NEUROSCIENCE

A central neural circuit for itch sensation

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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 the 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 suppressed pruritogen-induced scratching behavior. Thus, our studies reveal a central neural circuit that is critical for itch signal processing.

Itch sensation induces scratching behaviors, which could cause serious tissue damage in cases of chronic itch (*1*). Much progress has been made in identifying itch-selective molecules and neurons in the dorsal root ganglion and spinal cord (*2*–*6*). However, we know much less about the central circuit underlying itch processing (*7*–*10*). The spinothalamic tract has been implicated in itch processing (*11*, *12*). In addition, the spinoparabrachial pathway could also play important roles in various sensory processing, including itch sensation (*13*–*18*). However, the functional role of different ascending pathways in itch sensation remains unknown.

The activity of parabrachial nucleus (PBN)–projecting neurons in superficial dorsal spinal cord of mice was significantly elevated by a pruritic stimulus, as indicated by an increased expression of the immediate early gene c-Fos (Fig. 1, A to D). To further examine the function of the spinoparabrachial pathway in itch-induced scratching behavior, we infused an adeno-associated virus (AAV) carrying eNpHR3.0 or enhanced green fluorescent protein (EGFP) into the cervical dorsal spinal cord of mice and implanted optic fibers to the PBN bilaterally to selectively suppress the activity of spinal axon projections in the PBN (Fig. 1E and fig. S1, A to C and E to J). This significantly decreased the scratching behavior induced by both histamine and chloroquine (Fig. 1, F to H, 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-1,2,3,4-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 $c\text{-}Fos^+$ 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 $c\text{-}Fos^+$ 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 thus 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 $Vglut2^{f/f}$ mice (*25*) led to a selective reduction of *Vglut2* in the PBN (Fig. 3, A 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 S14A). 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).

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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*). Our study also highlights the importance of brain circuits in itch-induced scratching behavior in rodents, even though similar behavior (such as wiping) can be accomplished by spinal circuits

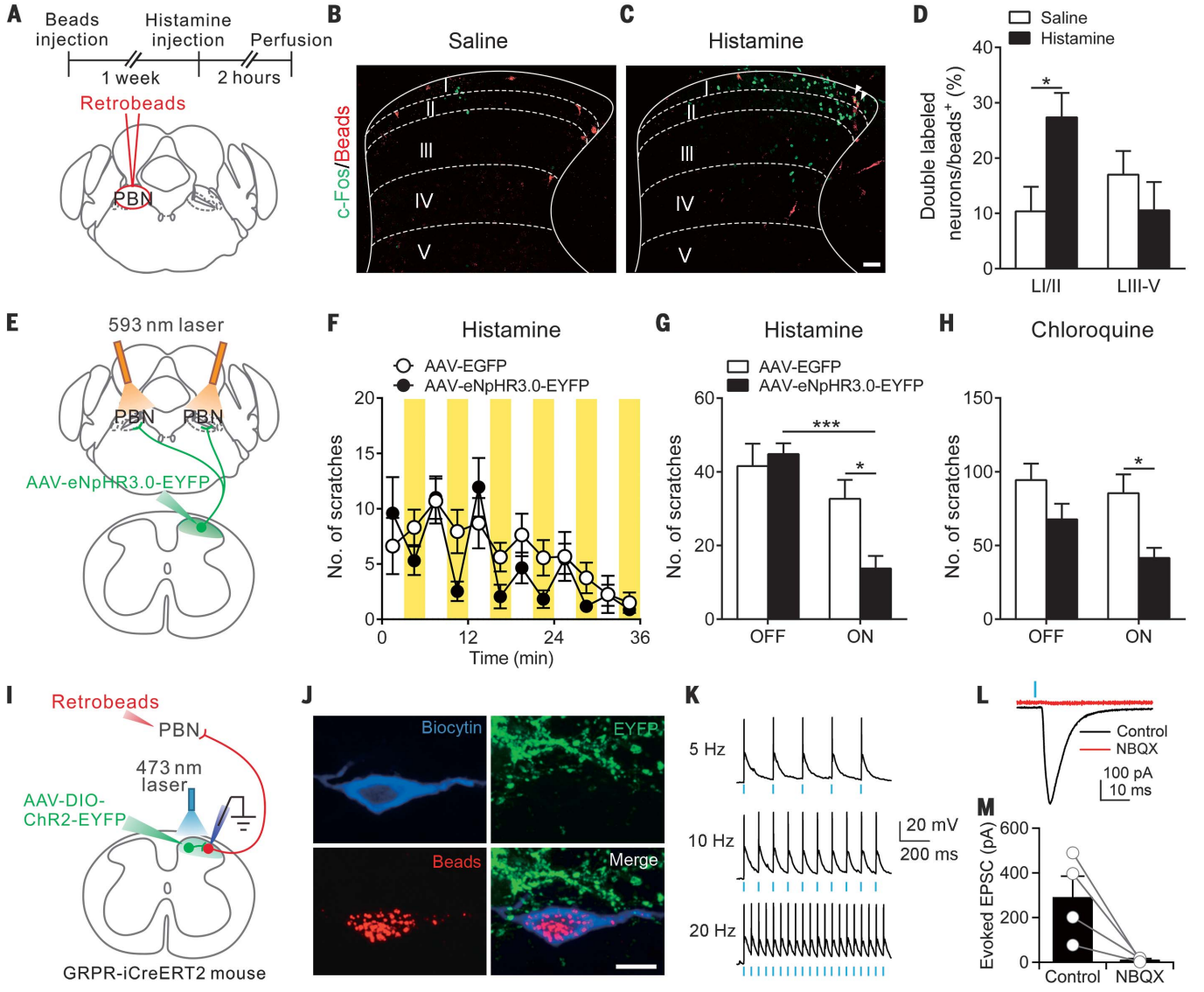

Fig. 1. Dissection of the spinoparabrachial pathway that mediates itch signal processing. (**A**) Schematic diagram for retrobeads injection and experimental timeline. (**B** and **C**) Representative images of c-Fos$^+$ and beads$^+$ neurons in the ipsilateral dorsal spinal cord after intradermal injection 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. Each point represents the number of scratching bouts in a 3-min light on (593 nm, 8 to 10 mW, yellow shaded) or light off period (n = 16 or 17 mice). (**G** and **H**) The total number of scratching bouts during light on or off phase 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 < 0.001. 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).

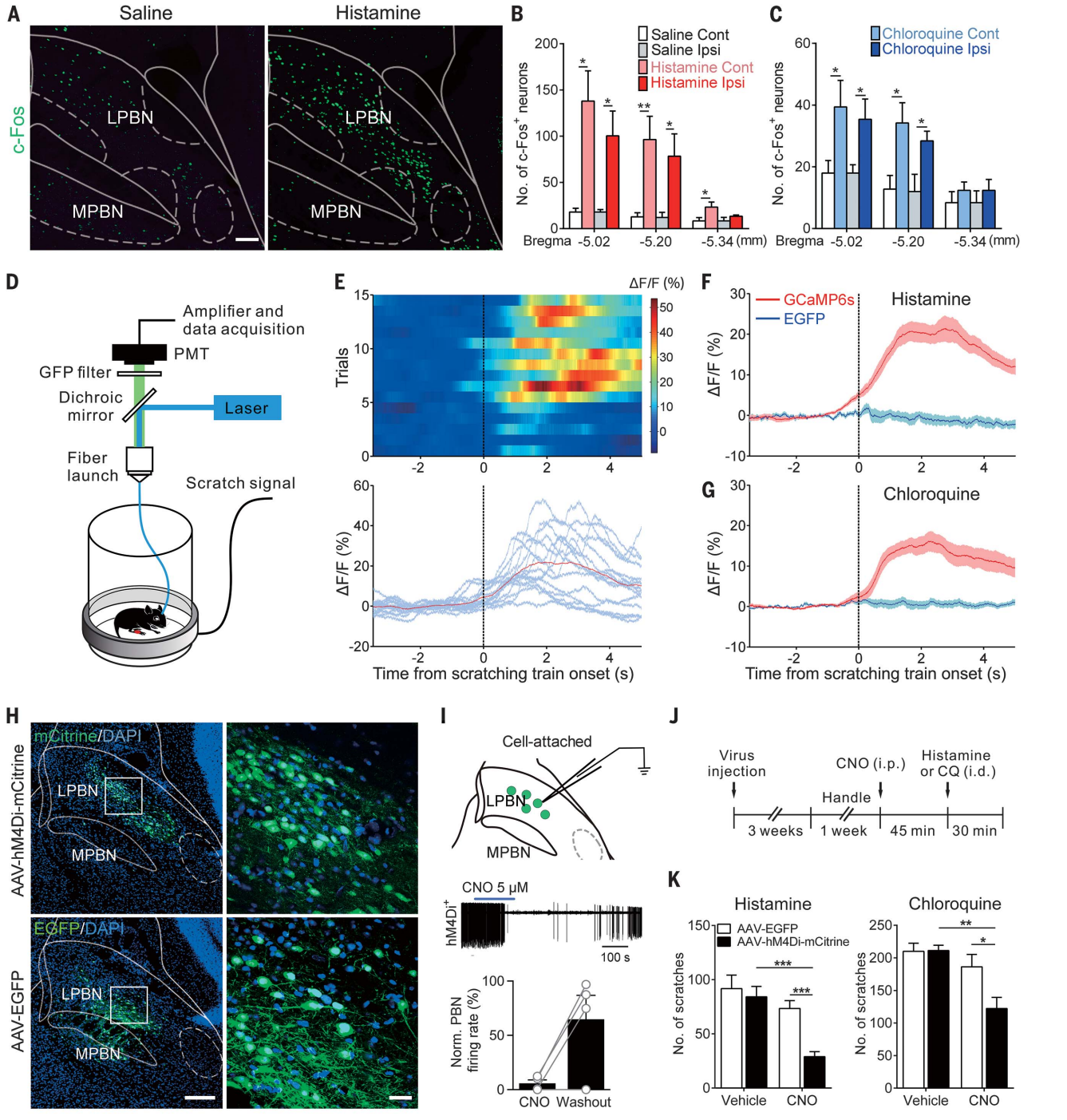

Fig. 2. Pharmacogenetic suppression of PBN neurons impaired itch-induced scratching behavior. (**A**) c-Fos expression in the PBN in response to histamine (right) or saline (left). Scale bar, 100 μm. (**B** and **C**) Number of c-Fos$^+$ neurons in different parts of PBN in response to (B) histamine 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 Ca^{2+} signal with fiber photometry and scratching behavior with a magnetic induction method simultaneously. (**E**) Ca^{2+} transients associated with scratching behavior induced by histamine. (Top) The heatmap illustrating Ca^{2+} signals aligned to the beginning of scratching trains. Each row plots Ca^{2+} signals corresponding to one scratching train. Color scale indicates $\Delta F/F$. (Bottom) Individual trial (light blue) and the averaged Ca^{2+} 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).

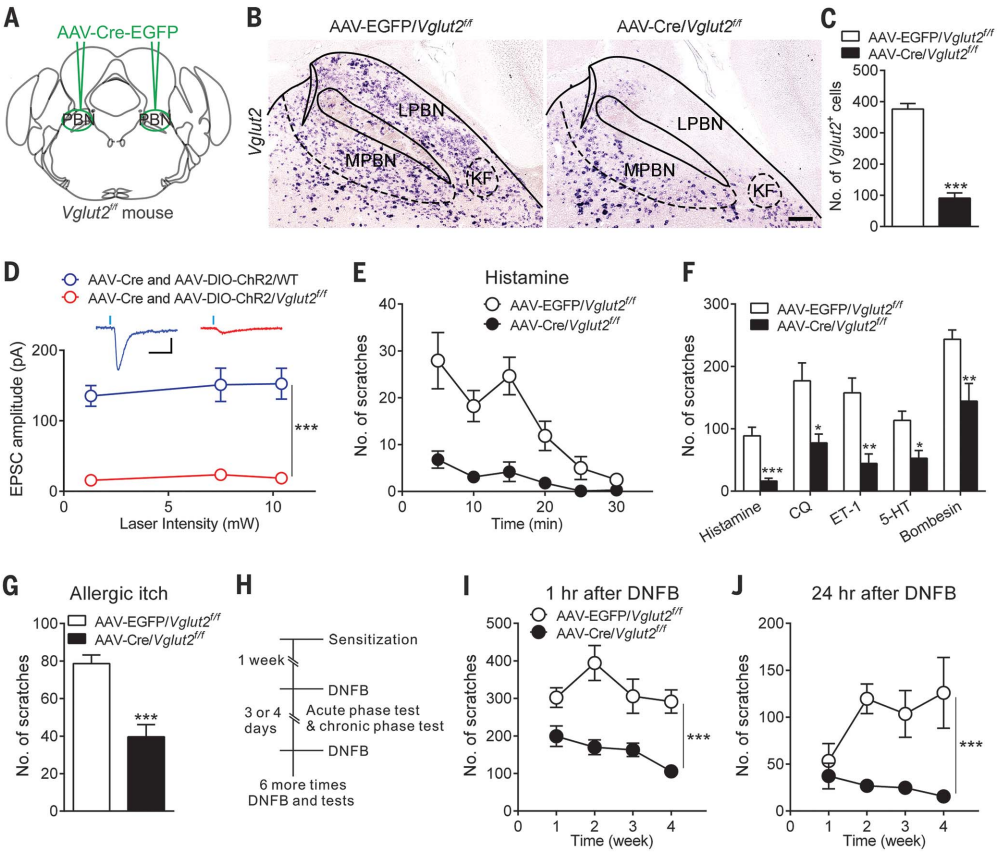

Fig. 3. Glutamatergic neurons in the PBN are required for itch-induced scratching behavior. (**A**) Bilateral injection of AAV-Cre-EGFP or AAV-EGFP into the PBN of *Vglut2*$^{f/f}$ mice. (**B** and **C**) In situ hybridization showing the reduction of *Vglut2*$^{+}$ neurons in the PBN of *Vglut2*$^{f/f}$ mice that received AAV-Cre-EGFP injection compared with those that received AAV-EGFP injection (n = 5 or 8 mice). Scale bar, 150 μm. (**D**) The EPSCs induced through photostimulation (473 nm, 1 ms, blue bars) in amygdala of wild-type and *Vglut2*$^{f/f}$ mice injected with AAV-Cre-EGFP and AAV-DIO-ChR2-mCherry in the PBN (n = 12 or 13 neurons). Scale bars, 10 ms and 40 pA. (**E**) The number of scratching bouts in response to histamine in *Vglut2*$^{f/f}$ mice injected with AAV-EGFP or AAV-Cre-EGFP in the PBN (n = 10 or 14 mice). (**F**) Summary showing the effect of genetic deletion of VGLUT2 in the PBN on scratching behaviors induced with different pruritogens (n = 5 to 14 mice). (**G**) Number of scratching bouts in allergic itch model (n = 5 or 8 mice). (**H**) Experimental timeline of the DNFB model. (**I** and **J**) Number of scratching bouts in chronic itch model induced with DNFB at (I) 1 hour and (J) 24 hours after DNFB treatment (n = 10 or 11 mice). Error bars represent SEM. *P < 0.05, **P < 0.01, ***P < 0.001. Unpaired t test for (C), (F), and (G); two-way ANOVA for (D), (I), and (J).

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.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/357/6352/695/suppl/DC1
Materials and Methods
Figs. S1 to S14
References (*31*–*42*)
Movie S1

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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.

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