A central neural circuit for itch sensation

Although itch sensation is an important protective mechanism for animals, chronic itch remains a challenging clinical problem. Itch processing has been studied extensively at the spinal level. However, how itch information is transmitted to the brain and what central circuits underlie itch-induced scratching behavior remain largely unknown. We found that the spinoparabrachial pathway was activated during itch processing and that optogenetic suppression of this pathway impaired itch-induced scratching behaviors. Itch-mediating spinal neurons, which express the gastrin-releasing peptide receptor, are disynaptically connected to the parabrachial nucleus via glutamatergic spinal projection neurons. Blockade of synaptic output of glutamatergic neurons in the parabrachial nucleus decreased the scratching behavior induced by histamine (Fig. 2D and fig. S4A) and fig. S1D), without affecting locomotive activity (fig. S1, K to N).

Next, we investigated the connections between the spinal itch-mediating neural network and the PBN. Neurons expressing gastrin-releasing peptide receptor (GRPR) in the spinal cord are essential for itch signal transmission (19) and serve as the downstream targets of other spinal itch-signaling neurons (20, 21). We first examined the possible direct projection of GRPR-expressing (GRPR+) neurons to the PBN by using a knock-in mouse line, with iCreERT2 inserted into the Grpr locus (fig. S2A). Anterograde tracing by selectively expressing enhanced yellow fluorescent protein (EYFP) in spinal GRPR+ neurons resulted in very few EYFP+ axons in the brain, including the PBN (fig. S2, B to G), confirming that spinal GRPR+ neurons are mostly local interneurons (22). In view of the large number of spinal axons projecting to the PBN (fig. S1J), we hypothesized that spinal GRPR+ neurons make synapses with PBN-projecting spinal neurons to transmit itch signals to the PBN. Recording from retrogradely labeled PBN-projecting neurons in spinal slices, we found that photostimulation of spinal GRPR+ neurons and their axons induced short-latency excitatory postsynaptic currents (EPSCs) (7 of 14 cells recorded) (fig. 1, I to L, and fig. S2H), which were blocked by the AMPA receptor antagonist NBQX (2,3-Dioxo-6-nitro-1,2,3-tetrahydrobenzo[f]quinoxaline-7-sulfonamide) (Fig. 1, L and M). The latency of light-induced EPSCs was 1.5 ± 0.2 ms with short jitter (fig. S2, I and J), indicating a monosynaptic glutamatergic connection between spinal GRPR+ neurons and PBN-projecting neurons. Whole-cell recording from PBN neurons in brain slices demonstrated monosynaptic glutamatergic connections made by spinal axon terminals (fig. S2, K to M).

We examined the involvement of PBN in itch signal processing. The number of c-Fos+ neurons in the PBN increased in response to histamine or chloroquine (Fig. 2, A to C, and fig. S3). Next, we measured the neural activities of PBN neurons during itch-induced scratching behavior in freely moving mice with fiber photometry by expressing the calcium indicator GCaMP6s in the PBN (Fig. 2D and fig. S4A) (23). Scratching behavior was recorded by using a magnetic induction method (fig. S5, movie S1, and supplementary materials, materials and methods). A cluster of scratching bouts was defined as a scratching train (fig. S5B). We aligned the calcium signal of PBN to the beginning of individual scratching trains (Fig. 2E and fig. S4B). The activity of PBN increased during scratching behavior induced with either histamine or chloroquine (Fig. 2, F and G, and fig. S4C). Consistently, we found that optogenetic activation of spinal GRPR+ neurons induced elevated activity of PBN neurons (fig. S4, D to F). These results are consistent with data obtained with extracellular recording, which demonstrated that a small percentage of recorded cells showed scratching-related responses (17.3% for chloroquine and 9.1% for histamine) (fig. S6).

To test the functional role of PBN in scratching behavior, we used a pharmacogenetic approach to suppress PBN activity. Bilateral PBN injection was made in wild-type mice with an AAV expressing hM4Di, a designer receptor exclusively activated by designer drugs (DREADD) (24), or EGFP as control (fig. 2H and fig. S7). The efficacy of hM4Di-mediated inhibition was confirmed with slice recordings (fig. 2I and fig. S8). Behaviorally, intraperitoneal injection of an hM4Di agonist, clozapine-N-oxide (CNO), to mice expressing hM4Di in the PBN significantly suppressed the scratching behavior in response to histamine or chloroquine (Fig. 2, J and K), as well as bombesin (fig. S9A). By contrast, this manipulation did not significantly affect motor functions and behavioral responses to thermal and mechanical stimuli nor induced overt distress (fig. S9, B to I).

Most c-Fos+ neurons in the PBN activated by histamine were glutamatergic neurons (fig. S10, A to C). Consistently, the activity of glutamatergic neurons in the PBN increased during the scratching behavior (fig. S10, D to G). We further examined the functional role of PBN glutamatergic neurons in itch-induced scratching behavior through genetic deletion of vesicular glutamate transporter 2 (VGLUT2). Bilateral injection of AAV-Cre-EGFP into the PBN of Vglut2−/− mice (25) led to a selective reduction of Vglut2 in the PBN (figs. 3A to C, and fig. S11) and resulted in blockade of synaptic output of PBN glutamatergic neurons (Fig. 3D and fig. S12). This manipulation significantly impaired the scratching behavior in response to both histamine-dependent and -independent pruritogens (Fig. 3, E and F, and figs. S13 and S14). It also significantly reduced the scratching behavior in the ovalbumin-induced allergic itch model (Fig. 3G) as well as in the 1-fluoro-2,4-dinitrobenzene (DNFB)-induced chronic itch model (Fig. 3, H to J). The same manipulation did not cause any significant change in body weight, motor activity, emotional responses, or behavioral responses to thermal and mechanical stimuli (fig. S14, B to M).
The central circuit responsible for the itch-induced scratching behavior has long been elusive (10, 20). Our findings suggest that the PBN represents a first central relay for itch sensation, and its activity regulates both acute and chronic itch-induced scratching. Spinal GRPR+ neurons are required for itch sensation (19), but their central target has been unknown. Here, we showed that GRPR+ neurons could activate PBN neurons via disynaptic excitatory connections. This spinoparabrachial pathway plays an important role in itch sensation, in addition to the contribution of the spinothalamic tract (8, 26, 27) and potential GRPR+ neuron-independent pathways (2).

Fig. 1. Dissection of the spinoparabrachial pathway that mediates itch signal processing. (A) Schematic diagram for retrobeads injection and experimental timeline. (B and C) Representative images of c-Fos+ and beads+ neurons in the ipsilateral dorsal spinal cord after intradermal injection of (C) histamine or (B) saline. Arrowheads indicate double-labeled neurons. Scale bar, 50 μm. (D) Percentage of beads+ cells expressing c-Fos in the dorsal spinal cord (n = 6 or 7 mice). (E) Schematic diagram for intraspinal cord viral injection and optical fiber implantation in the PBN. (F) Effect of optogenetic inhibition of the spinoparabrachial pathway on scratching behavior in response to histamine [(G), n = 16 or 17 mice] or chloroquine [(H), n = 16 or 17 mice]. (I) Schematic depicting virus and retrobeads injection, as well as recording configuration in spinal slices. (J) All recorded cells were filled with biocytin (blue) and were beads-positive (red). GRPR+ fibers were labeled with EYFP (green). Scale bar, 10 μm. (K) Action potentials induced through photostimulation (473 nm, 1 ms, blue bars) in spinal GRPR+ neurons. (L) Representative traces showing EPSCs evoked through photostimulation (473 nm, 1 ms) in a beads+ neuron in the spinal slice before and after NBQX (10 μM). (M) Summary data showing the amplitude of light-evoked EPSCs (n = 4 neurons). P = 0.056. Error bars represent SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Unpaired t test for (D); one-way analysis of variance (ANOVA) with Bonferroni’s correction for multiple comparisons test for (G) and (H); paired t test for (M).
Fig. 2. Pharmacogenetic suppression of PBN neurons impaired itch-induced scratching behavior. (A) c-Fos expression in the PBN in response to histamine (right) or saline (left). Scale bar, 100 μm. (B and C) Number of c-Fos* neurons in different parts of PBN in response to (B) histamine or (C) chloroquine as compared with control (n = 3 to 5 mice). Cont, contralateral; Ipsi, ipsilateral. (D) Schematic depicting the recording system for obtaining the Ca²⁺ signal with fiber photometry and scratching behavior with a magnetic induction method simultaneously. (E) Ca²⁺ transients associated with scratching behavior induced by histamine. (Top) The heatmap illustrating Ca²⁺ signals aligned to the beginning of scratching trains. Each row plots Ca²⁺ signals corresponding to one scratching train. Color scale indicates ∆F/F. (Bottom) Individual trial (light blue) and the averaged Ca²⁺ transients (red). (F and G) Mean fluorescent signal of mice injected with AAV-hSyn-GCaMP6s (red) or AAV-hSyn-EGFP (blue) in the PBN in response to histamine [(F), n = 5 or 7 mice] or chloroquine [(G), n = 5 or 6 mice], with shaded areas indicating SEM. (H) Expression of hM4Di-mCitrine or EGFP in the PBN. Scale bars, 200 μm (left), 25 μm (right). (I) (Top) hM4Di-mCitrine* cells were recorded in cell-attached mode in acute brain slices. (Middle) Effect of bath application of CNO on spikes of an example hM4DI-mCitrine* neuron in the PBN. (Bottom) Firing rate normalized to baseline (n = 4 neurons). (J) Timeline of the behavioral experiment. (K) Effect of pharmacogenetic inhibition of the PBN on the scratching behavior in response to histamine (n = 10 or 14 mice) or chloroquine (CQ) (n = 10 or 14 mice). Error bars represent SEM. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired t test for (B) and (C); one-way ANOVA with Bonferroni's correction for multiple comparisons test for (K).
in frogs (28). Given that the PBN receives dense projection from spinal cord in primates (29, 30), the spinoparabrachial pathway might also play a critical role in itch processing of humans. Our study paves the way for further dissection of central circuit mechanisms underlying itch sensation.

REFERENCES AND NOTES

25. Q. Tong et al., Cell Metab. 5, 383–393 (2007).

ACKNOWLEDGMENTS

We thank M. M. Poo and M. Beierlein for comments on the manuscript. We thank C. Luo, X. H. Xu, H. L. Hu, J. Yan, L. Yuan, X. J. Chen, Z. Li, J. Pan, K. Yang, and Y. J. Zhu for their technical support and all the laboratory members of Y.G.S. for helpful discussion. All data to understand and assess the conclusions of this study are available in the main text or supplementary materials. This work was supported by the National Natural Science Foundation of China (grants 31371122, 31371211, and 81322015), the Chinese Academy of Sciences Hundreds of Talents Program (to Y.G.S.), the Youth Thousand Plan (to Y.G.S.), and the Strategic Priority Research Program of the Chinese Academy of Sciences (grant XDB02010000).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/357/6352/695/suppl/DC1

Materials and Methods;
Figs. 51 to S14
References (31–42)
Movie S1

18 March 2017; accepted 7 July 2017
10.1126/science.aaf4918
A central neural circuit for itch sensation
Di Mu, Juan Deng, Ke-Fei Liu, Zhen-Yu Wu, Yu-Feng Shi, Wei-Min Guo, Qun-Quan Mao, Xing-Jun Liu, Hui Li and Yan-Gang Sun

Science 357 (6352), 695-699.
DOI: 10.1126/science.aaf4918

The circuits of itching and scratching
Itch is a major clinical problem with poor treatment options. In the past few years, much progress has been made in identifying itch-selective molecules and neurons. However, we know very little about the brain circuits underlying itch processing. Mu et al. found that a subpopulation of itch-processing neurons in the spinal cord directly excite other neurons that project to a brain stem structure called the parabrachial nucleus. Inhibition of this spino-parabrachial pathway reduced itching and scratching in mice.

Science, this issue p. 695

ARTICLE TOOLS http://science.sciencemag.org/content/357/6352/695
SUPPLEMENTARY MATERIALS http://science.sciencemag.org/content/suppl/2017/08/16/357.6352.695.DC1
RELATED CONTENT http://stm.sciencemag.org/content/scitransmed/6/223/223ra22.full
REFERENCES This article cites 42 articles, 9 of which you can access for free http://science.sciencemag.org/content/357/6352/695#BIBL
PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service