related to the contents of this article (10-2021-0180671 in Korea). The other authors declare no conflicts of interest.

Data and materials availability:

All software or algorithm used in this study is available and listed in the Key Resources Table. The All data and codes associated with this study are presented in the paper, supplementary materials. The data that support the findings of this study are available upon reasonable request from the lead contact

Fig.1: LC terminal activation in the cerebellar cortex by noxious stimuli

a. Schematic diagram showing virus injection for LC-specific mCherry expression.
a1-2. mCherry was expressed in LC and cerebellar cortex. mCherry (red), DBH (dopamine beta hydroxylase, Green). Scale bars, 100 μm.
Ratio of viral expression overlap with DBH positive cells in LC (83.39 ± 3.80 %; n = 2 slices of 1 mouse).

b. Schematic diagram showing *in vivo* two-photon calcium imaging using hind-paw electrical in LC-specific hM4Di and GECI expressing mouse.
b1. A coronal view of the z-stack image (red box, molecular layer, 40 μm from the surface).
b2. A dorsal view of the z-stack image, which represents the molecular layer (red box region of b1). Scale bars, 100 μm.

c. An example of ROI detection of LC terminals using the CNMF_E.

d. Representative traces of LC-terminal signals during various electrical stimuli (0, 1, 10 mA; 0.2 ms, 3 Hz, 10 sec) on the hindpaw (n = 16 ROIs in 3 mice)

e. Increased LC terminal calcium by hind-paw electrical stimuli and blocked by LC terminal hM4Di activation (n = 16 ROIs in 3 mice; 0 mA and 10 mA in saline group, ** $p = 0.0013$; 1 mA and 10 mA in saline group, **** $p < 0.0001$; 10 mA in saline group and 10 mA in c21 group, ** $p = 0.001$; Wilcoxon test)

Fig.2: NA released from LC terminal activates cerebellar BG

a. Schematic diagram showing *in vivo* two-photon calcium imaging using hind-paw electrical stimulus in BG and LC GECI expressing mouse.
An example of ROI detection of BG using grid pattern and LC terminal using the CNMF_E. Scale bar, 100 μm.

b. Traces of LC terminal and BG calcium signal during hind-paw noxious stimuli (0.2 ms, 10 mA, 3 Hz; BG, n = 256 ROIs in 4 mice; LC terminal, n = 17 ROIs in 4 mice; Mean for line, SEM for shade).

c. Increased BG and LC terminal calcium activity by hind-paw electrical stimulation (BG, n = 256 ROIs in 4 mice, **** $p < 0.0001$; LC terminal, n = 17 ROIs in 4 mice, ** $p = 0.0057$; paired t test).

d. Schematic diagram showing in-vivo virus injection for LC neuronal optogenetic modulation and BG Grab_NE imaging in cerebellar slice.
An example of ROI detection of BG global activity in molecular layer. Scale bar, 100 μm.

e. Trace of BG-specific NE sensor signal during LC optogenetic activation (593.5 nm, 5 mW, 5 Hz, 5 sec; n = 10 slices in 5 mice; Mean for line, SEM for shade).

f. Increased BG Grab_NE activity by laser stimulation (n = 6 slices; * $P = 0.0313$; Wilcoxon test).

g. Latency of BG Grab_NE peak formation by laser stimulation (n = 6 slices; Mean ± SEM = 3.82 ± 0.05 s).

h. Schematic diagram showing virus injection for LC neuronal optogenetic modulation and BG calcium slice imaging.
An example of ROI detection of BG global activity in molecular layer. Scale bar, 100 μm.

i. Trace of BG calcium signal during LC optogenetic activation or control (ChrimsonR, n = 6 slices in 5 mice; Control, n = 3 slices in 2 mice; Mean for line, SEM for shade).

j. Increased BG calcium activity by laser stimulation (n = 6 slices; * $P = 0.313$; Wilcoxon test).

k. Latency of calcium peak formation by laser stimulation (n = 6 slices; Mean ± SEM = 6.82 ± 0.23 s)

Fig.3: Capsaicin induces global BG calcium response (flare) associated with pain behavior

a. Licking behavior in capsaicin-induced pain (n = 5 mice in vehicle group; n = 10 mice in capsaicin group; *** p = 0.0001; Two-way ANOVA).

b. An example of ROI detection of BG using grid pattern. (8 by 8, 64 ROIs).

c. Representative capsaicin-induced BG calcium response (above, vehicle; below, capsaicin; flare activity is indicated by red arrow head).
BG calcium synchronous response as a "Flare" activity in capsaicin-induced pain (n = 7 mice in each group; **** p < 0.0001; Two-way ANOVA). Scale bar, 100 μm.

d. Licking behavior in capsaicin-induced pain after BG hM4Di activation (n = 7 mice in each group; ** p = 0.0086; Two-way ANOVA).

e. Schematic diagram showing *in vivo* two-photon imaging of BG calcium in response to hind-paw capsaicin injection in BG hM4Di expressing mouse.

f. Representative capsaicin-induced BG calcium response (above, saline group; below, CNO group; flare activity indicated by red arrow).
Flare activity in capsaicin-induced pain (n = 5 mice in each group; **** p < 0.0001; Two-way ANOVA).

g. Licking behavior in capsaicin-induced pain after LC terminal hM4Di activation (n = 9 mice in each group; ** p = 0.0014; Two-way ANOVA).

h. Schematic diagram showing *in vivo* two-photon imaging of BG calcium in response to hind-paw capsaicin injection in LC hM4Di expressing mouse.

i. Representative of capsaicin-induced BG calcium response (above, saline group; below, C21 group; flare activity indicated by red arrow).
Flare activity in capsaicin-induced pain (n = 5 mice in each group; **** p < 0.0001; Two-way ANOVA).

Fig.4: α1-AR mediates capsaicin-induced BG calcium flare and pain behavior

a. Schematic diagram showing in vivo two-photon BG calcium imaging and drug application using the cannula simultaneously.

b. Representative of capsaicin-induced BG calcium response (Saline, left above; Prazosin (PRZ), left below; Yohimbine (YHB), right above; Propranolol (PROP), right below; flare activity indicated by red arrowheads).
Flare activity in capsaicin-induced pain (n = 6 mice in each group; *** p = 0.0003; Two-way ANOVA).

c. Licking behavior in capsaicin-induced pain (n = 9 mice in saline group, n = 10 mice in prazosin group; **** p < 0.0001; Two-way ANOVA).

d. Schematic showing slice imaging of BG calcium in response to LC terminal optogenetic modulation during prazosin bath application.

e. Trace of BG calcium signal during LC optogenetic activation or control (ACSF, n = 6 slice in 5 mice; Prazosin, n = 4 slice in 2 mice; mean for line, SEM for shade; using 539.5 nm, 10 mW, 5 Hz, 5 sec).

f. Decreased BG calcium activity by prazosin application (* p = 0.0146; Unpaired t-test).

g. Schematic diagram showing *in vivo* two-photon imaging of BG calcium in response to capsaicin-injection in BG-specific genetic manipulation model mice.

h. Representative of capsaicin-induced BG calcium response (above, scrambled group; below, knockdown group; flare activity indicated by red arrow head).
Flare activity in capsaicin-induced pain (n = 5 mice in each group; **** p < 0.0001; Two-way ANOVA).

i. Licking behavior in Capsaicin-induced pain (n = 7 mice in each group; * p = 0.0012; Two-way ANOVA).

Fig.5: Manipulation of BG calcium regulates Purkinje cell activity and pain behavior

a. Schematic diagram showing measurement of PC spontaneous firing using in vivo electrophysiology and drug application using cannula simultaneously.

b. Representative of PC spontaneous firing in capsaicin-induced pain (Red, Prazosin application group; Black, ACSF application group).

c. Changes in PC firing after capsaicin injection.

d. Schematic diagram showing measurement of PC attached recording using BG chemogenetic manipulation.

e. PC firing rate affected by BG activity modulated by CNO application (n = 8 slices in mCherry group; n = 5 slices in hM3Dq group; n = 8 slices in hM4Di group).

f. PC firing rate after 5 min of CNO application (hM3Dq and mCherry, ** p = 0.0085; hM3Dq and hM4Di, ** p = 0.0039; hM4Di and mCherry, N.S. p = 0.9113; One-way ANOVA).

g. Increase in DCN activity during capsaicin-induced pain was confirmed by c-fos expression (n = 11 slices of 3 mice in vehicle group; n = 10 slices of 3 mice in capsaicin group; ** p = 0.0038; Unpaired t-test).

h. Decrease in DCN activty during capsaicin-induced pain by local prazosin injection for cerebellar lobule 4/5 was confirmed by c-fos expression (n = 17 slices of 3 mice in saline group; n = 18 slices of 3 mice in prazosin group; *** p = 0.0001; Unpaired t-test).

i. Capsaicin-induced licking behavior after saline or muscimol injection in medial DCN (n = 7 mice for each group; *** p = 0.0003; Two-way ANOVA).

Fig.1: LC terminal activation in the cerebellar cortex by noxious stimuli

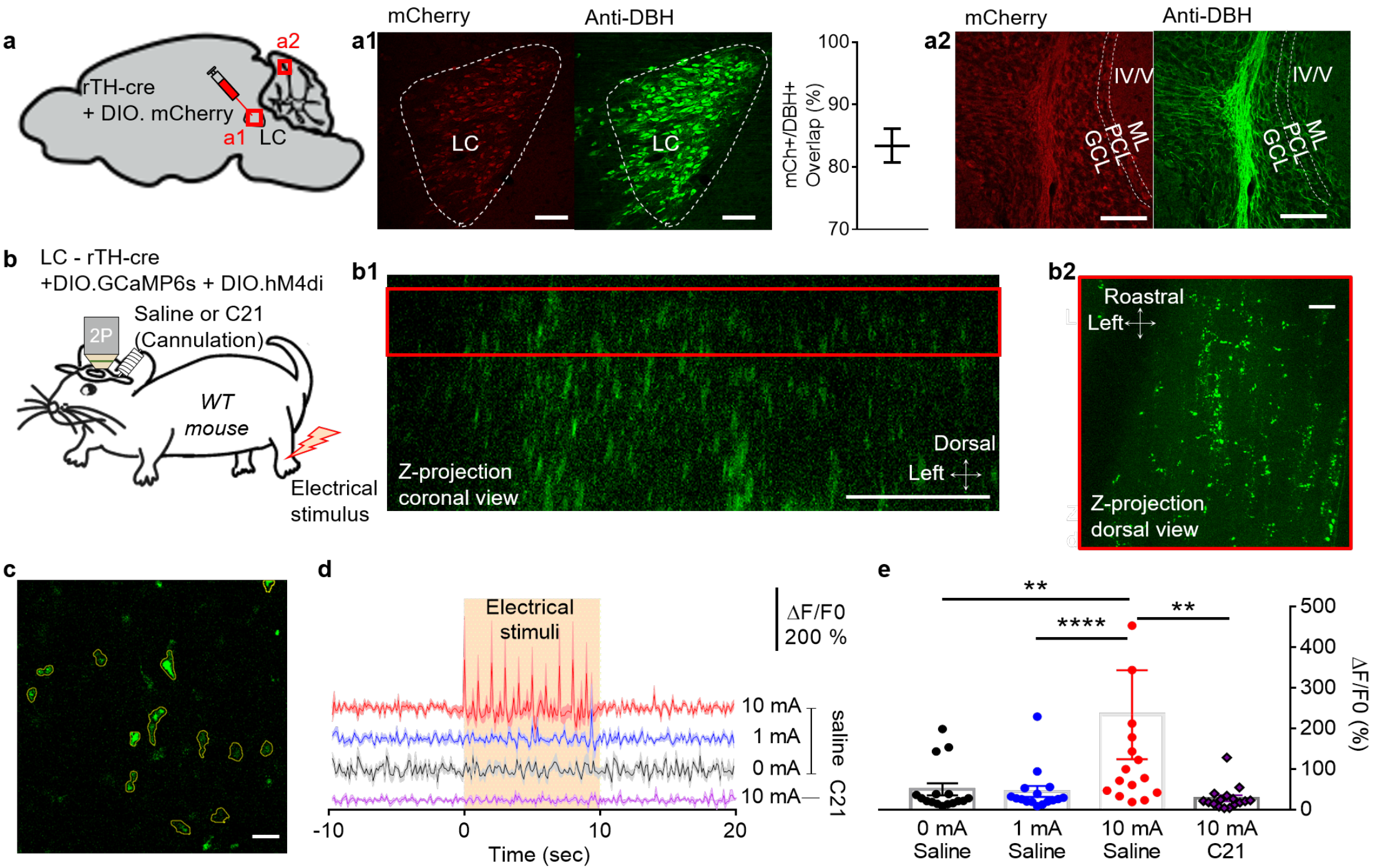


Fig.2: NA released from LC terminal activates cerebellar BG

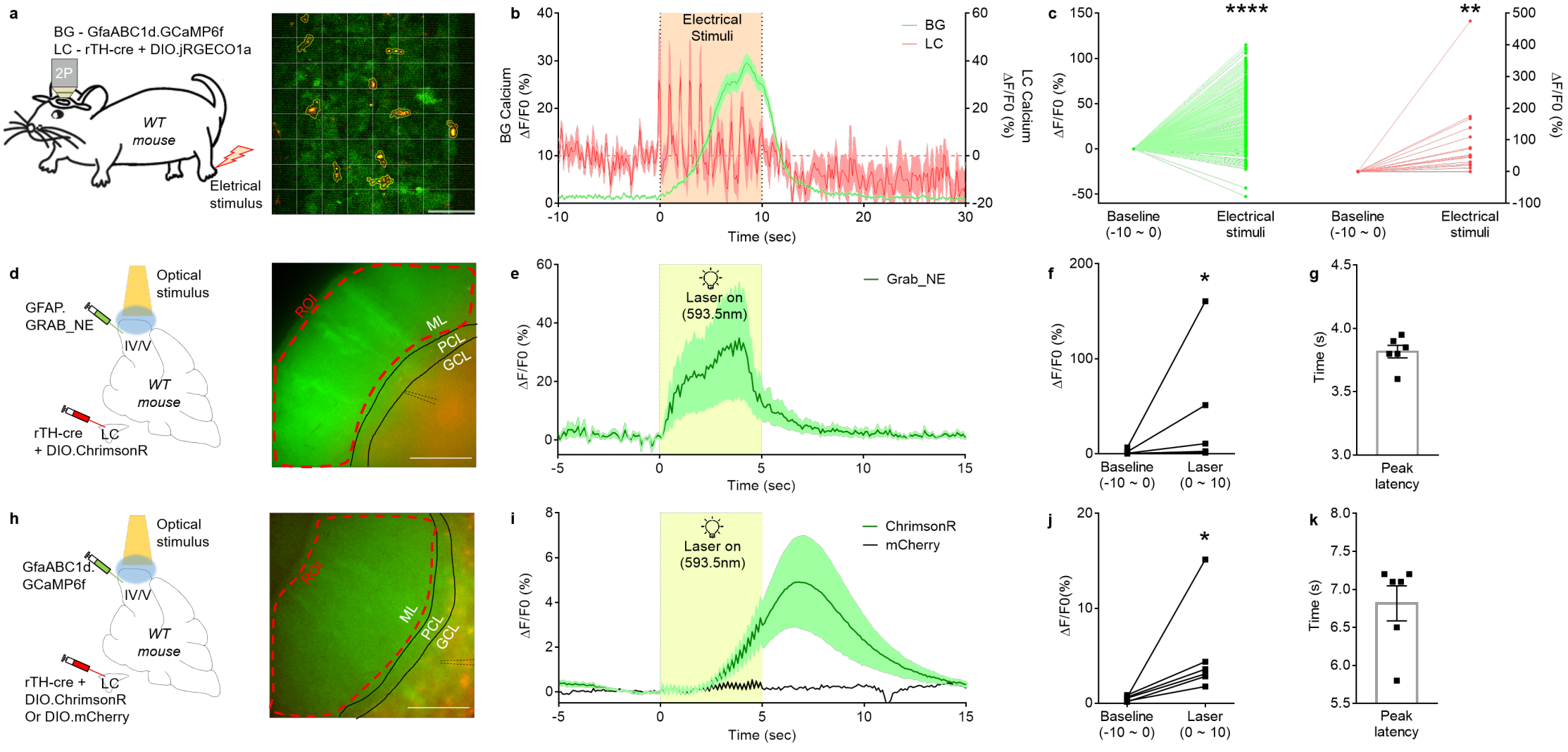


Fig.3: Capsaicin induces global BG calcium response (flare) associated with pain behavior

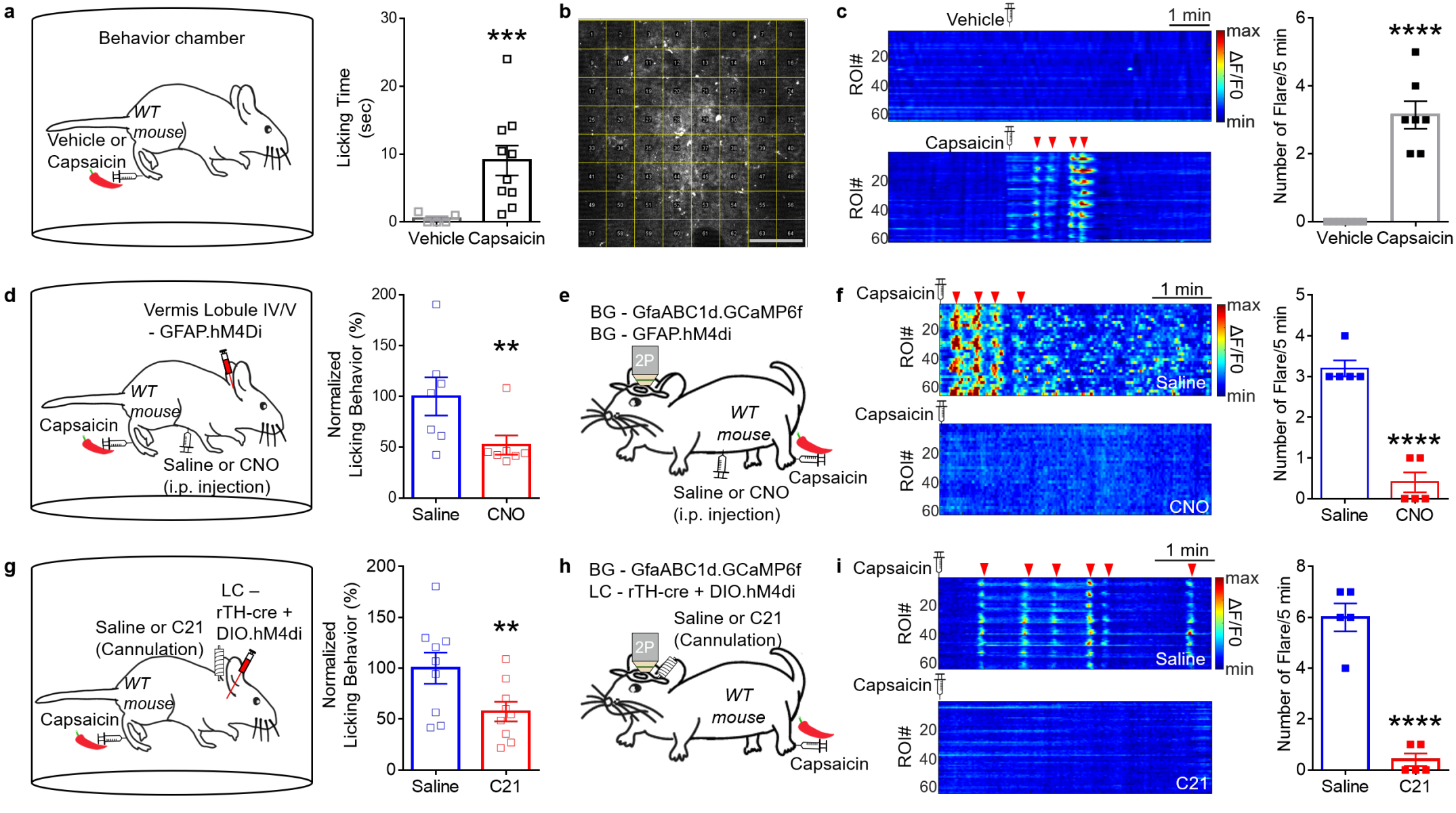


Fig.4: α1-AR mediates capsaicin-induced BG calcium flare and pain behavior

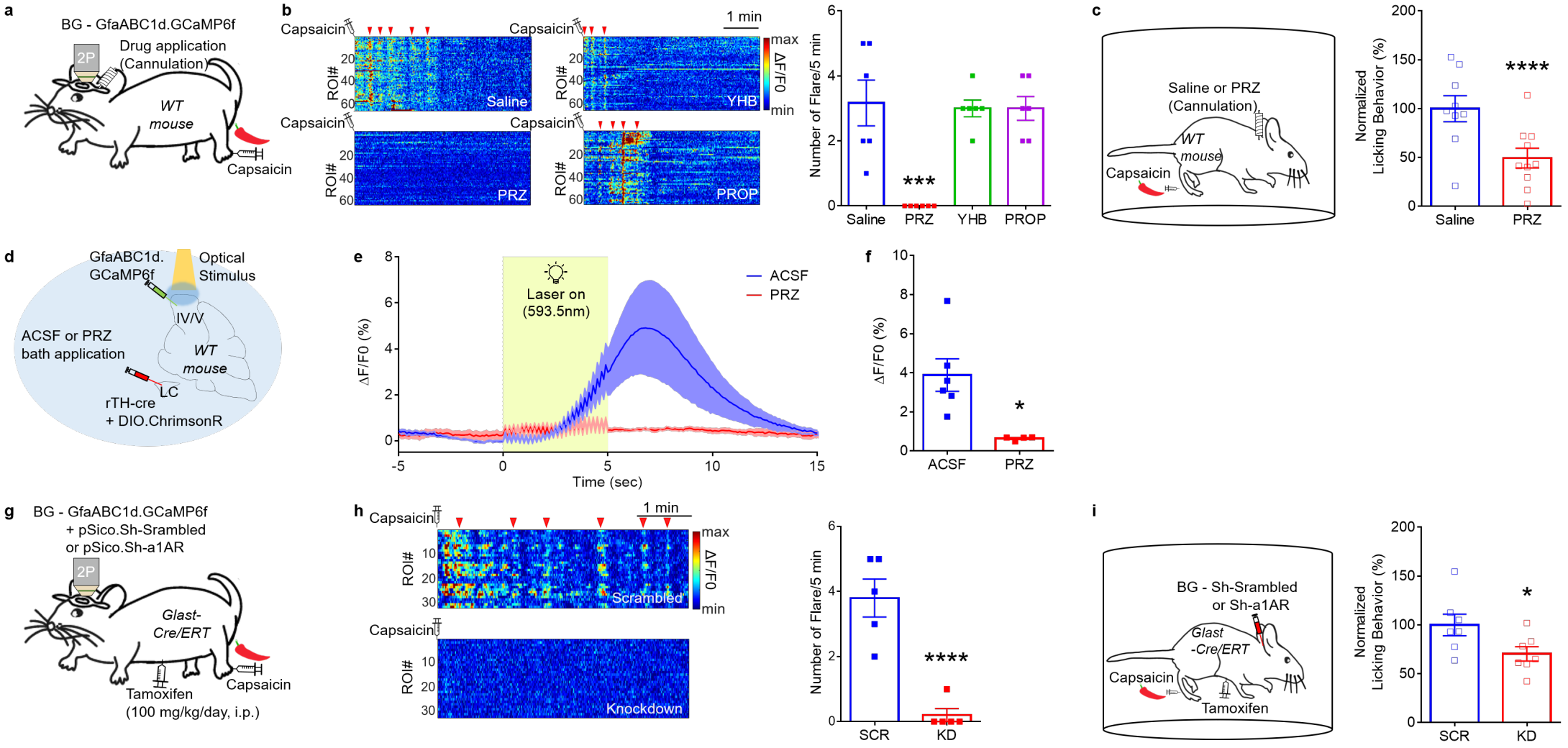
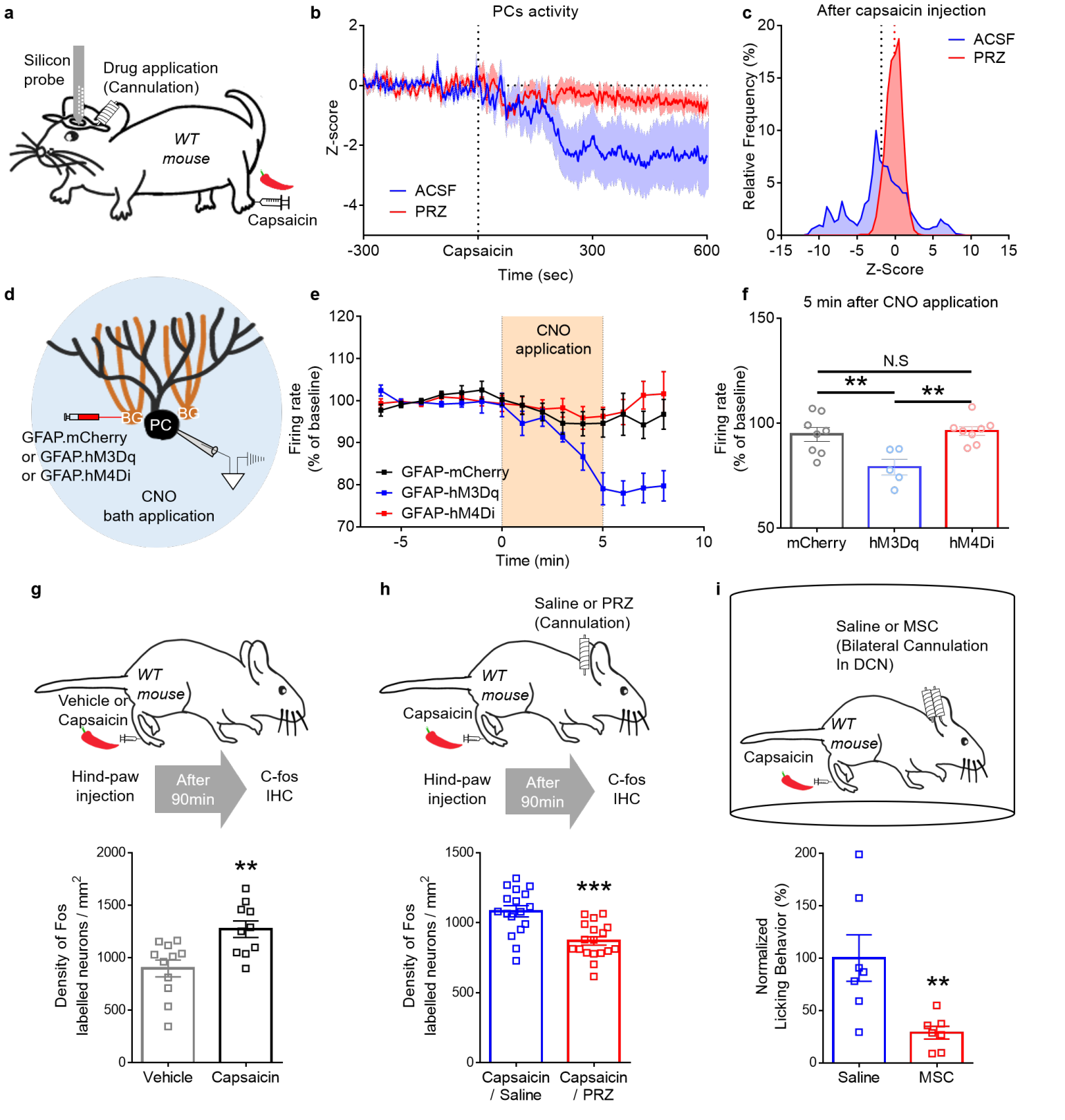


Fig.5: Manipulation of BG calcium regulates Purkinje cell activity and pain behavior



Materials and methods

Key resources table

Reagent type(species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, Strain background *Mus Musculus*	Tg(Slc1a3-cre/ERT)1Nat/J GLAST-CreER	Jackson Laboratory	RRID: IMSR_JAX:012586	Stock no: 012586
Strain, Strain background *Mus Musculus*	Glast-CreERT2	doi: 10.1002/glia.20350. [1]		
Virus	AAV5.gfaABC1D.lck-GCaMP6f	Addgene	RRID: Addgene_52924	Plasmid #52924
Virus	AAV1.CAG.Flex.Nes-jRGECO1a.WPRE.SV40	Addgene	RRID: Addgene_100852	Plasmid #100852
Virus	AAV1.CAG.Flex.GCaMP6s.WPRE.SV40	Addgene	RRID: Addgene_100842	Plasmid #100842
Virus	AAV9.rTH.PI.Cre.SV40	Addgene	RRID: Addgene_107788	Plasmid #107788
Virus	AAV5.Syn.FLEX.rc[ChrimsonR-tdTomato]	Addgene	RRID: Addgene_62723	Plasmid #62723
Virus	AAV5.GFAP104.mCherry	Addgene	RRID: Addgene_58909	Plasmid #58909
Virus	AAV9.hSyn.DIO.mCherry	Addgene	RRID: Addgene_50459	Plasmid #50459
Virus	AAV5.GFAP.hM3D(Gq).mCherry	Addgene	RRID: Addgene_50478	Plasmid #50478
Virus	AAV5.GFAP.hM4D(Gi).mCherry	Addgene	RRID: Addgene_50479	Plasmid #50479
Virus	AAV9.hSyn.DIO.hM4D(Gi).mCherry	Addgene	RRID: Addgene_44362	Plasmid #44362
Virus	AAV5.pSico.α1AR.shRNA.tdTomata	KIST virus facility		Fig. S6
Virus	AAV.GFAP104.GRAB_NE3.1	IBS virus facility		
Antibodies	Recombinant Anti-Dopamine beta Hydroxylase(DBH) antibody	Abcam		Cat no: ab209487
Antibodies	Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	RRID: AB_143165	Cat no: A11008
Antibodies	c-Fos (9F6) Rabbit mAb #2250	Cell Signaling Technology	RRID: AB_2247211	Cat no: 2250
Antibodies	ABC-HRP Kit, Peroxidase (Rabbit IgG)	VECTOR Laboratories	RRID: AB_2336810	Cat no: PK-4001

Antibodies	DAB Substrate Kit, Peroxidase (HRP), with Nickel, (3,3'-diaminobenzidine)	VECTOR Laboratories	RRID: AB_2336382	Cat no: SK-4100
Antibodies	Anti-alpha 1 Adrenergic Receptor antibody (adra1)	abcam	RRID: AB_2747703	Cat no: ab166925
Antibodies	Anti-Glyceraldehyde-3-Phosphate Dehydrogenase Antibody, clone 6C5 (GAPDH)	Santa Cruz	RRID: AB_627679	Cat no: SC-32233
Antibodies	Donkey Anti-Goat IgG H&L (HRP)	abcam		Cat no: ab205723
Antibodies	Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP	invitrogen	RRID: AB_2536527	Cat no: G-21040
Chemical compound, drug	Zoletil	Virvac		
Chemical compound, drug	Rompun	Bayer		
Chemical compound, drug	Dexamethasone	Samyang Phamaceutical		
Chemical compound, drug	Meloxicam	Boehringer Ingelheim		
Chemical compound, drug	DREADD agonist 21(Compound 21)	Hellobio	CAS no: 2250025-92-2	Cat no: HB6124
Chemical compound, drug	Clozapine N-oxide (CNO) dihydrochloride	Hellobio	CAS no: 2250025-93-3	Cat no: HB6149
Chemical compound, drug	Capsaicin	Sigma Aldrich	CAS no: 404-86-4	Cat no: HB1179
Chemical compound, drug	Prazosin hydrochloride	Sigma Aldrich	CAS no: 19237-84-4	Cat no: P7791
Chemical compound, drug	Yohimbine hydrochloride	Sigma Aldrich	CAS no: 65-19-0	Cat no: Y3125
Chemical compound, drug	Propranolol hydrochloride	Sigma Aldrich	CAS no: 318-98-9	Cat no: P0884
Chemical compound, drug	Muscimol	Sigma Aldrich	CAS no: 2763-96-4	Cat no: M1523
Chemical compound, drug	Tamoxifen	Sigma Aldrich	CAS no: 10540-29-1	Cat no: T5648
Chemical compound, drug	Sunflower seed oil	Santa Cruz Biotechnology	CAS no: 8001-21-6	Cat no: sc-215936
Software, algorithm	ZEN	Zeiss	RRID: SCR_013672	

Software, algorithm	VisiView	Visitron Systems		
Software, algorithm	MATLAB	Mathwroks Inc	RRID: SCR_001622	
Software, algorithm	CNMF_E	https://github.com/zhoupc/CNMF_E		
Software, algorithm	Fiji	http://fiji.sc	RRID: SCR_002285	
Software, algorithm	Qupath0.3.0	https://qupath.github.io/	RRID: SCR_018257	
Software, algorithm	Patchmaster	HEKA	RRID: SCR_000034	
Software, algorithm	Igor Pro	WaveMetrics	RRID: SCR_000325	
Software, algorithm	Mountainsort4	https://github.com/magland/mountainsort4		
Software, algorithm	phy	https://github.com/cortex-lab/phy		
Software, algorithm	GraphPad Prism	GraphPad	RRID: SCR_002798	
Software, algorithm	Ethovision XT 11.5	Noldus	RRID: SCR_000441	
Software, algorithm	LabChart 7	ADinstruments		Powerlab software

Animals, craniotomy

The experimental processes were approved by the Seoul National University Institutional Animal Care and Use Committee and performed under the guidelines of the National Institutes of Health and the Animal Care Committee of Yamanashi University (Chuo, Yamanashi, Japan). Seven- to ten-week-old wild-type or astrocyte-specific Tamoxifen inducible cre expressing mouse line, TG(Slca3-cre/ERT)1Nat/J (Jackson Laboratory, ME, USA) as known as Glast-CreER. Glast-CreERT2 mice were provided by Dr. Magdalena Götz (Helmholz Center Munich, Institute of Stem Cell Research, Munich, Germany) and by Dr. K. Tanaka (Tokyo Medical and Dental University, Tokyo, Japan) [1]. mice were anesthetized with intraperitoneal injections of Zoletil/Rompun mixture (30 mg / 10 mg/kg) or under 1.5-2 % isoflurane. A small craniotomy was made over lobule 4/5 of the cerebellar vermis/paravermis according to previous descriptions but with some modifications [2, 3]. In short, after placing the anesthetized mouse on a stereotaxic frame (Narishige, Tokyo, Japan), the skin was incised, and bone was removed with a no.11 surgical blade. To minimize edema and related inflammation, dexamethasone (0.2 mg/kg) and meloxicam (20 mg/kg) were administered by subcutaneous injection. A metal ring for head fixation was attached with Superbond dental cement (Sun Medical, Japan). Then, a 1.4 × 2.3 mm size glass coverslip (Matsunami, Japan) was tightly placed on the cortex and fixed by applying cyanoacrylate glue (Vetbond, 3M), as previously described [3].

Viral Expression and injection

For BG-specific GECI expression, 100-200 nl virus solution of 3-5 × 10^{10} genome copies containing AAV5.gfaABC1d.Lck-GCaMP6f (Addgene, MA, USA) were injected at two or three sites in the craniotomy site at the cerebellar cortex (Vermis Lobule 4/5) of the WT mice with a beveled glass pipette (5 MΩ). For GECI expression in LC, the virus was injected into the bilateral LC by making a small hole in the skull (posteriorly 5.4 mm and bilaterally 1.1 mm from bregma) with a dental drill. The glass pipette was set vertical angle for the skull. After approaching a 3.5 mm depth, virus solution containing 100-200 nl mixture of AAV9.rTH.PI.Cre.SV40 and AAV1.CAG.GCaMP6s.WPRE.SV40 or AAV1.CAG.Flex.Nes-jRGECO1a.WPRE.SV40 (Addgene, MA, USA) was injected with a Picopump at 5 nl/sec. The pipettes were left in place for 10 min before they were removed to minimize backflowing. For red-shift GECI expression in BG, AAV1.CAG.Flex.Nes-jRGECO1a.WPRE.SV40 was injected into Glast-CreER (Jackson Laboratory, ME, USA) mice. For NE sensor expression in BG, AAV.GFAP104.GRAB_NE3.1 (IBS virus facility, Daejeon, Korea) was injected into wild-type mice. Grab_NE working mechanism has been described previously [4]. For Chemogenetic expression in BG, AAV5.GFAP.hM3D(Gq).mCherry or AAV5.GFAP.hM4D(Gi).mCherry was injected into wild-type mice. For mCherry expression in BG, AAV5.GFAP104.mCherry was injected into wild-type mice. For Chemogenetic expression in LC, a mixture of AAV9.rTH.PI.Cre.SV and AAV9.hSyn.DIO.hM4D(Gi).mCherry was injected into wild-type mice. For Optogenetic expression in LC, a mixture of AAV9.rTH.PI.Cre.SV and AAV5.Syn.FLEX.rc[ChrimosonR-tdTomato] was injected into wild-type mice. For mCherry expression in LC, a mixture of AAV9.rTH.PI.Cre.SV40 and AAV9.hSyn.DIO.mCherry was injected into wild-type mice. For α1-AR knockdown expression in BG, AAV5.pSico. α1AR.shRNA.tdTomato (KIST virus facility, Seoul, Korea) was was injected into cre-activated Glast-CreER or Glast-CreERT2 mice by repeating tamoxifen (100 mg/kg) for 5 days.

Two-photon microscopy and chronic window imaging

In vivo imaging experiments of genetic manipulation model using Glast-CreERT2 were performed using an Olympus Fluoview FVMPE-RS two-photon laser scanning microscope (Olympus, Tokyo, Japan) equipped with Maitai HP DS-OL (Spectra-Physics, Santa Clara, CA) and InSight DS-OL (Spectra-Physics). We used the laser at 920 nm and a 495-540 nm bandpass pass emission filter for GCaMP6f and the laser at 1100 nm and a 575-645 bandpass emission filter for tdTomato. Images were gathered with a 25 × water immersion lens (XPLN25XWMP2) with a numerical aperture of 1.05 (Olympus). All other experiments were performed with confocal microscopy was performed with a laser scanning multiphoton microscope (Zeiss LSM 7 MP, Carl Zeiss, Jena, Germany) equipped with non-descanned detectors (NDD). Excitation was carried out with a mode-locked titanium:sapphire laser system (Chameleon, Coherent, Santa Clara, CA, USA) operating at a 900 nm wavelength for GCaMP6 and GRAB_NE using GFP filter and 980 nm for jRGECO1a and mCherry using RFP filter. Generally, objective W Plan-Apochromat 20×, 1.0 numerical aperture (Carl Zeiss) was used. Images were acquired using ZEN software (Zeiss Efficient Navigation, Carl Zeiss) and processed using a custom-written MATLAB (MathWorks) script. High-resolution of 512 × 512-pixel reference images were acquired at a rate of 8 s per frame in the molecular layer (around 20–60 μm from the dura). For BG Ca^{2+} imaging, 2-5 Hz speed time-lapse scanning was performed with a 512 × 512 resolution window at 50-100 μm from the dura. For LC terminal Ca^{2+} imaging, 5-8 Hz speed time-lapse scanning was performed with a 512 × 256 or 512 × 512 resolution window at 50-100 μm from the dura. For chronic window imaging, 14-21 days after chronic window surgery, the mice were imaged under 1-1.5 % isoflurane anesthesia with head-fixed using a clamp and custom-made metal rings.

BG and LC ROI detection and calcium analysis

Since it is difficult to specify each BG process, an ROI was constructed with 64 grids for 512-512-pixel images and 32 grids for 512-256-pixel images and analyzed [5]. CNMF_E was used to select ROIs for the LC terminal in the cerebellar vermis lobule 4/5 [6] and coordinate ROI values obtained through CNMF_E were confirmed through image j, and ROIs in the same location were manually combined (Fig. S8). Customized scripts in MATLAB were used to analyze the calcium transient signals. Calcium signal amplitudes were calculated as $\Delta F/F0$ ($\Delta F = F - F0$) for each cell. F0 means the baseline fluorescence signal calculated by averaging the lowest 10 % of all fluorescence signals from individual traces. The change value of the maximum value of calcium signal from noxious electrical stimulation was confirmed by comparing it with the baseline. To characterize the flare, BG calcium activation was confirmed through noxious electrical stimuli before capsaicin-induced pain experiments. After confirming the calcium response, the period of at least 60 % synchronization and calcium activation as much as the amount of calcium from electrical stimulation of 5-10 seconds or more is specified as a flare.

Noxious Stimulation

To deliver noxious electrical stimulation, a needle with a blunt tip is inserted into the plantar side of the hind paw, and stimulation with 3 Hz frequency, 0.2 ms duration, and 0, 1, 10 mA power is provided for 10 seconds using Powerlab 4/25T (ADInstruments, New South Wales, Australia) device.

For actual pain stimulation, 0.01% capsaicin solution dissolved in 20 % ethanol (in PBS) was used. Licking behavior was observed when 10 μl capsaicin solution was injected into the hind paws, which disappeared within 10 minutes each, respectively.

Drug Application

A unilateral cannula was installed to inject the drug only into the target site, cerebellar vermis lobule 4/5 (-6.0 mm anteroposterior from bregma, midline). Drugs such as AR blocker (Prazosin, 100 μM; yohimbine, 100 μM; propranolol, 100 μM) or C21 (1 mM) used in the experiment were injected through this cannula with a syringe pump (Pump 11 Elite Infusion Only; Harvard apparatus), and 0.5 μl was slowly injected for about 10 minutes.

A bilateral cannula was installed to inject the drug only into the target site, deep cerebellar nuclei (-6.2 mm anteroposterior from bregma, ±1 mm mediolateral from bregma, -3.2 mm dorsoventral from the surface of skull). Drugs such as GABA A agonist (Muscimol, 100 μM) used in the experiment were injected through this cannula with a syringe pump (Pump 11 Elite Infusion Only; Harvard apparatus), and 0.5 μl was slowly injected for about 10 minutes.

Chemogenetic modulation

For BG chemogenetic modulation, BG-specific virus AAV5.GFAP.hM4D(Gi).mCherry, was injected into cerebellar vermis lobule 4/5. To prevent systemic activation by intraperitoneal injection of CNO (5 mg/kg), the virus was locally injected into the cerebellar vermis lobule 4/5 and acted only on the expressed lobules. Behavioral experiments and calcium imaging were performed 1hr after CNO intraperitoneal injection by validation of hM4Di activation (Fig. S2).

For LC chemogenetic modulation, LC-specific virus, mixture AAV9.rTH.PI.CRE.SV40 and AAV9.hSyn.hM4D(Gi).mCherry, was injected into LC. To prevent systemic activation by C21, a cannula

was installed in the cerebellar vermis lobule 4/5, and C21 (1 mM, 0.5 μl) was locally infused through the cannula. Then, the virus is expressed in the whole brain, but the effect of C21 appears only locally in the cerebellum. Behavioral experiments and calcium imaging were performed 10 min after C21 infusion.

To determine the effect on PC firing through BG chemogenetic modulation, we measured spontaneous PC firing rate from cerebellar slices with CNO application. CNO (10 μM) was perfused for 5 min following a 5 min baseline period.

For BG-specific Gq activation of cerebellar vermis lobule 4/5, AAV5-GFAP.hM3D(Gq).mCherry, was injected. In the same way as BG hM4Di activation, CNO was administered intraperitoneally, and behavioral experiments were performed 1 hour later (Fig. S4).

For BG-specific Gq activation of other regions in the cerebellum, we selected crus 1/2 in the cerebrocerebellar region and cerebellar vermis lobule 9/10 in the vestibulocerebellar region and injected the virus into each of these regions. As with the above BG chemogenetic modulation, the behavioral experiment was performed 1 hour after CNO intraperitoneal administration (Fig. S5).

Behavioral test

Capsaicin-induced pain test

To measure capsaicin-induced licking behavior, mice were placed in a cylindrical chamber with only the bottom transparent, and the bottom surface was acquired with a camera. After putting a mouse in the chamber and measuring the baseline for 10 minutes, the mouse was taken out and capsaicin is quickly injected into the hind paw, then put back into the chamber and taken for an additional 20 minutes. The first 10 minutes of the 20 minutes indicate the capsaicin phase, and after the 10 minutes indicate the recovery phase. We watched the video we recorded and manually recorded how many licks the injected paw was.

Open Field Test

Place the mouse in a chamber 40 cm x 40 cm, acquired video for 20 minutes, and measure the total distance traveled between various measurable indicators. In this case, the mouse should be the first time to experience the chamber. Mice behavior was recorded using a mouse-tracking software (EthoVision XT 11.5, Noldus, Netherlands)

Gait test, and Ledge test

This method was used with a slight modification of the previously described method [7]. The behavioral patterns of mice were scored on a scale of 0-3, with 3 as normal to 0 as abnormal. First, for measuring the Gait, place the mouse on a flat surface with its head facing away from the investigator and observe the mouse from behind as it walks. Scoring is done according to the following criteria. Score 3 – the mouse moves normally, with its body weight supported on all limbs, with its abdomen not touching the ground, and with both hindlimbs participating evenly, Score 2 - it shows a tremor or appears to limp while walking, Score 1 - it shows a severe tremor, severe limp, lowered pelvis, or the feet point away from the body during locomotion ("duck feet"), Score 0 – the mouse has difficulty moving forward and drags its abdomen along the ground. Next, for measuring the Ledge test, Place the mouse on the cage's ledge and observe the mouse as it walks along the cage ledge and lowers itself into its cage. Scoring is done according to the following criteria. Score 3 – walk along the ledge without losing its balance, and will lower itself back into the cage gracefully, using its paws, Score 2 – the mouse loses its footing while walking along the ledge, but otherwise appears coordinated, Score 1 – it does not effectively use its hind legs, or lands on its head rather than its paws when descending into the cage, Score 0 – it falls off the

ledge, or nearly so, while walking or attempting to lower itself, or shakes and refuses to move at all despite encouragement

Behavioral indicators were normalized according to capsaicin and each control group. All experiments were performed in a blinded manner, comparing the control group with the experimental group, respectively (Fig. S7).

Slice electrophysiology, optogenetic modulation, and imaging

An acute brain slice preparation and an electrophysiological experiment were carried out as previously described [8]. 5 to 9-week-old mice were anesthetized by isoflurane and briefly decapitated. Then, 250 μm-thick sagittal slices of the cerebellar vermis were obtained from mice using a vibratome (VT1200S, Leica). The ice-cold cutting solution contained 75 mM sucrose, 75 mM NaCl, 2.5 mM KCl, 7 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 25 mM glucose with bubbled 95% O_2 and 5% CO_2. The slices were immediately moved to artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM glucose with bubbled 95 % O_2 and 5 % CO_2. Then, they were recovered at 32 °C for 30 min and at room temperature for 1 hr. All recordings were performed within 8 hr after recovery.

The brain slices were placed in a submerged chamber with perfusion of ACSF for at least 10 min before recording. Cell-attached recordings were made at 29.5-30 °C. We used recording pipettes (3–4 MΩ) filled with (in mM): 9 KCl, 10 KOH, 120 K-gluconate, 3.48 $MgCl_2$, 10 HEPES, 4 NaCl, 4 Na_2ATP, 0.4 Na_3GTP, and 17.5 sucrose (pH 7.25). Electrophysiological data were acquired using an EPC9 patch-clamp amplifier (HEKA Elektronik) and PatchMaster software (HEKA Elektronik) with a sampling frequency of 20 kHz, and the signals were filtered at 2 kHz. All electrophysiological recordings were acquired in lobule 4/5 of the cerebellar central vermis. The spontaneous firing rate was analyzed using Igor Pro (WaveMetrics) and normalized by the average firing rate during the baseline period.

For BG Ca^{2+} or NE sensor imaging with LC optogenetic modulation and validation, slices were prepared from mice that had AAV5.gfaABC1d.Lck-GCaMP6f or AAV.GFAP104.GRAB_NE3.1 injected into their cerebellar vermis lobule 4/5, and AAV9.rTH.PI.Cre.SV40 and AAV5.Syn.FLEX.rc[ChrimsonR-tdTomato] was injected into their LC 3-4 weeks before the preparation. Using OptoPatcher (A-M systems) for slice recording with optogenetic manipulation, a 593.5 nm laser was used with 0-10 mW power, 1-5 Hz frequency, 5 sec duration, and then the signal from BG was acquired at 5 Hz with a scientific CMOS camera (Prime, Photometrics). All experiments were performed in duplicate or triplicate and traces of each manually selected cell were analyzed. Fluorescence images were acquired with VisiView software (Visitron Systems GmbH, Germany). The ROIs were manually detected in a field of view (Molecular layers within the image), and signals were extracted and converted to a normalized value (dF/F0). The value of F0 was calculated using the median value of the 5 and 15 percentile fluorescence in a sliding time window by each ROI.

In Vivo Electrophysiology

In vivo recordings were performed using anesthetized, head-fixed mice. Cranial window (1 x 3 mm) was made at least 1 week after cannula and ring implant under 1-1.5 % isoflurane. After head fixation, a custom-made 32-channel silicon probe was gradually inserted into vermis lobule 4/5 using a single-axis manipulator (SOLO, Sutter instrument). After confirming PC activities by complex spikes, ACSF or

CNO was injected through a cannula. After at least 30 min of stable recording, capsaicin was injected at the hind paw ipsilateral side of the recording site. Single units were isolated using Mountainsort4 and manually curated using phy. Two types of band-pass filters were used for detecting simple spikes and complex spikes, 300-6000 Hz and 10-200 Hz, respectively. The firing rate was normalized to baseline activity before capsaicin injection using a z-score transformation. Increase or decrease units were defined as an average z-score after capsaicin injection > 3 or < -3.

Immunohistochemistry

Anesthetized LC specific mCherry expressing mice were perfused with PBS and again with 4 % paraformaldehyde (PFA; T&I). Brains were taken out and fixed in 4 % PFA overnight. After embedding the Frozen Section Compound FSC; LeicaBiosystems), we obtained 30-μm-thick sagittal slices on slides by using Cryotome (HM525 NX; Thermo Fisher Scientific). FSC was removed with PBS. Afterward, slices were permeabilization with a PBS-T (0.3 % Triton X-100) for 3 repetitions of 10 min at room temperature. Next, slices were blocked with a serum solution containing PBS-T and 5 % Bovine Serum Albumin BSA; Sigma Aldrich) for 3 repetitions of 1hr at room temperature. The slices were then incubated overnight at 4 °C with diluted primary antibody, anti-Dopamine beta-hydroxylase (anti-Rabbit, 1:500; Abcam). After washing in PBS-T, a fluorescence-conjugated secondary antibody, Alexa Fluor-488 (anti-rabbit, 1:500; Invitrogen), was used to treat the slices for 2 hr at room temperature. Primary antibodies and secondary antibodies were diluted in a blocking solution, respectively. Images were acquired and processed using a confocal microscope (Zeiss LSM 7 MP, Carl Zeiss, Jena, Germany) and Zen software (Zeiss).

Estimation of c-fos expression

To measure the c-fos expression in the DCN, DAB staining was performed following the manufacturer's instructions (Vector Laboratories). It is mostly similar to the above-described immunohistochemistry, but there are some differences. First, Mice used for capsaicin-induced pain and prazosin cerebellar application experiments were perfused with PBS after 90 minutes of capsaicin injection with maximally increased c-fos. Second, the slices were then incubated for 48 hr at 4 °C with diluted primary antibody for c-fos (9F6) (anti-rabbit, 1:1000; Cell Signaling). Third, Signals were developed with the Vectastain ABC kit (PK-4001, Vector Laboratories), and DAB reagents (SK-4100, Vector Laboratories). Last, after washing in PBS and blocking with BSA, a biotinylated secondary antibody (anti-rabbit, 1:200; Vector Laboratories), was used to treat the slices for 24 hr at room temperature. Images were acquired and processed using a fluorescence microscope (IX53, Olympus), and scanned images were analyzed automatically using QuPath [9]. The measured image was 400 pixels (px) per 100 μm. The detection of nuclei was performed with the following settings: Requested pixel size Background radius 5 px, Median filter radius 3 px, Sigma 1.5 px, Minimum area 1 px^2, Maximum area 400 px^2, Intensity threshold 0.1, max background intensity 1.5-2, cell expansion 2 px split by shape. The numbers of cells and fluorescent spots were calculated through QuPath scripts. Estimation of the c-fos expression in the DCN was performed by counting the c-fos-positive cells per unit area (mm^2).

Western blotting

Cerebellar vermis lobule 4/5 was dissected in a BG-specific α1-AR KD model mouse (Fig. S6d-e). Each cerebellar vermis lobule 4/5 was homogenized in 75 μl lysis buffer containing 10 mM Tris-HCl (pH 6.8) buffer, 1.6 % SDS, protease inhibitor, and phosphatase inhibitors. Samples were run on a 12 % bis tris gel and transferred to a PVDF membrane for other proteins. After blocking in 3 % BSA in 0.1 % TBST,

membranes were probed with primary antibody (goat anti-ADRA1, 1:2000, Abcam, ab166925; mouse anti-GAPDH, 1:1,000, Santa Cruz Biotechnology, sc32233) overnight at 4 °C. After washing 3 times in 0.1 % TBST, membranes were probed with horseradish peroxidase-conjugated secondary IgG for 1 h at room temperature. Signals from membranes were detected by using an ECL chemiluminescence substrate kit (Thermo Pierce). Proteins were normalized to GAPDH, and phosphorylated proteins were normalized to their respective total proteins.

Statistics

Graph plotting and statistical analysis were carried out with GraphPad Prism (GraphPad Software Inc, CA). The hypothesis was tested by two-way ANOVA followed by post hoc Tukey's test, for multiple comparisons using either GraphPad Prism or MATLAB. Paired and unpaired t-tests between sample pairs were carried out. Results were considered significant if the P-value was below 0.05. Asterisks denoted in the graph indicate the statistical significance. * means p-value < 0.05, ** < 0.01, *** < 0.001, and **** < 0.0001. The test name and statistical values are presented in each figure legend.

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Fig. S1. Validation of optogenetic modulation in LC neuron-specific manner

a. Schematic diagram showing virus injection for validation of LC somatic optogenetic modulation. (n = 15 ROIs).

b. Validation of laser stimulation (593.5nm, 5 Hz, 5 sec) strength through LC soma activation and calcium response.

c. AUC according to laser stimulation power.

d. Peak of LC somatic calcium response according to laser stimulation power.

e. Validation of laser stimulation frequency (593.5nm, 10 mW, 5 sec) through LC soma activation and calcium response.

f. AUC according to laser stimulation frequency.

g. Peak of LC somatic calcium response according to laser stimulation frequency.

h. Schematic diagram showing virus injection for validation of LC terminal optogenetic modulation. (n = 13 ROIs).

i. Validation of laser stimulation frequency (593.5nm, 10 mW, 5 sec) through LC terminal activation and calcium response.

j. AUC according to laser stimulation frequency.

k. Peak of LC terminal calcium response according to laser stimulation frequency.

Fig. S2: Validation of BG hM4Di activation

a. Representative BG calcium response before and after (~3 hr) CNO i.p. injection during hind-paw electrical stimuli.

b. Schematic diagram showing *in vivo* two-photon BG calcium imaging using hind-paw electrical stimulation in BG hM4Di expressing mouse.

c. Virus expression in cerebellar vermis lobule 4/5.

d. Imaging schedule before and after CNO injection.

e and f. Changes in calcium response before and after CNO injection (using grid pattern; 8 by 4; n = 64 ROIs in 2 mice; **** p = 0.0001; One-way ANOVA).

Fig. S3: No change in PC firing rate through prazosin application

a. Plot showing PC spontaneous firing rate before (blue dots) and after prazosin application (red dots) (n = 1 slice).

b. Bar graph showing the Z-score of PC spontaneous firing rate before (blue) and after prazosin application (red) in vivo.

Fig. S4: Validation of BG-specific genetic manipulation

a-c. α1-AR KD development.

a. Representative calcium traces of glial cells of Scrambled group by PE and TFLLR application.

b. Representative calcium traces of glial cells of α1a-shRNA group by PE and TFLLR application.

c. Summarizing bar graph showing calcium transient amplitude of glial cells (n = 20 glial cells in Scrambled group; n = 45 glial cells in α1a-shRNA group; PE 100 μM, ** p = 0.0376; TFLLR 30 μM, N.S; Two-way A

NOVA).

d. A diagram for experimental design. BG-specific α1-AR KD virus was delivered and genetic knockdown was validated by western blot analysis. Cerebellar lobule 4/5 where the virus was delivered were selectively dissected.

e. Example images of dissection of cerebellar lobule 4/5.

f. Representative images (top) and its summarizing bar graph showing significant decrease of ADRA1 expression in KD compared to scrambled group. (Difference between groups = 0.67 ± 0.06; ** p = 0.0015; Unpaired t test).

Fig. S5: Locomotion was compatible among experimental groups

There was no significance in all experiments, and each group was as follows (Two-way ANOVA).

a. C21 local application of LC hM4Di group (n = 9 mice saline or C21).

b. CNO i.p. injection of BG hM4Di in cerebellar vermis lobule 4/5 group (n = 7 mice in saline or CNO group).

c. CNO i.p. injection of BG hM3Dq in cerebellar vermis lobule 4/5 group (n = 8 mice in saline group; n = 7mice in CNO group).

d. CNO i.p. injection of BG hM4Di in cerebellar Crus 1/2 group (n = 6 mice in saline or CNO group).

e. CNO i.p. injection of BG hM4Di in cerebellar vermis lobule 9/10 group (n = 7 mice in saline or CNO group).

f. BG specific α1-AR knockdown group (n = 8 mice in scrambled group; n = 7 mice in Knockdown group).

g. Prazosin application in cerebellar vermis lobule 4/5 (n = 6 mice in saline or Prazosin group).

h. Musimol application in DCN (n = 7 mice in saline or Muscimol group).

Fig. S6: Capsaicin-induced pain behavior during BG hM3Dq activation

a. A diagram for experimental design. Normalized percentage of capsaicin-induced licking behavior of saline and BG hM3Dq groups (Virus expression in cerebellar vermis lobule 4/5).

b. Virus expression in cerebellar vermis lobule 4/5.

c. Capsaicin-induced licking behavior in the presence of saline or CNO (n=9 mice for saline group; n=10 mice for CNO group; N.S., p=0.2985; Unpaired t-test). Scale bar, 500 μm.

Fig. S7: Capsaicin-induced pain behavior during BG hM4Di activation in other cerebellar regions

a. A diagram for experimental design. Normalized percentage of capsaicin-induced licking behavior of saline and BG hM4Di groups (Virus expression in cerebellar crus 1/2).

b. Virus expression in cerebellar crus 1/2.

c. Capsaicin-induced licking behavior in the presence of saline or CNO (n = 6 mice for each group; N.S., p = 0.6985; Unpaired t-test). Scale bar, 500 μm.

d. A diagram for experimental design. Normalized percentage of capsaicin-induced licking behavior of saline and BG hM4Di groups (Virus expression in cerebellar vermis lobule 9/10).

e. Virus expression in cerebellar vermis lobule 9/10.

f. Capsaicin-induced licking behavior in the presence of saline or CNO (n = 7 mice for each group; N.S., p =

0.7851; Unpaired t-test). Scale bar, 500 μm.

Fig. S8: ROI detection of LC terminal using the CNMF_E

Fig. S1. Validation of optogenetic modulation in LC neuron-specific manner

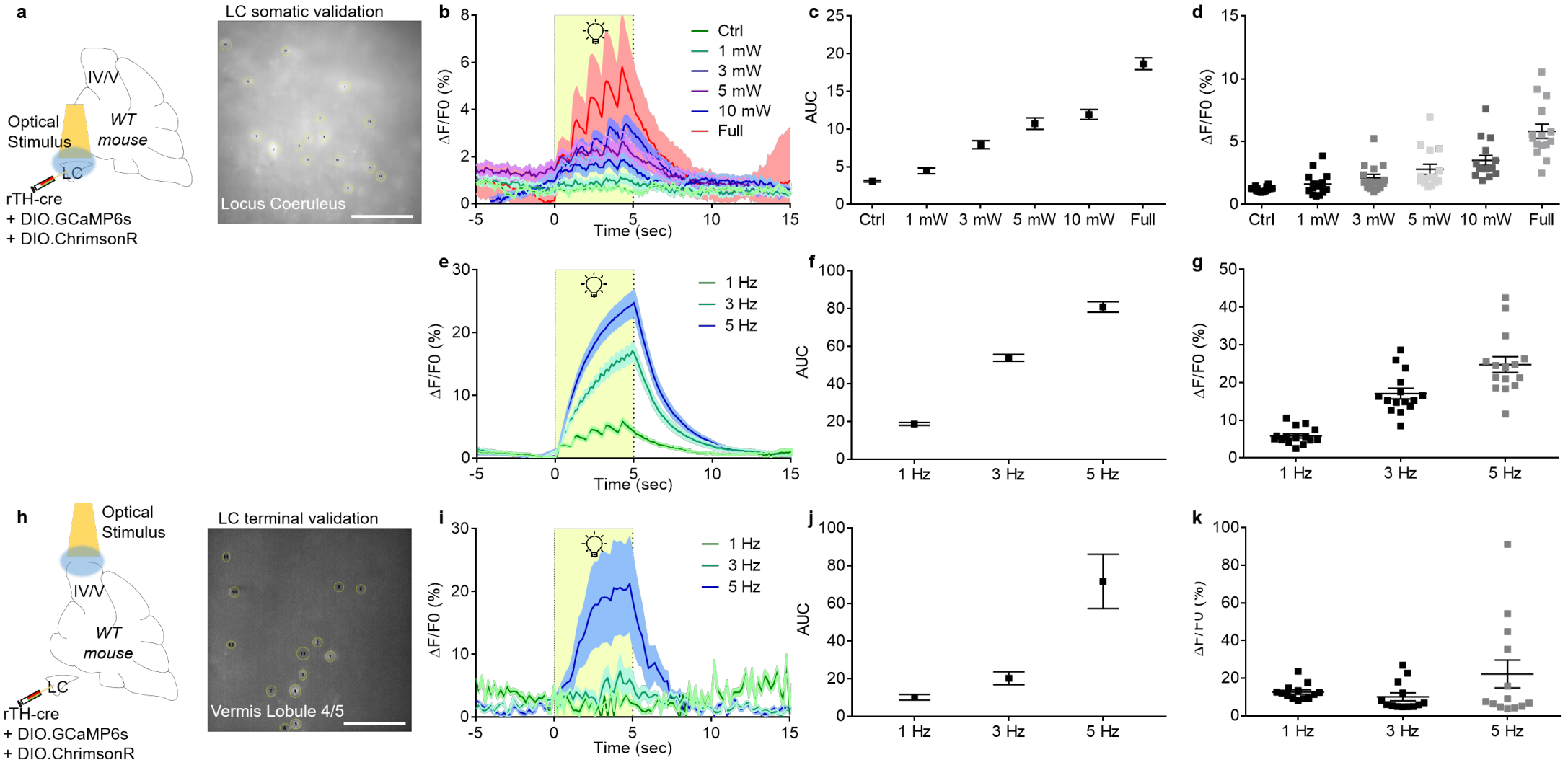


Fig. S2: Validation of BG hM4Di activation

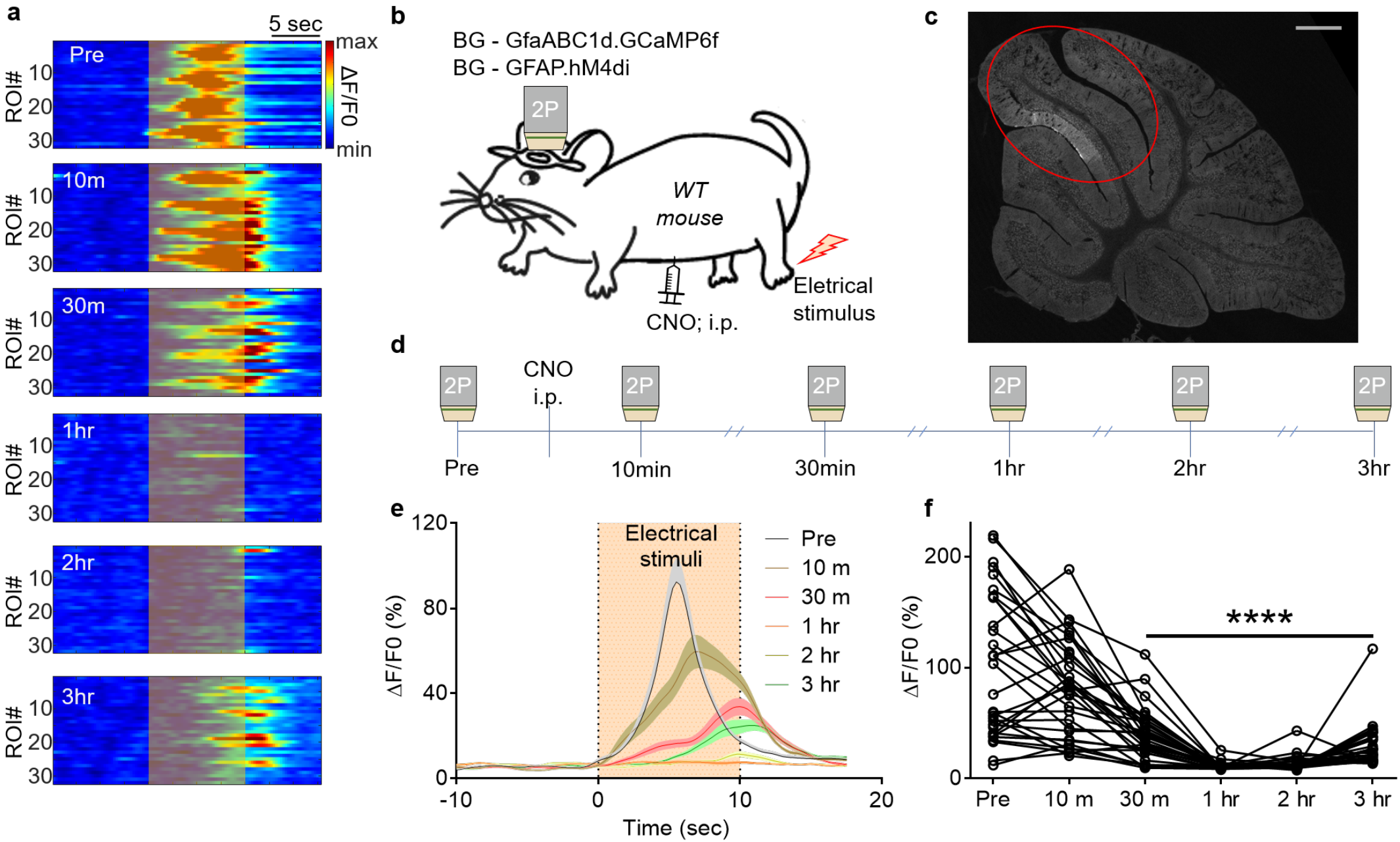


Fig. S3: No change in PC firing rate through prazosin application

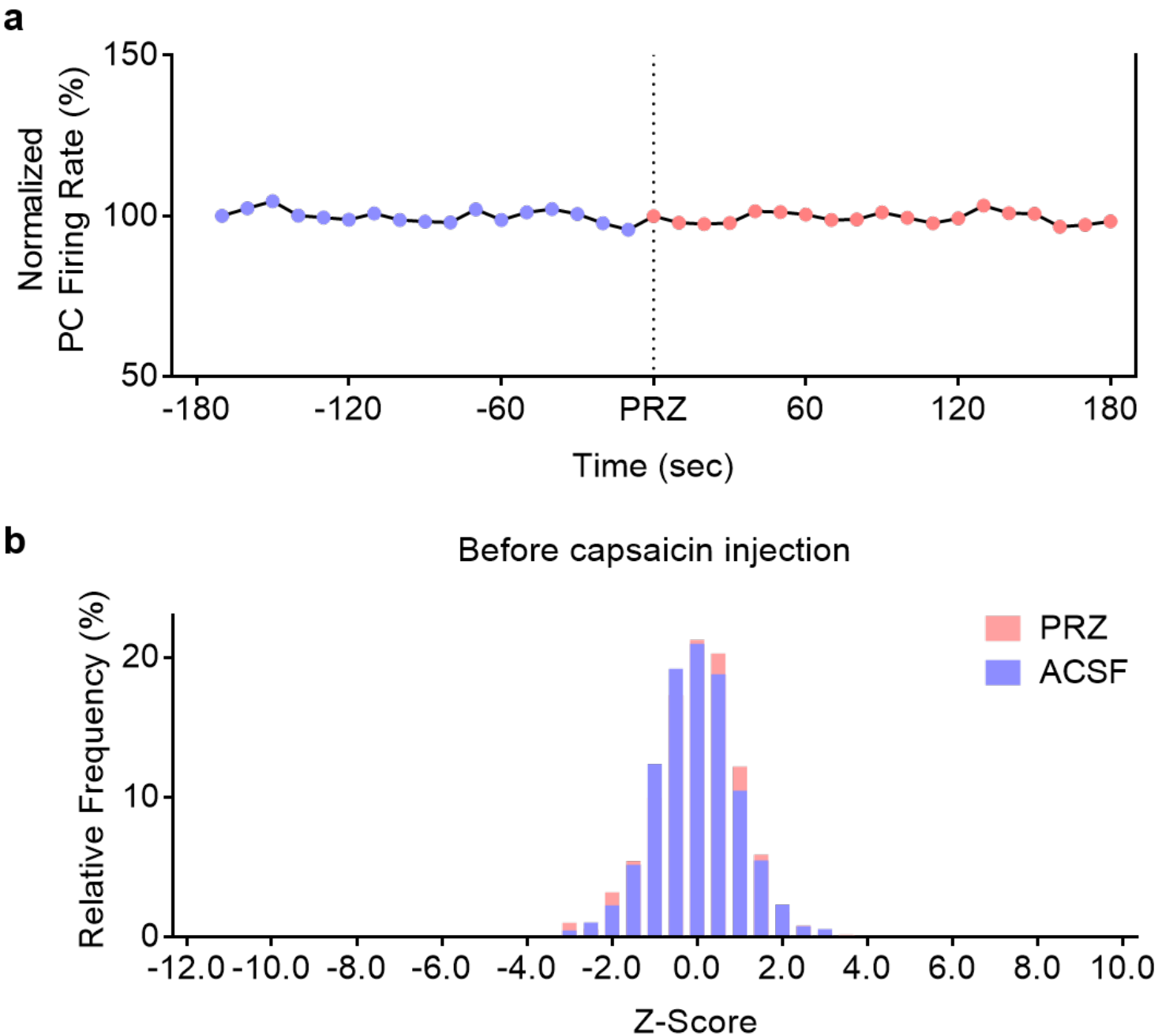
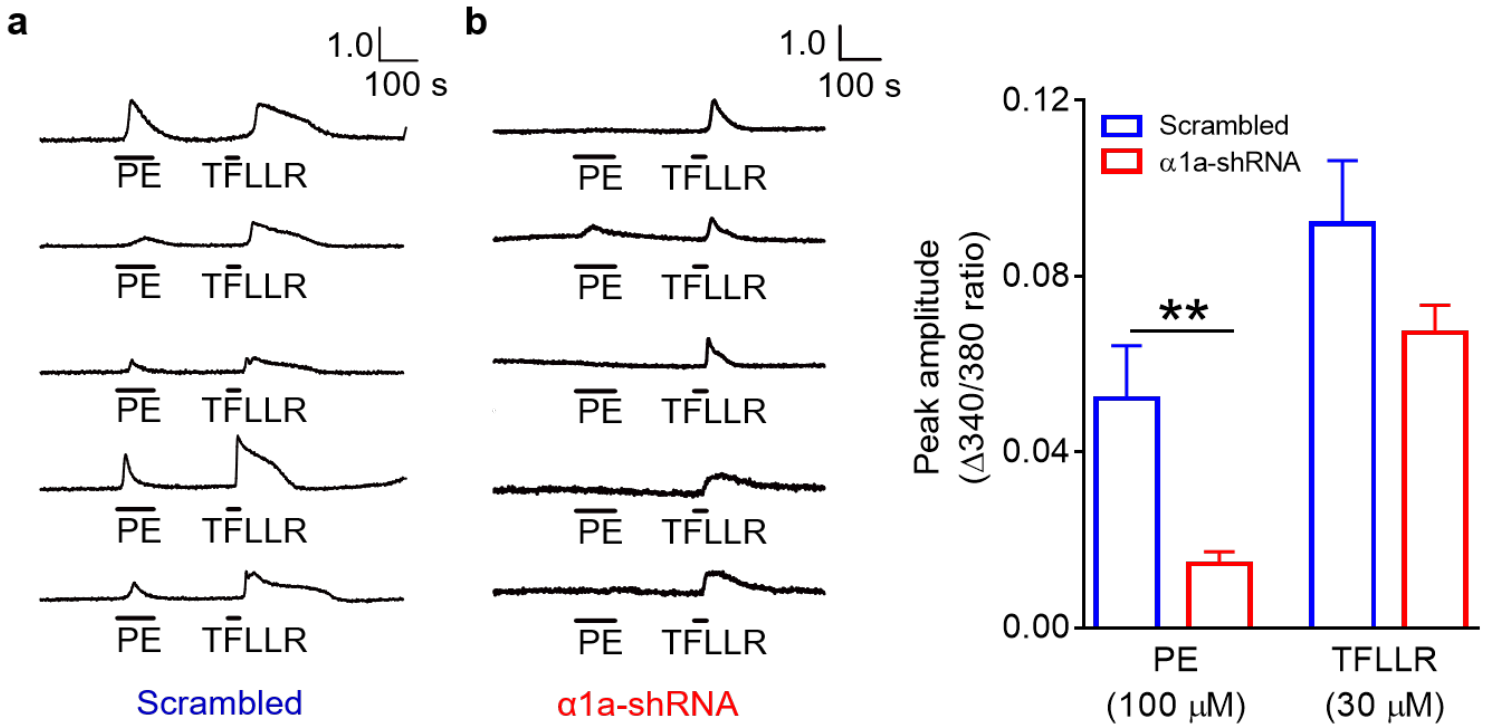
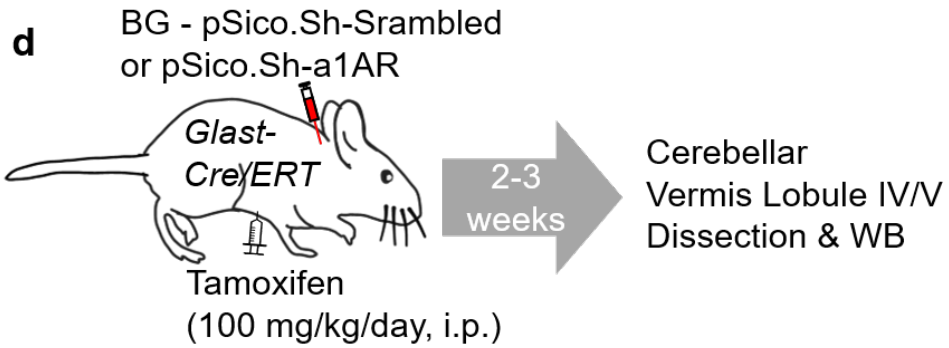
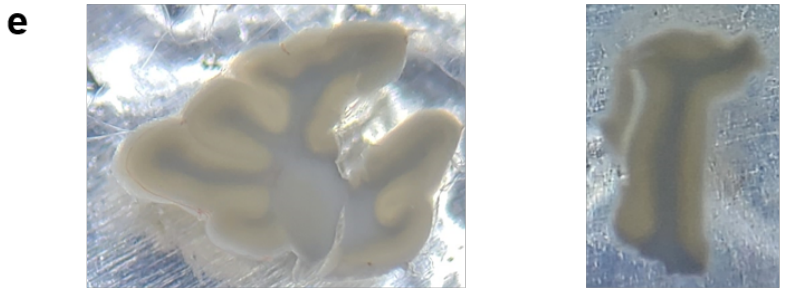


Fig. S4: Validation of BG-specific genetic manipulation







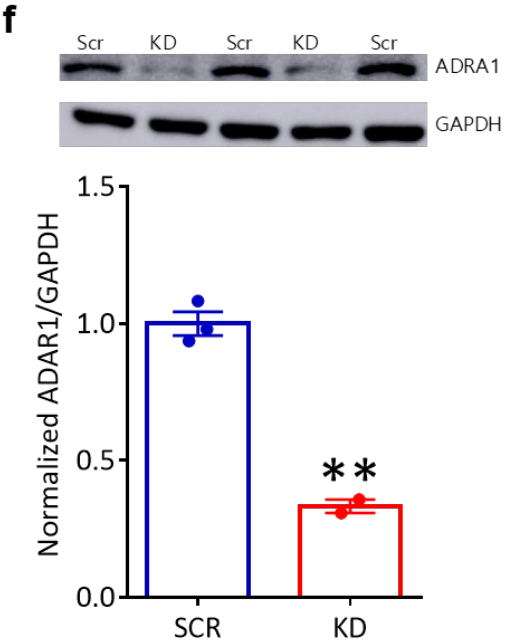


Fig. S5: Locomotion was compatible among experimental groups

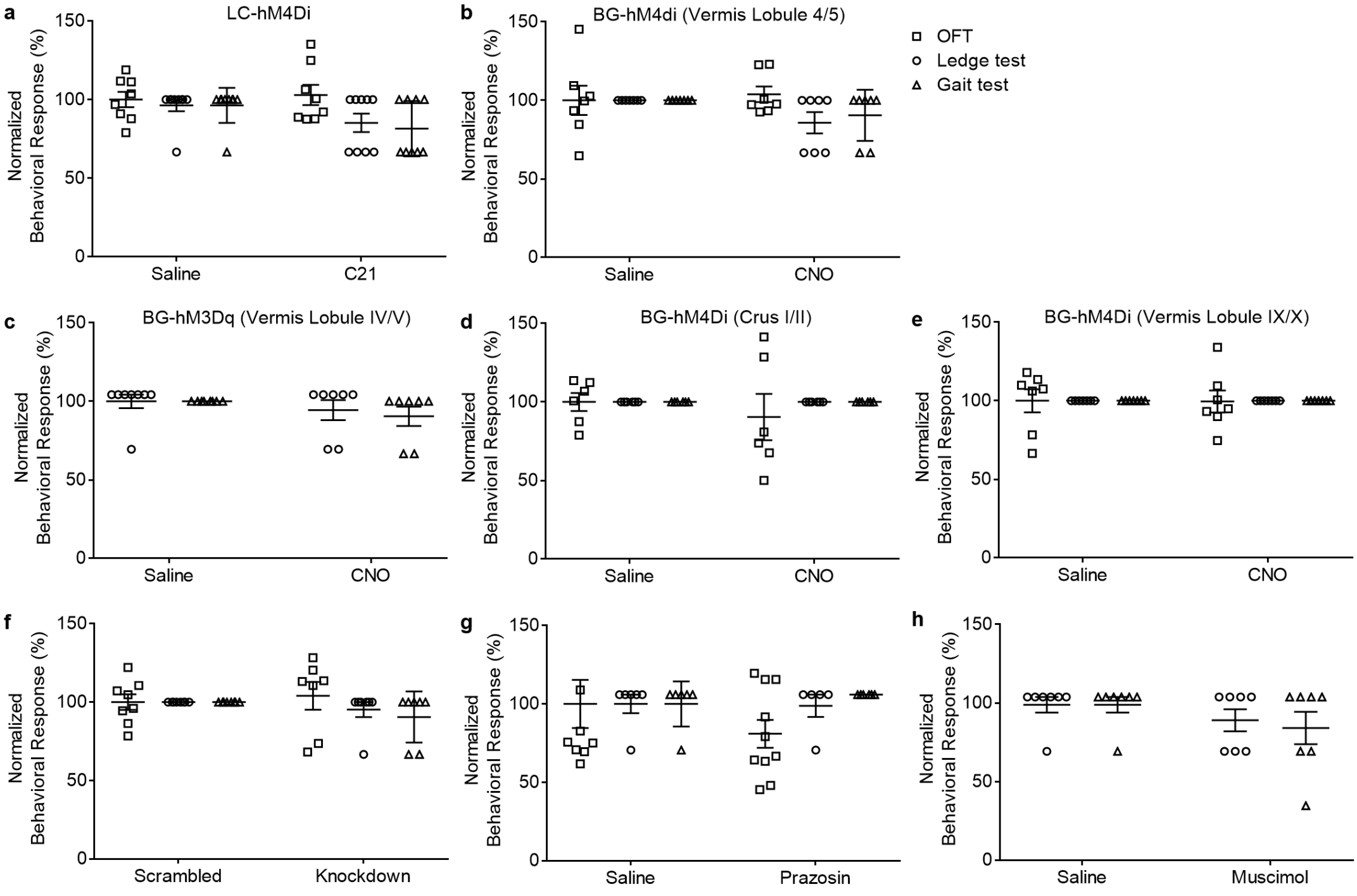


Fig. S6: Capsaicin-induced pain behavior during BG hM3Dq activation

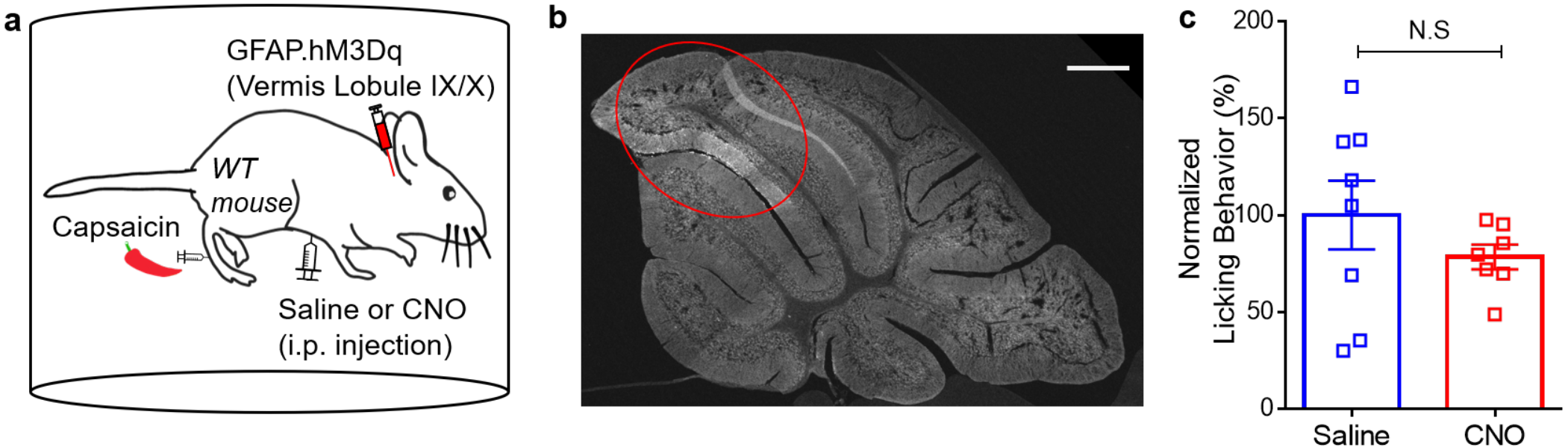
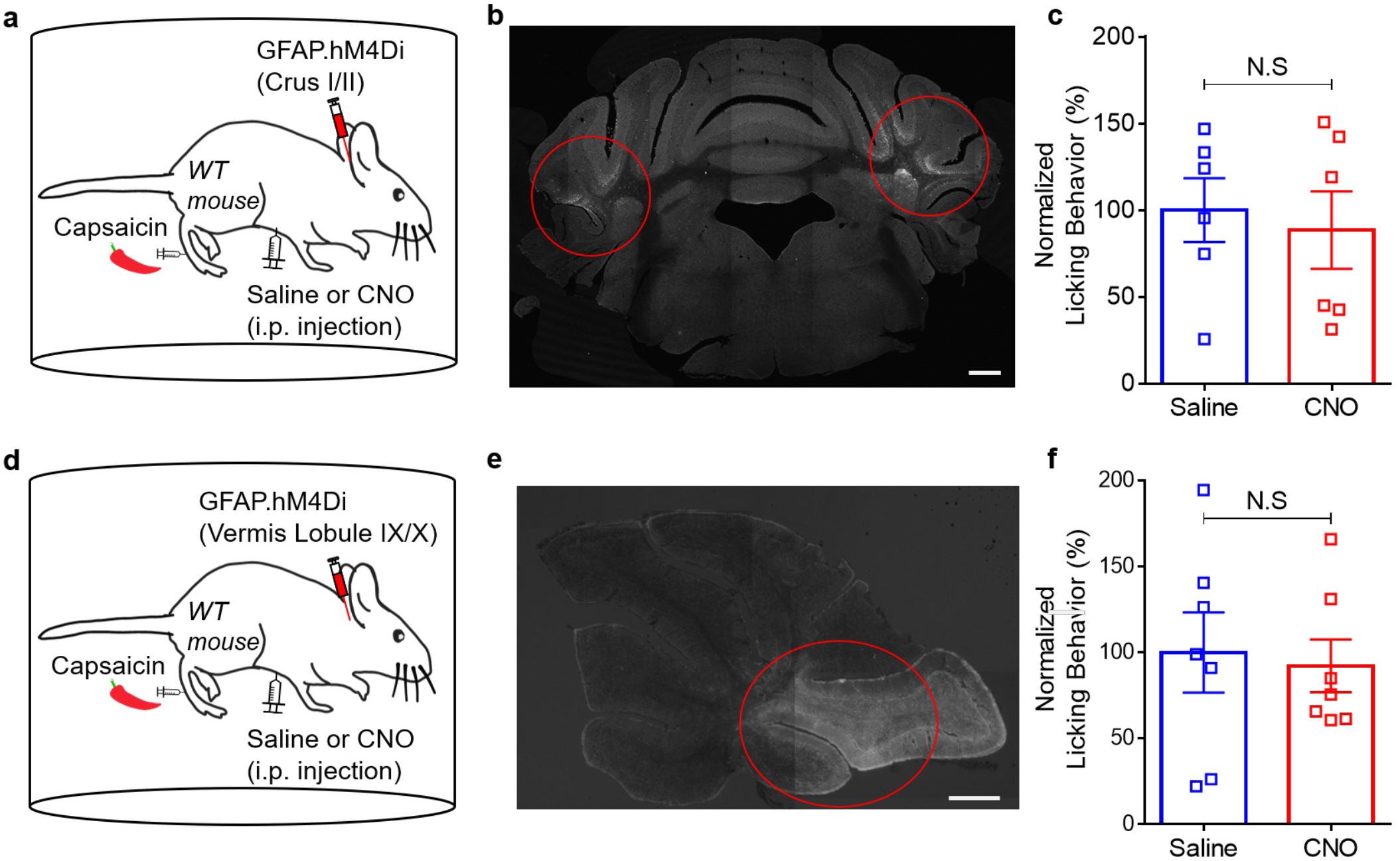
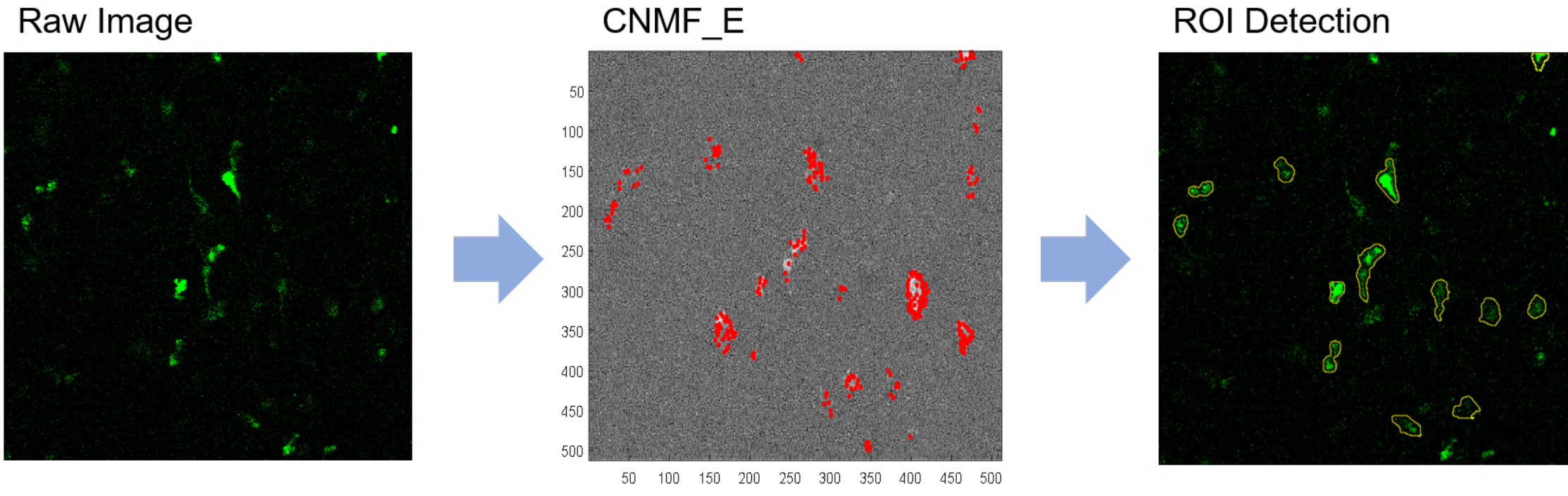


Fig. S7: Capsaicin-induced pain behavior during BG hM4Di activation in other cerebellar regions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