Neuroscience Letters 671 (2018) 7–12

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Cholinergic neurons in medial septum maintain anxiety-like behaviors induced by chronic inflammatory pain

Ying-Ying Jiang[a], Yu Zhang[a], Shuang Cui[a], Feng-Yu Liu[a], Ming Yi[a,*], You Wan[a,b,c,*]

[a] Neuroscience Research Institute and Department of Neurobiology, School of Basic Medical Sciences, Peking University, Beijing 100083, PR China
[b] Key Laboratory for Neuroscience, Ministry of Education/National Health and Family Planning Commission, Peking University, Beijing 100083, PR China
[c] Department of Neurobiology, Peking University, 38 Xueyuan Road, Beijing 100083, PR China

ARTICLE INFO

Keywords:
Chronic inflammatory pain
Anxiety
Cholinergic neuron
Medial septum

ABSTRACT

Cholinergic neurons in the medial septum (MS) participate in various cognitive and emotional behaviors, including innate anxiety. Chronic pain involves perceptual, cognitive and emotional components. Whether MS cholinergic system modulates pain-induced anxiety and the underlying neural circuits are involved remain unclear. In the present study, we showed that chemogenetic (DREADD) inhibition of MS cholinergic neurons relieved pain-induced anxiety-like behaviors in open field and elevated plus maze tests. Inhibiting the MS–rostral anterior cingulate cortex (rACC), but not the MS–ventral hippocampal CA1 pathway, achieved anxiolysis. These findings indicate the involvement of MS cholinergic system in modulating pain-induced anxiety-like behaviors.

1. Introduction

Acetylcholine has a broad range of neuromodulatory influences on neuronal properties and plays an active role in information processing and behaviors such as working memory and motivation [1–3]. Medial septum and diagonal band complex (MS), an important part of basal forebrain cholinergic system, is the major source of cholinergic (as well as non-cholinergic) projections to cingulate cortex, entorhinal cortex and hippocampus [4].

Anxiety is a common co-morbidity in various neurological disorders including chronic pain [5–7]. Though MS cholinergic system maintains innate anxiety in several rodent behavioral paradigms [8], whether MS cholinergic system modulates pathological anxiety and the underlying neural circuits are involved remain unclear.

In the present study, we examined the potential involvement of MS cholinergic neurons in pain-induced anxiety-like behaviors with chemogenetics [9].

2. Materials and methods

2.1. Animals

Adult male ChAT (choline acetyltransferase)-Cre transgenic mice (8–10 weeks of age, Jax Lab #006410, subsequently referred to as ChAT mice) were housed in 4–6 cohorts with stable room temperature (22–23 °C), humidity (40–60%), and circadian cycle (12 h light/dark cycles, starting at 07:00). All experimental procedures followed the Guidelines of the Committee for Research and Ethical Issues of International Association for the Study of Pain [10]. Mice were handled for at least three days before experiments. All behavioral testing was performed in a blind manner.

2.2. Virus injection

For intracranial virus injection, mice were anaesthetized with 1% pentobarbital sodium, positioned in a stereotaxic instrument (RWD, Shenzhen, China), and injected with 0.35 μl AAV5-hSyn-DIO-hM4D (Gi)-mCherry virus [11] solution (1×10^{12} virus particles/ml, the University of North Carolina Vector Core Facilities) into MS (1.2 mm anterior-posterior (AP), 0.73 mm medial-lateral (ML), −4.13 mm dorsal-ventral (DV) from bregma, 10° angle towards the midline) at a rate of 0.1 μl/min for 5 min through a 1-μL Hamilton microsyringe. After injection, needles were left in place for an additional 5 min before it was slowly withdrawn to minimize spread of virus solution. For chemogenetic manipulation (designer receptors exclusively activated by designer drugs, DREADD) of the MS, 1 mg/kg clozapine *N*-oxide (CNO, 0.2 mg/ml dissolved in normal saline, Tocris) [8,12] was injected intraperitoneally 30 min before behavioral testing. For MS–rostral anterior cingulate cortex (rACC) or MS–ventral hippocampal CA1 (vCA1) pathway manipulation, ACC (+1.6 mm AP, +0.4 mm ML, −0.7 mm DV) or vCA1 (-3.0 mm AP, +/−3.2 mm ML, −2.7 mm DV) were implanted with metal cannula (RWD, Shenzhen, China) for CNO

* Corresponding authors at: Neuroscience Research Institute and Department of Neurobiology, School of Basic Medical Sciences, Peking University, Beijing 100083, PR China.
E-mail addresses: mingyi@bjmu.edu.cn (M. Yi), ywan@hsc.pku.edu.cn (Y. Wan).

https://doi.org/10.1016/j.neulet.2018.01.041
Received 1 December 2017; Received in revised form 18 January 2018; Accepted 22 January 2018
Available online 02 February 2018
0304-3940/

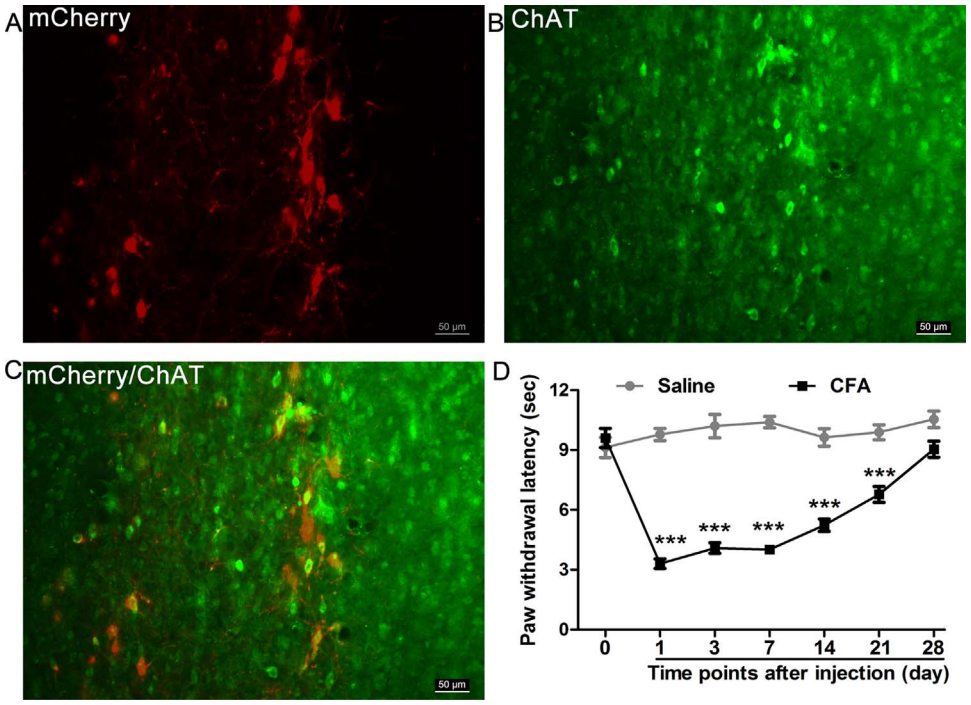

Fig. 1. Validation of the chemogenetics system in the MS and establishment of CFA-induced inflammatory pain model in mice. (A) mCherry (red) stands for the AAV5-hSyn-DIO-hM4D(Gi)-mCherry virus expression. (B) ChAT (green). (C) mCherry/ChAT merge. It is noted that restricted expression of AAV5-hSyn-DIO-hM4D(Gi)-mCherry virus in the MS of ChAT-Cre mice. A large proportion (86.5 ± 3.1%) of infected MS neurons (red) (A) were co-labelled with ChAT (green), a marker for cholinergic neurons (B, C). (D) CFA injection produced thermal hyperalgesia that lasted approximately 3 weeks. ***p < 0.001, two-way ANOVA with Bonferroni's test, n = 9 animals/group.

(3.0 μM in aCSF, 0.5 μl) delivery. All virus injection sites were verified histologically.

2.3. Establishment of CFA-induced inflammatory pain

The complete Freund's adjuvant (CFA) model of chronic pain in mice was established as previously described [13]. Three to 4 weeks after virus injection, mice were anaesthetized with 1% sodium pentobarbitone, and the left hindpaw was intraplantarly injected with 40 μl CFA to induce inflammatory pain. Equal volumes of normal saline (NS) were used as the control.

2.4. Assessment of thermal hyperalgesia

Each mouse was handled for 10 min, and adapted in a Plexiglas cube for 30 min for 3 days before experiments. Thermal pain threshold was measured while the animal stayed calm and awake. Paw withdrawal latencies (PWLs) to thermal stimuli were measured with a focused radiant heat (15 W of power) applied onto the central region of the hind paw (Hargreaves Method, IITC 390) [13]. PWLs were measured 3 times at minimal 5-min intervals and averaged. To avoid tissue damage, a cut-off time of 20 s was imposed.

2.5. Open field test

Twenty-eight days after CFA or saline injection, each mouse was placed in a 60 cm × 60 cm × 60 cm open field chamber with 30 lx illumination and allowed to explore for 5 min freely. The area was divided into 16 quadrants (4 central and 12 peripheral). Time, entries and distance in the central zone and total distance travelled during the test were recorded using a digital video camera and measured using the SMART software (v2.5.21, Panlab, Harvard Apparatus. SMART Video-tracking, RRID:SCR_002852). The apparatus was cleaned using 75% ethanol after each exploration [8,13].

2.6. Elevated plus maze test

The elevated plus maze test was carried out two days after open field test. The maze was made of clear Plexiglas with two open arms and two identical closed arms (5 cm × 30 cm each arm, and 15 cm wall height for closed arms) and was placed 50 cm above the floor in a room (30 lx illumination). Each mouse was placed onto the center area with the head toward one open arm, and allowed to explore the maze for 5 min recorded with a digital video camera. Time in open arms, and entries into open and closed arms were analyzed with the SMART software. The apparatus was cleaned using 75% ethanol after each exploration [8,13,14].

2.7. Immunostaining

Mice were deeply anaesthetized with 1% pentobarbital (100 mg/kg, *i.p.*) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.01 M phosphate buffer (PB), pH 7.4. After removal from the skull, the brain was post-fixed for 4–6 h, and dehydrated in graded sucrose (20% to 30%). After embedding in optimal cutting temperature compound, the brain was sectioned coronally at 50 μm on a freezing microtome (Model 1950, Leica Instrument Co, Ltd).

For the ChAT immunofluorescent staining, sections were rinsed with 0.5% Triton-X in 0.1 M PBS and blocked with 10% BSA for 1 h. Sections were then incubated with the primary antibody (rabbit anti-ChAT, 1: 200, Millipore AB143) in 0.5% PBST for 30 h at 4 °C. Sections were rinsed, incubated with Alexa Fluor 488-conjugated goat anti-rabbit antibody (1: 500, Abcam, 1 h at 24 °C), rinsed in PBS, mounted and coverslipped. Images were taken by a fluorescence microscope (Leica DMI4000B) [13].

2.8. Data analysis

Data were expressed as means ± SEMs (standard error of means). All data were analyzed and plotted using the GraphPad Prism 5 software. Statistical analysis was performed using two-way ANOVA with Bonfferoni *post hoc* test among groups, and unpaired Student's *t*-test between two groups. Numbers of animals used were indicated by n. Probability values of $P < 0.05$ were considered to represent significant differences.

3. Results

3.1. Chemogenetic inhibition of MS cholinergic neurons relieves pain-induced anxiety

We chemogenetically inhibited the activity of cholinergic neurons in the MS. The virus expression was restricted to the MS (Fig. 1A), and showed significant colocalization with ChAT (86.5 ± 3.1%), the marker of cholinergic neurons (Fig. 1B, C). The inhibitory effect of Gi has been confirmed with whole cell patch clamp in our previous work: the number of action potentials significantly decreased after incubation of CNO in brain slices expressing Gi virus [8].

After virus expression, CFA or saline was injected into left hindpaw. Mice in the CFA group showed significantly lower paw withdrawal latencies to heat stimuli in the left hindpaw from days 1–21 than those in the saline group (interaction: $F_{(6,96)} = 26.82$, $p < 0.001$, time effect: $F_{(6,96)} = 22.19$, $p < 0.001$, CFA effect: $F_{(1,96)} = 221.60$, $p < 0.001$, two-way ANOVA with Bonferroni's test, Fig. 1D), which indicated successful establishment of chronic inflammatory pain model of mice.

We examined anxiety-like behaviors of these mice 28 days after CFA or saline injection when thermal hyperalgesia had recovered, to exclude influences from pain. CFA chronic pain mice had fewer numbers of entries (CFA effect: $F_{(1,32)} = 5.25$, $p < 0.05$; treatment effect: $F_{(1,32)} = 4.34$, $p < 0.05$; interaction: $F_{(1,32)} = 5.91$, $p < 0.05$, Fig. 2A), less time spent (CFA effect: $F_{(1,32)} = 16.08$, $p < 0.001$; treatment effect: $F_{(1,32)} = 2.52$, $p > 0.05$; interaction: $F_{(1,32)} = 4.26$, $p < 0.05$, Fig. 2B), and less distance travelled (CFA effect: $F_{(1,32)} = 7.91$, $p < 0.01$; treatment effect: $F_{(1,32)} = 1.52$, $p > 0.05$; interaction: $F_{(1,32)} = 4.40$, $p < 0.05$, Fig. 2C) in the central zone of the open field, as well as less time spent (CFA effect: $F_{(1,32)} = 1.06$, $p > 0.05$; treatment effect: $F_{(1,32)} = 2.91$, $p > 0.05$; interaction: $F_{(1,32)} = 17.68$, $p < 0.001$, Fig. 2E) and fewer numbers of entries (CFA effect: $F_{(1,32)} = 5.21$, $p < 0.05$; treatment effect: $F_{(1,32)} = 8.16$, $p < 0.01$; interaction: $F_{(1,32)} = 34.79$, $p < 0.001$, two-way ANOVA with Bonferroni's test, Fig. 2F) in open arms of the elevated plus maze. By contrast, the total distance (CFA effect: $F_{(1,32)} = 0.66$, $p > 0.05$; treatment effect: $F_{(1,32)} = 0.0002$, $p > 0.05$; interaction: $F_{(1,32)} = 0.03$, $p > 0.05$, Fig. 2D) in the open field, and entries into closed arms (CFA effect: $F_{(1,32)} = 0.05$, $p > 0.05$; treatment effect: $F_{(1,32)} = 0.34$, $p > 0.05$; interaction: $F_{(1,32)} = 1.37$, $p > 0.05$, Fig. 2G) and total entries (CFA effect: $F_{(1,32)} = 2.04$, $p > 0.05$; treatment effect: $F_{(1,32)} = 0.51$, $p > 0.05$; interaction: $F_{(1,32)} = 2.87$, $p > 0.05$, two-way ANOVA with Bonferroni's test, Fig. 2H) in the elevated plus maze remained unchanged, indicating normal locomotion. These findings suggest that chronic pain-induced anxiety is relieved by inhibiting the MS cholinergic system.

3.2. Chemogenetic inhibition of MS–rACC pathway relieves pain-induced anxiety

Two major targets of MS cholinergic efferents are rACC and ventral hippocampus, both of which participate in anxiety-like behaviors [15]. To elucidate the neural pathway involved in the anxiolytic effect of MS inhibition, AAV5-hSyn-DIO-hM4D(Gi)-mCherry virus was injected into the MS of ChAT-Cre mice, with CNO injected into bilateral rACC or ventral hippocampal CA1, respectively.

Inhibiting the MS–rACC pathway produced similar effects to inhibiting MS. Mice in the CNO group increased the number of entries ($t_{(15)} = 2.47$, $p < 0.05$, Fig. 3A), time spent ($t_{(15)} = 2.83$, $p < 0.05$, Fig. 3B) and distance travelled ($t_{(15)} = 2.48$, $p < 0.05$, Fig. 3C) in the central zone of the open field, as well as time spent ($t_{(15)} = 2.17$, $p < 0.05$, Fig. 3E), number of entries ($t_{(15)} = 4.07$, $p < 0.001$, Fig. 3F) and total entries ($t_{(15)} = 3.51$, $p < 0.05$, Fig. 3H) into open arms in the elevated plus maze. Total distance ($t_{(15)} = 0.89$, $p > 0.05$, Fig. 3D) in the open field, and entries into closed arms ($t_{(15)} = 2.09$, $p > 0.05$, Fig. 3G) in the elevated plus maze remained unchanged.

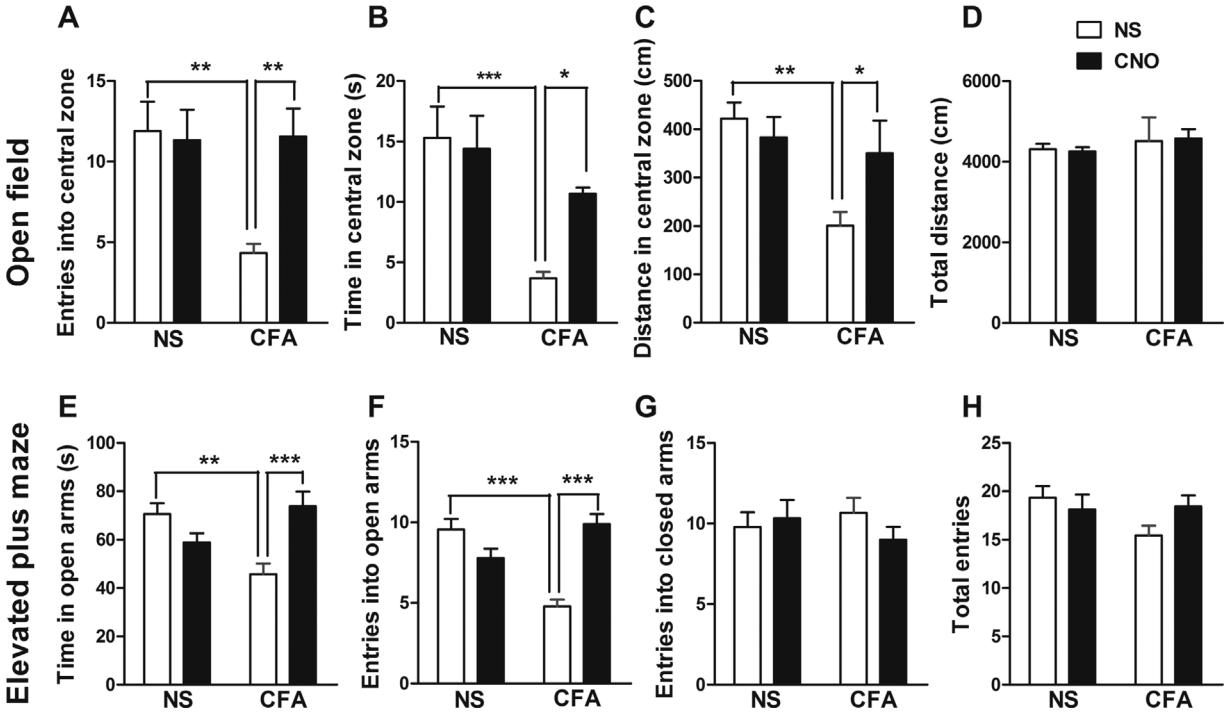

Fig. 2. Chemogenetic inhibition of MS cholinergic neurons relieved pain-induced anxiety. (A–D) Chemogenetical inhibition of MS cholinergic neurons relieved pain-induced anxiety in the open field test. CFA chronic pain mice exhibited fewer entries (A, left), less time spent (B, left) and less distance travelled in the central zone (C, left), all of which were reversed by inhibition of MS cholinergic neurons (A, B, C, right). The total distance in the open field remained unchanged (D). (E–H) Chemogenetical inhibition of MS cholinergic neurons relieved pain-induced anxiety in the elevated plus maze test. CFA chronic pain mice spent less time and had fewer entries into the open arms (E, F, left). Inhibition of MS cholinergic neurons significantly increased the time spent, and the number of entries into open arms (E, F, right). Both the number of entries into closed arms and total entries remained unchanged (G, H). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-way ANOVA with Bonferroni's test, n = 9 animals/group.

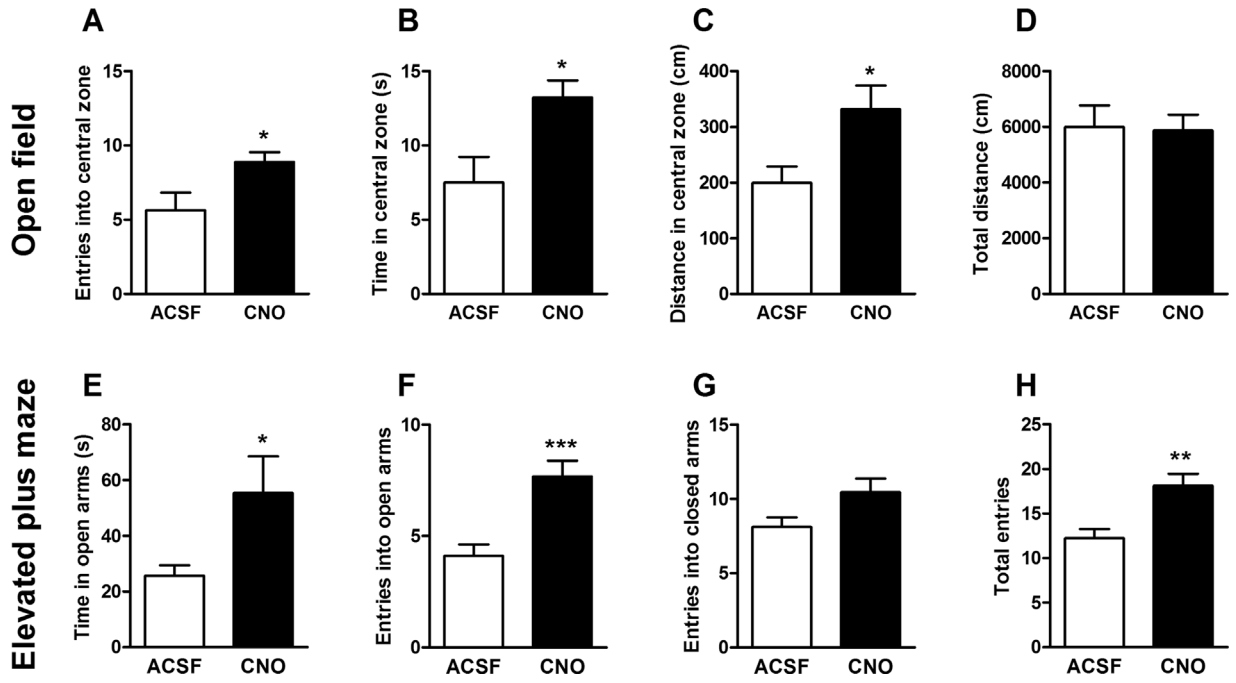

Fig. 3. Chemogenetic inhibition of MS–rACC pathway relieved pain-induced anxiety. (A–D) Chemogenetical inhibition of MS–rACC pathway relieved pain-induced anxiety in the open field test. CFA chronic pain mice with CNO microinjection into rACC exhibited more entries (A), time spent (B) and travelled distance in central zone (C), but similar total distance in the open field (D). (E–H) Chemogenetical inhibition of MS–rACC pathway relieved pain-induced anxiety in the elevated plus maze. CFA chronic pain mice with CNO microinjection into rACC exhibited more time spent (E) and entries (F) into open arms and total entries (H), but similar closed arm entries (G). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test, n = 8–9 animals/group.

3.3. Chemogenetic inhibition of MS–vCA1 pathway does not affect pain-induced anxiety

By sharp contrast, anxiolytic effects were not observed under inhibition of the MS–vCA1 pathway, indicated by similar numbers of entries ($t_{(13)} = 0.78$, $p > 0.05$, Fig. 4A), time spent ($t_{(13)} = 0.46$, $p > 0.05$, Fig. 4B) and distance travelled ($t_{(13)} = 0.22$, $p > 0.05$, Fig. 4C) into central zone in open field test, and time spent ($t_{(13)} = 0.18$, $p > 0.05$, Fig. 4E) and number of entries ($t_{(13)} = 1.16$, $p > 0.05$, Fig. 4F) into open arms in the elevated plus maze.

These results indicate that MS cholinergic efferents to the rACC, but not to the vCA1, participate in anxiogenesis in CFA-induced pain.

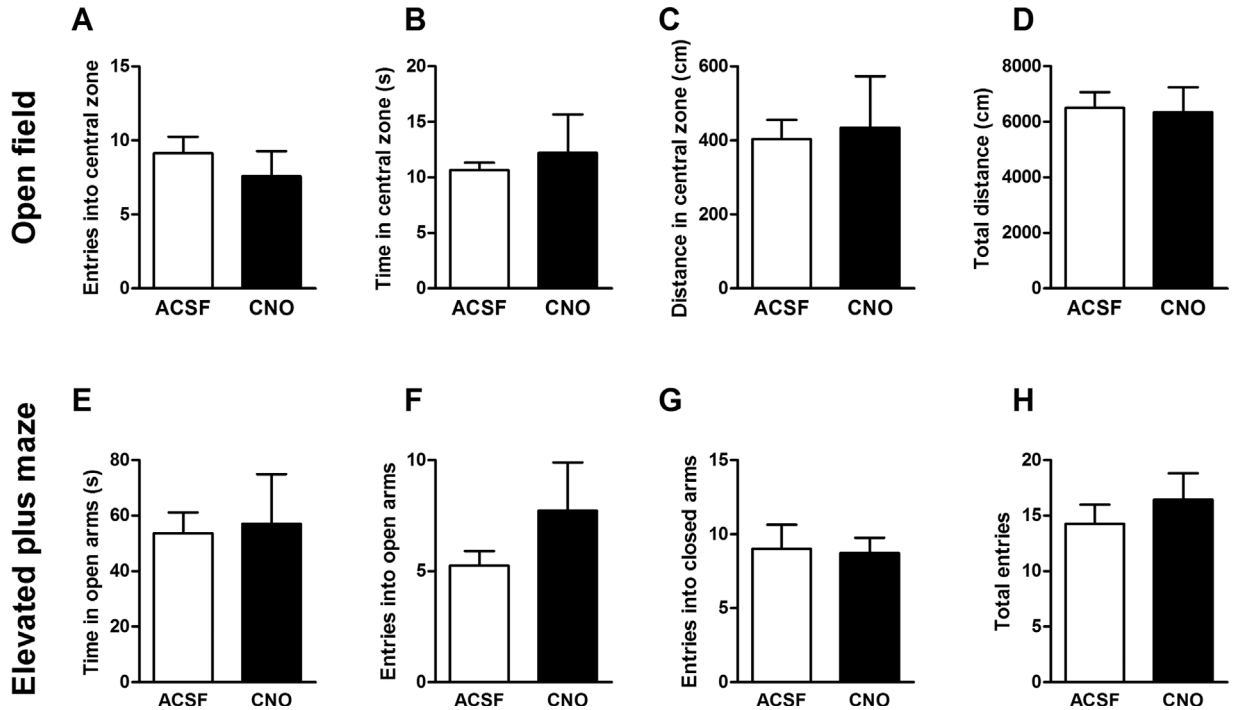

Fig. 4. Chemogenetic inhibition of MS–vCA1 pathway did not affect pain-induced anxiety. (A–D) Chemogenetical inhibition of MS–vCA1 pathway did not affect pain-induced anxiety in the open field. None of the parameters including entries (A), time spent (B), travelled distance in central zone (C) and the total distance (D) showed significant difference. Elevated plus maze test showed similar results. Time spent (E), number of entries into open (F) and closed arms (G), and total entries (H) remained unchanged. n = 7–8 animals/group.

4. Discussion

More than 50% of MS neurons are excited by noxious stimuli from widespread peripheral regions, as shown by extracellular recording [16]. The spino-septal neurons locate in the deep dorsal horn, where nociceptive neurons have been identified [17,18]. Cholinergic neurons in the MS further project to a broad range of pain-modulatory sites in the neocortex and the hippocampus [4]. Selective ablation of MS cholinergic neurons with 192 IgG-saporin (SAP) reduces sensitivity to nociceptive heat stimulation, supporting a role of MS cholinergic system in pain modulation [19,20].

We and others have demonstrated a significant role of the MS cholinergic system in maintaining innate anxiety [8,21]. Anxiety frequently co-occurs with chronic pain [22–26], and is sometimes referred as generalized anxiety disorder (GAD) [27–29]. In the CFA chronic pain model used in the present study, previous work has shown that anxiety-like behaviors occur as early as 3 or 7 days after pain induction [23], maintain 10–15 days after CFA injection [26,30,31], and can be observed even 21 or 28 days after the model establishment [22,25,32]. In the present study, we did not observe significant changes in anxiety-like behaviors in saline-treated mice under MS cholinergic inhibition (Fig. 2). This is in contrast to our previous report of anxiolytic effects of MS cholinergic inhibition in innate anxiety [8]. One significant difference between these two studies is the experimental setting. The present study adopted 30 lx illumination, which significantly promoted exploration in normal animals than our previous study (300 lx) [8]. The choice of this low illumination level stemmed from our failure to detect pain-induced anxiety-like behaviors (data not shown), which had been repeatedly demonstrated in previous studies [13,22,25,32], with 300 lx due to the floor effect.

Besides MS, many other brain regions are also involved in anxiety-like emotion, including ACC [33], amygdala [23] and hippocampus [13]. Ventral hippocampal lesions [34], deletion of the NR1 subunit of NMDA receptors from dentate granule cells [35], and promoting neurogenesis in the ventral hippocampus via overexpressing brain-derived neurotrophic factor [13] all reduce anxiety. The involvement of ACC in anxiety is anatomically documented, in that ACC neurons receive direct and indirect projections from the amygdala, a key area for different forms of anxiety [36]. Inhibiting ACC neuronal activities significantly attenuates anxiety-like behaviors in mice [33]. In addition, presynaptic long-term potentiation (pre-LTP) was associated with anxiety induced by chronic pain in behavioral test, and inhibition of pre-LTP in ACC by HCN channel inhibitors reduced enhanced anxiety induced by chronic pain [36]. Finally, the protein kinase Mzeta (PKMzeta)-GluR1 pathway is involved in anxiety-like behaviors of chronic inflammatory pain induced by CFA [37].

In the present study, we show that compared with saline group, CFA mice exhibit anxiety-like emotion in both open field and elevated plus maze tests. Temporally inhibiting cholinergic neurons in the MS with chemogenetics alleviates pain-induced anxiety and this effect is achieved possibly through the MS–rACC, but not the MS–vCA1, pathway. Distinct responses of rACC and hippocampal neurons are observed in chronic pain. Pain increases neuronal activities of ACC neurons, as revealed by immunohistochemistry [38], *in vivo* local field potential recording [39] and patch clamping experiments [40–42]. By contrast, the excitability of ventral hippocampal pyramidal neurons is depressed by pain, as indicated by reduced hippocampal neurogenesis in inflammatory pain [13], reduction in short-term plasticity in CA3–mossy fiber pathway, increased of neonatal neuronal apoptosis in chronic inflammatory pain, and decreases in hippocampal volume in human chronic pain patients [43].

How do MS cholinergic neurons affect rACC and hippocampal excitability? The effect of acetylcholine on hippocampal excitability is complex and relates to the concentration of acetylcholine or its analogue [44], the timing of the input relative to the Schaffer collateral stimulation [45], and the type of acetylcholine receptors [46]. Previous studies demonstrate that acetylcholine through nicotinic acetylcholine receptors facilitates the excitation of pyramidal neurons in CA1, which is the main output location of information in the hippocampus [47]. However, our study fails to identify an obvious effect of inhibiting MS–vCA1 pathway on pain-induced anxiety. This might result from the persistent pain-induced suppression of hippocampal activities [13,43], which could not be further affected by MS cholinergic inhibition because of floor effects. Much less is known about the effect of MS inhibition on rACC neurons. One study showed decreased power of ACC field potentials after intraperitoneal injection of scopolamine, a muscarinic acetylcholine receptor antagonist [48]. It would not be surprising that inhibiting MS cholinergic system attenuates anxiety by suppressing abnormal ACC activities in chronic pain.

In conclusion, our study suggests that chemogenetic inhibition of MS cholinergic neurons alleviates pain-induced anxiety, and that rACC is one efferent target mediating this effect.

Conflict of interests

The authors declare no conflict of interests.

Authors' contributions

YYJ and YZ performed the experiment; FYL, MY and YW designed the experiment; All authors wrote the manuscript.

Acknowledgements

This research was supported by grants from the National Basic Research Program of Ministry of Science and Technology of China (2014CB548200 and 2015CB554503), the National Natural Science Foundation of China (91732107, 81521063, 81571067 and 21305005), the Beijing Municipal Natural Science Foundation (5182013), Key Project of Chinese Ministry of Education (109003) and the "111" Project of Ministry of Education of China (B07001).

References

[1] F. Khakpai, M. Nasehi, A. Haerirohani, A. Eidi, M.R. Zarrindast, Septo-hippocampo-septal loop and memory formation, Basic Clin. Neurosci. 4 (2013) 5–23.
[2] I. Avila, S.C. Lin, Distinct neuronal populations in the basal forebrain encode motivational salience and movement, Front. Behav. Neurosci. 8 (2014) 421.
[3] I. Klinkenberg, A. Sambeth, A. Blokland, Acetylcholine and attention, Behav. Brain Res. 221 (2011) 430–442.
[4] S. Paul, W.K. Jeon, J.L. Bizon, J. Han, Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment, Front. Aging Neurosci. 7 (2015) 43.
[5] K. Koga, G. Descalzi, T. Chen, H. Ko, J. Lu, S. Li, J. Son, T. Kim, C. Kwak, R.L. Huganir, M. Zhao, B. Kaang, G.L. Collingridge, M. Zhuo, Coexistence of two forms of LTP in ACC provides a synaptic mechanism for the interactions between anxiety and chronic pain, Neuron 85 (2015) 377–389.
[6] M.K. Jastrowski, School anxiety in children and adolescents with chronic pain, Pain Res. Manage. 2017 (2017) 8328174.
[7] S. Kadimpati, E.L. Zale, M.W. Hooten, J.W. Ditre, D.O. Warner, Associations between neuroticism and depression in relation to catastrophizing and pain-related anxiety in chronic pain patients, PLoS One 10 (2015) e126351.
[8] Y. Zhang, Y.Y. Jiang, S. Shao, C. Zhang, F.Y. Liu, Y. Wan, M. Yi, Inhibiting medial septal cholinergic neurons with DREADD alleviated anxiety-like behaviors in mice, Neurosci. Lett. 638 (2017) 139–144.
[9] G.M. Alexander, S.C. Rogan, A.I. Abbas, B.N. Armbruster, Y. Pei, J.A. Allen, R.J. Nonneman, J. Hartmann, S.S. Moy, M.A. Nicolelis, J.O. McNamara, B.L. Roth, Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors, Neuron 63 (2009) 27–39.
[10] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.
[11] C. Anaclet, N.P. Pedersen, L.L. Ferrari, A. Venner, C.E. Bass, E. Arrigoni, P.M. Fuller, Basal forebrain control of wakefulness and cortical rhythms, Nat. Commun. 6 (2015) 8744.
[12] C.S. Chiou, C.C. Chen, T.C. Tsai, C.C. Huang, D. Chou, K.S. Hsu, Alleviating bone cancer-induced mechanical hypersensitivity by inhibiting neuronal activity in the anterior cingulate cortex, Anesthesiology 125 (2016) 779–792.
[13] J. Zheng, Y.Y. Jiang, L.C. Xu, L.Y. Ma, F.Y. Liu, S. Cui, J. Cai, F.F. Liao, Y. Wan, M. Yi, Adult hippocampal neurogenesis along the dorsoventral axis contributes differentially to environmental enrichment combined with voluntary exercise in

alleviating chronic inflammatory pain in mice, J. Neurosci. 37 (2017) 4145–4157.
[14] L.K. Fonken, A.D. Gaudet, K.R. Gaier, R.J. Nelson, P.G. Popovich, MicroRNA-155 deletion reduces anxiety- and depressive-like behaviors in mice, Psychoneuroendocrino 63 (2016) 362–369.
[15] E.J. Bubb, L. Kinnavane, J.P. Aggleton, Hippocampal–diencephalic–cingulate networks for memory and emotion: an anatomical guide, Brain Neurosci. Adv. 1 (2017) 1102101704.
[16] P. Dutar, Y. Lamour, A. Jobert, Activation of identified septo-hippocampal neurons by noxious peripheral stimulation, Brain Res. 328 (1985) 15–21.
[17] H.M. Newman, R.T. Stevens, A.V. Apkarian, Direct spinal projections to limbic and striatal areas: anterograde transport studies from the upper cervical spinal cord and the cervical enlargement in squirrel monkey and rat, J. Comp. Neurol. 365 (1996) 640–658.
[18] J.L. Li, T. Kaneko, R. Shigemoto, N. Mizuno, Distribution of trigeminohypothalamic and spinohypothalamic tract neurons displaying substance P receptor-like immunoreactivity in the rat, J. Comp. Neurol. 378 (1997) 508–521.
[19] F. Zheng, S. Khanna, Selective destruction of medial septal cholinergic neurons attenuates pyramidal cell suppression, but not excitation in dorsal hippocampus field CA1 induced by subcutaneous injection of formalin, Neuroscience 103 (2001) 985–998.
[20] S.T. Ang, M.Z. Ariffin, S. Khanna, The forebrain medial septal region and nociception, Neurobiol. Learn. Memory 138 (2017) 238–251.
[21] M. Zarrindast, R. Tajik, M. Ebrahimi-Ghiri, M. Nasehi, A. Rezayof, Role of the medial septum cholinoceptors in anxiogenic-like effects of nicotine, Physiol. Behav. 119 (2013) 103–109.
[22] D. Wang, Z. Tian, Y. Guo, H. Guo, W. Kang, S. Li, Y. Den, X. Li, B. Feng, D. Feng, J. Zhao, G. Liu, M. Zhao, Anxiolytic-like effects of translocator protein (TSPO) ligand ZBD-2 in an animal model of chronic pain, Mol. Pain 11 (2015) s12915–s12990.
[23] J. Chen, Y. Song, J. Yang, Y. Zhang, P. Zhao, X. Zhu, H. Su, The contribution of TNF-α in the amygdala to anxiety in mice with persistent inflammatory pain, Neurosci. Lett. 541 (2013) 275–280.
[24] L.K. Refsgaard, J. Hoffmann-Petersen, M. Sahlholt, D.S. Pickering, J.T. Andreasen, Modelling affective pain in mice: effects of inflammatory hypersensitivity on place escape/avoidance behaviour, anxiety and hedonic state, J. Neurosci. Methods 262 (2016) 85–92.
[25] M. Narita, C. Kaneko, K. Miyoshi, Y. Nagumo, N. Kuzumaki, Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala, Neuropsychopharmacology 3 (2006) 739–750.
[26] S.B. Liu, Z. Tian, Y.Y. Guo, N. Zhang, B. Feng, Activation of GPR30 attenuates chronic pain-related anxiety in ovariectomized mice, Psychoneuroendocrinology 53 (2015) 97–104.
[27] B. Bandelow, Generalized anxiety disorder and pain, Modern Trends Pharmacopsychiatry 30 (2015) 153–165.
[28] E. Likhtik, J.M. Stujenske, M.A. Topiwala, A.Z. Harris, J.A. Gordon, Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety, Nat. Neurosci. 17 (2013) 106–113.
[29] K. Janzen, B. Peters-Watral, Treating co-occurring chronic low back pain & generalized anxiety disorder, Nurse Pract. 41 (12–18) (2016) 18–19.
[30] G.C. Do Nascimento, C.R.A. Leite-Panissi, Time-dependent analysis of nociception and anxiety-like behavior in rats submitted to persistent inflammation of the temporomandibular joint, Physiol. Behav. 125 (2014) 1–7.
[31] J. Tian, Z. Tian, S.L. Qin, P.Y. Zhao, X. Jiang, Anxiolytic-like effects of α-asarone in a mouse model of chronic pain, Metab. Brain Dis. 32 (2017) 2119–2129.
[32] H.L. Guo, Y. Xiao, Z. Tian, X.B. Li, D.S. Wang, Anxiolytic effects of sesamin in mice with chronic inflammatory pain, Nutr. Neurosci. 19 (2015) 231–236.
[33] S.S. Kim, H. Wang, X.Y. Li, T. Chen, V. Mercaldo, G. Descalzi, L.J. Wu, M. Zhuo, Neurabin in the anterior cingulate cortex regulates anxiety-like behavior in adult mice, Mol. Brain 4 (2011) 6.
[34] D.M. Bannerman, J.N. Rawlins, S.B. McHugh, R.M. Deacon, B.K. Yee, T. Bast, W.N. Zhang, H.H. Pothuizen, J. Feldon, Regional dissociations within the hippocampus–memory and anxiety, Neurosci. Biobehav. Rev. 28 (2004) 273–283.
[35] C. Barkus, S.B. McHugh, R. Sprengel, P.H. Seeburg, J.N. Rawlins, D.M. Bannerman, Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion, Eur. J. Pharmacol. 626 (2010) 49–56.
[36] M. Zhuo, Neural mechanisms underlying anxiety-chronic pain interactions, Trends Neurosci. 39 (2016) 136–145.
[37] J. Du, J. Fang, C. Wen, X. Shao, Y. Liang, J. Fang, The Effect of Electroacupuncture on PKMzeta in the ACC in regulating anxiety-like behaviors in rats experiencing chronic inflammatory pain, Neural. Plast. 2017 (2017) 1–13.
[38] M.Q. Zhang, T.X. Wang, R. Li, Z.L. Huang, W.J. Han, X.C. Dai, Y.Q. Wang, Helicid alleviates pain and sleep disturbances in a neuropathic pain-like model in mice, J. Sleep Res. 26 (2017) 386–393.
[39] A.L. Harris-Bozer, Y.B. Peng, Inflammatory pain by carrageenan recruits low-frequency local field potential changes in the anterior cingulate cortex, Neurosci. Lett. 632 (2016) 8–14.
[40] K. Koga, X. Li, T. Chen, H.W. Steenland, G. Descalzi, M. Zhuo, In vivo whole-cell patch-clamp recording of sensory synaptic responses of cingulate pyramidal neurons to noxious mechanical stimuli in adult mice, Mol. Pain 6 (2010) 62.
[41] K.R. Gong, F.L. Cao, Y. He, C.Y. Gao, D.D. Wang, H. Li, F.K. Zhang, Y.Y. An, Q. Lin, J. Chen, Enhanced excitatory and reduced inhibitory synaptic transmission contribute to persistent pain-induced neuronal hyper-responsiveness in anterior cingulate cortex, Neuroscience 171 (2010) 1314–1325.
[42] X.Y. Li, N. Wang, Y.J. Wang, Z.X. Zuo, K. Koga, F. Luo, M. Zhuo, Long-term temporal imprecision of information coding in the anterior cingulate cortex of mice with peripheral inflammation or nerve injury, J. Neurosci. 34 (2014) 10675–10687.
[43] A.A. Mutso, D. Radzicki, M.N. Baliki, L. Huang, G. Banisadr, Abnormalities in hippocampal functioning with persistent pain, J. Neurosci. 32 (2012) 5747–5756.
[44] R.T. Betterton, L.M. Broad, K.T. Atanasova, J.R. Mellor, Acetylcholine modulates gamma frequency oscillations in the hippocampus by activation of muscarinic M1 receptors, Eur. J. Neurosci. 45 (2017) 1570–1585.
[45] J.L. Yakel, Nicotinic ACh receptors in the hippocampus: role in excitability and plasticity, Nicotine Tob. Res. 14 (2012) 1249–1257.
[46] C.A. Thorn, M. Popiolek, E. Stark, J.R. Edgerton, Effects of M1 and M4 activation on excitatory synaptic transmission in CA1, Hippocampus 27 (2017) 794–810.
[47] B.Y. Chung, W. Bignell, D.L. Jacklin, B.D. Winters, C.D. Bailey, Postsynaptic nicotinic acetylcholine receptors facilitate excitation of developing CA1 pyramidal neurons, J. Neurophysiol 116 (2016) 2043–2055.
[48] A.M.P. Miller, B.J. Frick, D.M. Smith, J. Radulovic, K.A. Corcoran, Network oscillatory activity driven by context memory processing is differently regulated by glutamatergic and cholinergic neurotransmission, Neurobiol. Learn. Memory 145 (2017) 59–66.