Activity-dependent silencing reveals functionally distinct itch-generating sensory neurons

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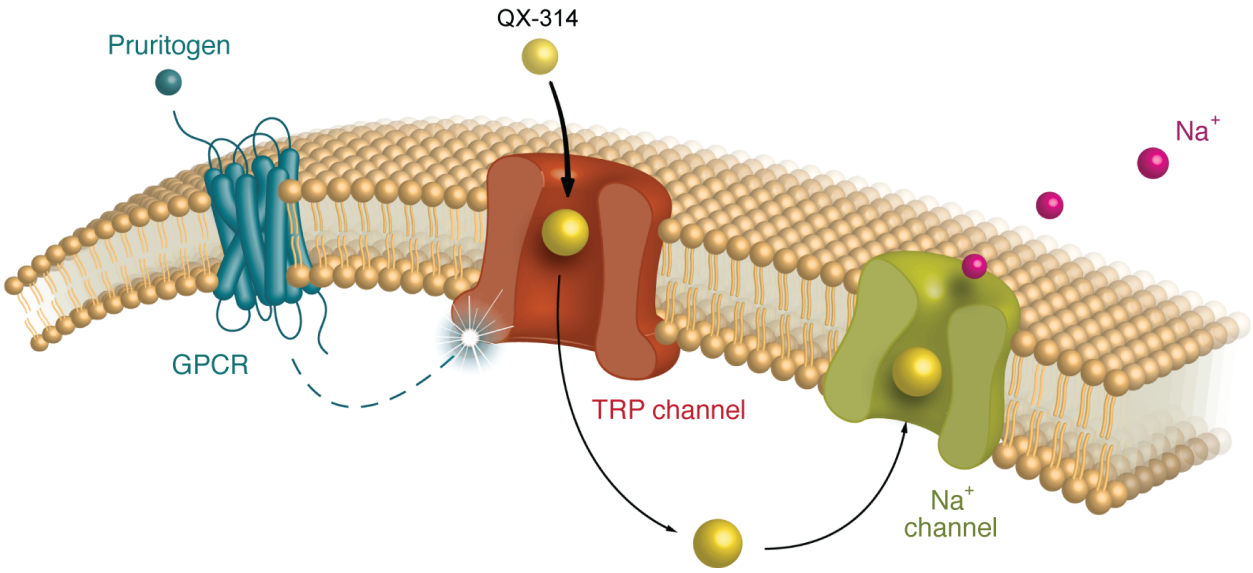
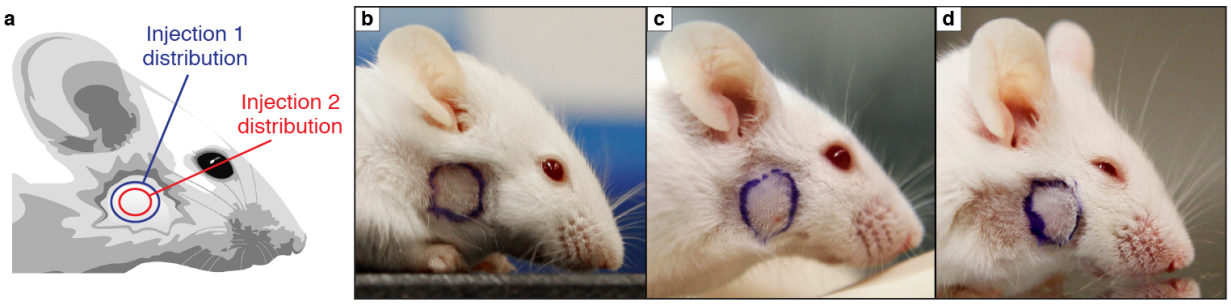
SUPPLEMENTARY FIGURES

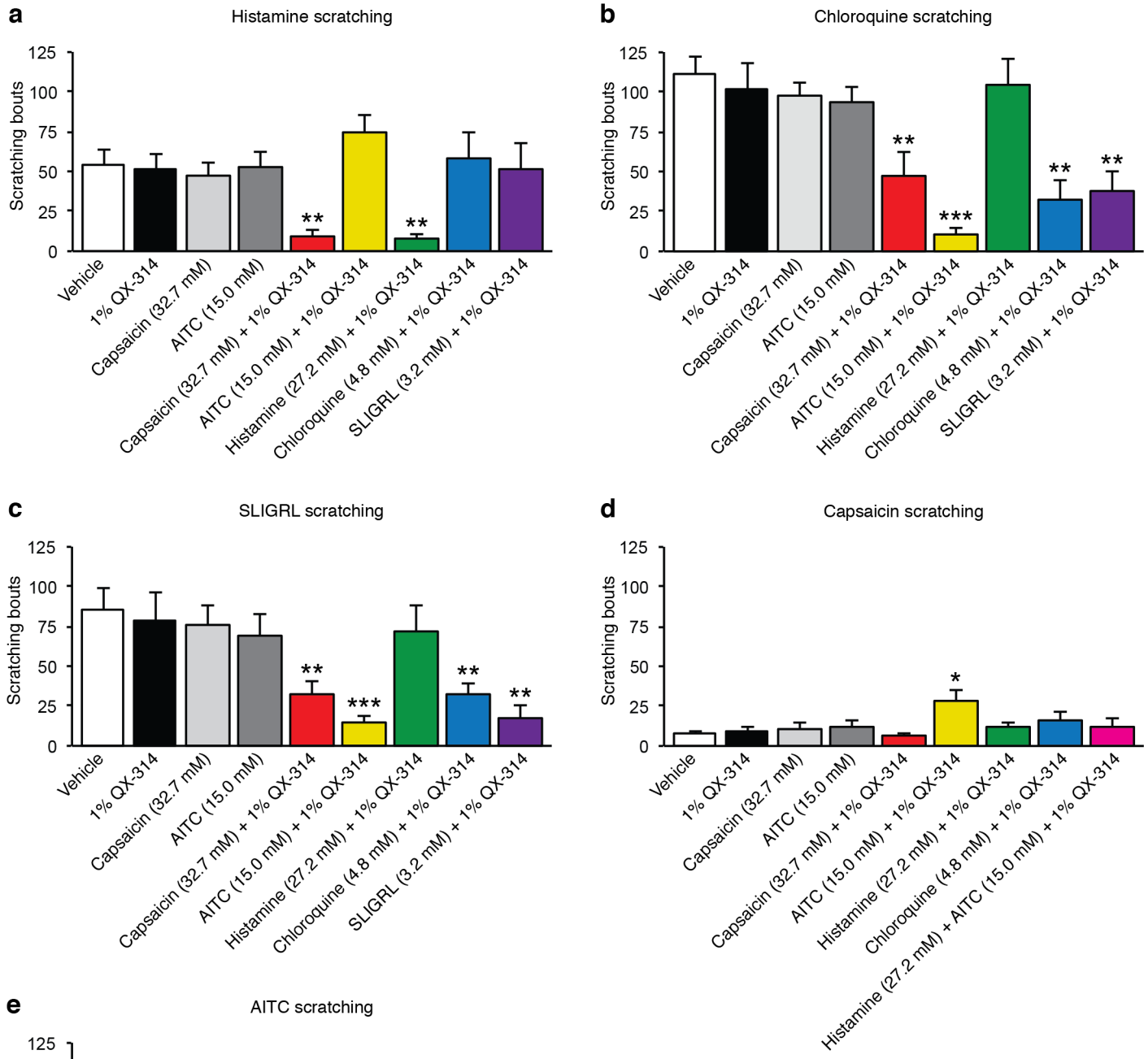


Supplementary Figure 1. Model of pruritogen mediated targeted delivery of QX-314

Pruritogen mediated GPCR activation initiates opening of large-pore ion channels (e.g., TRPV1, TRPA1) to allow entry of the membrane-impermeant sodium channel blocker, QX-314. Once inside the nerve fiber, QX-314 has access to its binding site on the sodium channel and blocks thereby conduction only in fibers activated by the administered pruritogen.



Supplementary Figure 2. Parameters for proper sequential intradermal injections **(a)** Conditioning injections (Injection 1, blue oval) were delivered in 20µl of vehicle while test injections (Injection 2, red oval) were administered in 10µl of vehicle so that the area of distribution of injection 2 was within the cutaneous area of distribution of injection 1. **(b)** No bulla formed = injection too deep. **(c)** Slightly domed bulla = proper injection. **(d)** Semi-translucent, high-domed bulla = injection too shallow.



Supplementary Figure 3. Pruritogen and algogen-mediated scratching measured 30 minutes after conditioning injections (indicated at the x-axes)
Data from Fig. 4 and Fig. 7 are presented again here for ease of comparison with data from the control experiments. **(a)** Histamine-evoked hindlimb scratching bouts. **(b)** Chloroquine-evoked hindlimb scratching bouts. **(c)** SLIGRL-evoked hindlimb scratching bouts. **(d)** Capsaicin-evoked hindlimb scratching bouts. **(e)** AITC-evoked hindlimb scratching bouts. All values represent total cheek scratching bouts in thirty minutes beginning thirty minutes after intradermal cheek conditioning injection. P-value annotations represent comparison of value of annotated column to vehicle conditioning injection value (No annotation, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Error bars represent SEM.

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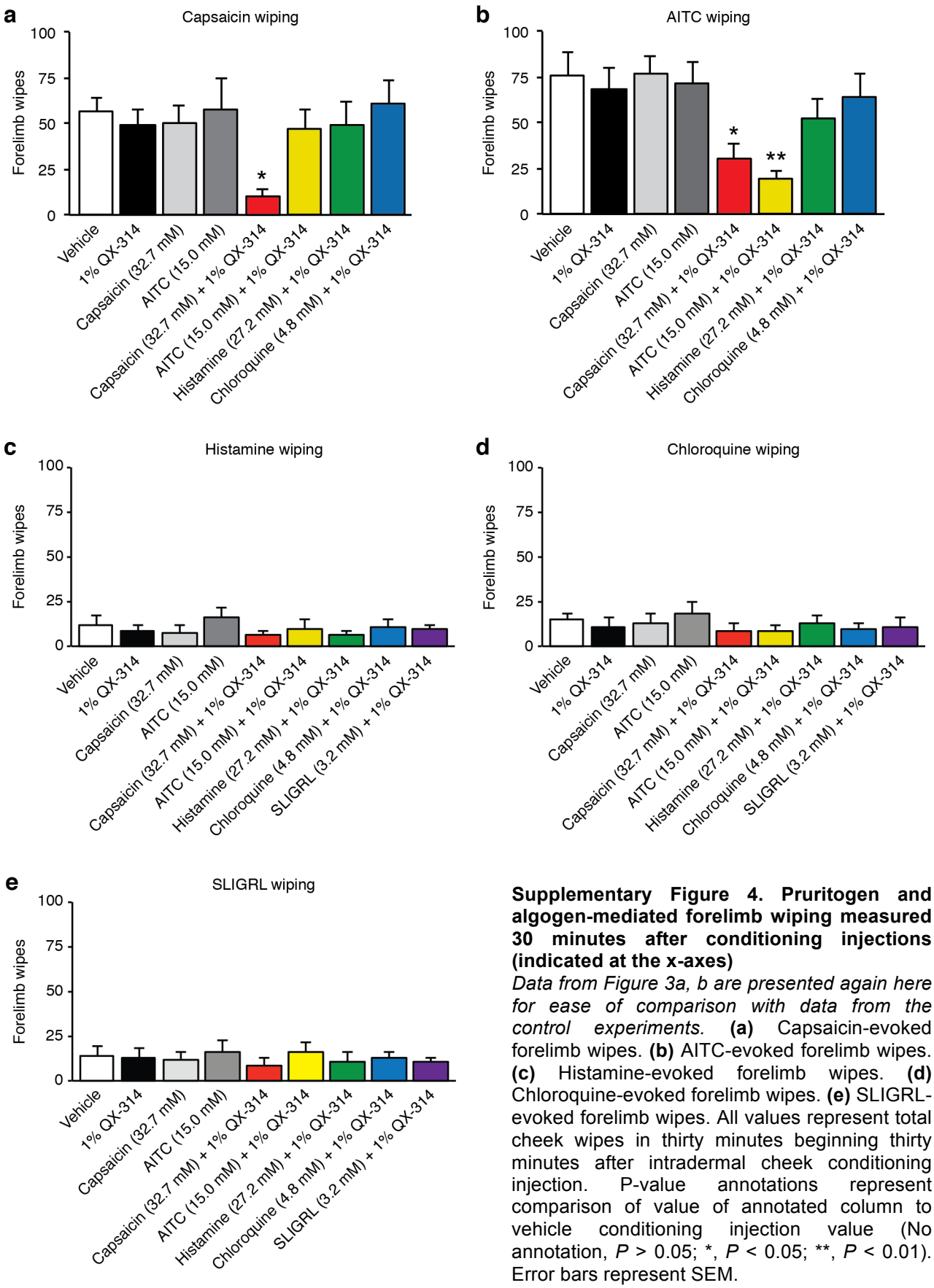
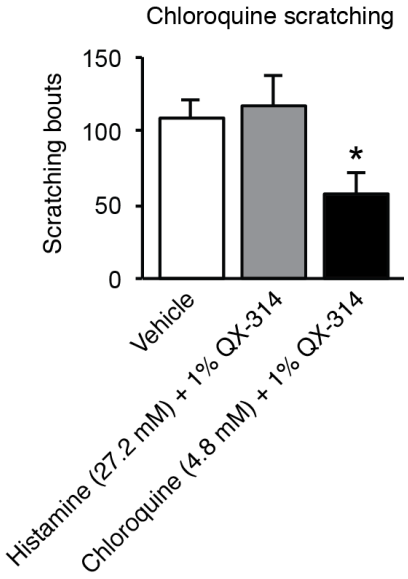


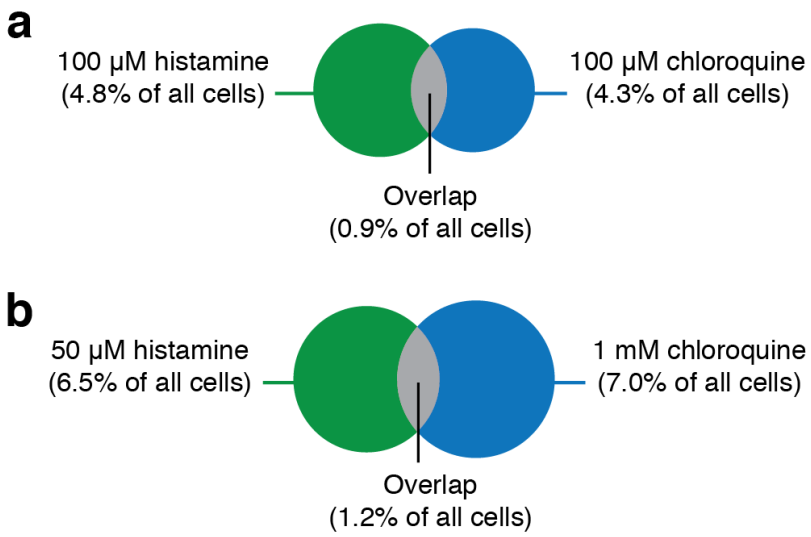
Supplementary Figure 4. Pruritogen and algogen-mediated forelimb wiping measured 30 minutes after conditioning injections (indicated at the x-axes)

Data from Figure 3a, b are presented again here for ease of comparison with data from the control experiments. **(a)** Capsaicin-evoked forelimb wipes. **(b)** AITC-evoked forelimb wipes. **(c)** Histamine-evoked forelimb wipes. **(d)** Chloroquine-evoked forelimb wipes. **(e)** SLIGRL-evoked forelimb wipes. All values represent total cheek wipes in thirty minutes beginning thirty minutes after intradermal cheek conditioning injection. P-value annotations represent comparison of value of annotated column to vehicle conditioning injection value (No annotation, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$). Error bars represent SEM.



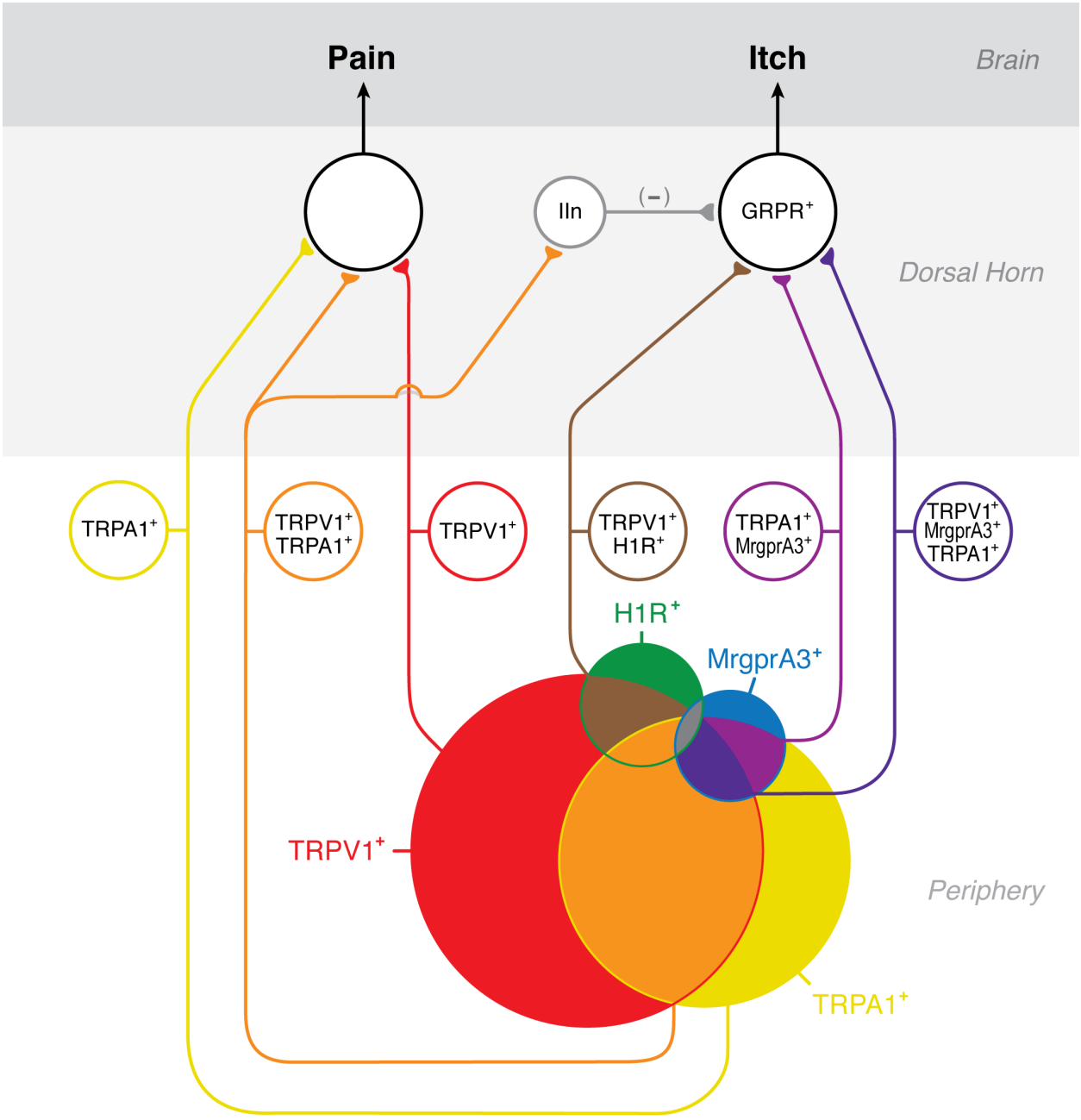
Supplementary Figure 5. Intradermal rostral back injection of chloroquine together with QX-314, but not histamine together with QX-314, prevents scratching following test injection of chloroquine

Chloroquine-evoked scratching bouts thirty minutes after intradermal rostral back conditioning injection of vehicle (white column), histamine (27.2 mM) together with 1% QX-314 (green column) and chloroquine (4.8 mm) with 1% QX-314 (blue column). P-value annotation represents comparison of value of Histamine + 1% QX-314 conditioning injection group to value of vehicle conditioning injection group (*, $P < 0.05$). Error bars represent SEM.



Supplementary Figure 6. Venn representation of relative populations of trigeminal and DRG neurons defined by their calcium responses to histamine and chloroquine

(a) Histamine (100 μM) activated 10 of 208 trigeminal neurons, while 9 responded to 100 μM chloroquine. Among histamine-activated cells 8 of 10 responded to histamine but not chloroquine. For chloroquine-responsive neurons, 7 of 9 responded to chloroquine alone. 2 cells responded to both histamine and chloroquine. **(b)** Among 417 cultured DRG neurons, 32 cells responded 50 μM histamine and 34 were activated by 1 mM chloroquine. Among histamine responsive cells, 27 of 32 responded to histamine alone. Chloroquine, but not histamine, activated 29 of 34 chloroquine responders. 5 cells responded to histamine and chloroquine.



Supplementary Figure 7.
Hypothetical model of itch and pain circuitry based on primary afferent neuron subsets associated with transmission of pain and itch

Venn representation of afferents associated with the peripheral transmission of pain and itch are defined by expression of TRPV1 (capsaicin-responsive neurons, red circle), TRPA1 (AITC-responsive neurons, yellow circle), H1R (histamine-responsive neurons, green circle) and MrgprA3 (chloroquine-responsive neurons, blue circle). Our data suggest that peripheral terminals responding to capsaicin and histamine (brown shaded area) mediate histaminergic itch, while non-histaminergic itch is transmitted by two groups of fibers: fibers expressing TRPV1, TRPA1 and MrgrprA3 (purple shaded area), and fibers that express only TRPA1 and MrgprA3 (fuchsia shaded area). We propose that distinct populations of primary afferents, histamine itch-generating fibers ($H1R^+/TRPV1^+$) and non-histamine itch-generating fibers ($MrgprA3^+/TRPA1^+/TRPV1^{+/-}$), transmit signals to itch-related sensory neurons located in the spinal cord dorsal horn. Finally, our observation that algogens can evoke scratching (itch) following silencing of contra-algogen-responsive fibers implies existence of a population of $TRPV1^+/TRPA1^+$ fibers (from within orange shaded area) that initiate an algogen-mediated inhibition or masking of itch probably via central inhibitory interneurons (IIn).

Supplementary Tables

	Vehicle		**Histamine+ QX-314** (histamine-positive neurons)		**Histamine +QX-314** (histamine-negative neurons)		**Histamine + QX-314 + capsazepine** (histamine-positive neurons)	
	Relative Peak Current (Treatment/ control) (%)	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**
Before	100	0	100	0	100	0	100	0
2.5 min	86.6	5.8	59.7	11.8	96.99	3.2	93.7	4.2
5 min	79.2	4.7	47.8	17.6	86.8	6.1	86.9	4.1
7.5 min	69.4	5.2	30.1	9.3	76.1	11.8	71.3	4.2
10 min	63.2	9.9	14.8	9.3	63.5	19.1	53.4	4.2

Supplementary Table 1. The time course of the effect of histamine + QX-314 and histamine + QX-314 + capsazepine on relative amplitude of peak sodium current in histamine-positive (n=3) and histamine-negative (n=3) cells. Note, the current rundown when only vehicle was applied (n=4, both histamine-positive and histamine-negative cells). The significance of the treatments was obtained by comparison between different treatment groups (see Supplementary table 2).

	Vehicle vs. Histamine+ QX-314 (histamine-positive neurons)		**Vehicle vs. Histamine+ QX-314** (histamine-negative neurons)		**Vehicle vs. Histamine + QX-314 + capsazepine** (histamine-positive neurons)		**Histamine + QX-314** (histamine-positive neurons) **vs. Histamine +QX-314** (histamine-negative neurons)		**Histamine + QX-314** (histamine-positive neurons) **vs. Histamine + QX-314 + capsazepine** (histamine-positive neurons)		**Histamine + QX-314** (histamine-negative neurons) **vs. Histamine + QX-314 + capsazepine** (histamine-positive neurons)	
	p-value	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**
Before	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0
2.5 min	> 0.05	2.7	> 0.05	0.9	> 0.05	1.9	< 0.01	3.4	< 0.001	4.4	> 0.05	0.9
5 min	< 0.05	2.7	> 0.05	0.7	> 0.05	1.9	< 0.05	3.2	< 0.001	4.3	> 0.05	1.1
7.5 min	< 0.01	3.4	> 0.05	0.6	> 0.05	1.4	< 0.01	3.7	< 0.001	4.5	> 0.05	0.7
10 min	< 0.001	4.2	> 0.05	0.02	> 0.05	0.06	< 0.01	3.5	< 0.01	3.9	> 0.05	0.07

Supplementary Table 2. Comparison of the effects of histamine + QX-314 and histamine + QX-314 + capsazepine on the amplitude of sodium currents in histamine-positive (n=3) and histamine-negative (n=3) TG neurons, calculated using two-way ANOVA non-parametric test with post-hoc Bonferroni test. The difference considered significant if p-value is below 0.05. t – degree of freedom.

	Vehicle		Chloroquine + QX-314 (chloroquine-positive neurons)		Chloroquine +QX-314 (chloroquine-negative neurons)		Chloroquine + QX-314 + HC-030031 (chloroquine-positive neurons)	
	Relative Peak Current (Treatment/ control) (%)	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**	**Relative Peak Current (Treatment/ control) (%)**	**SEM**
Before	100	0	100	0	100	0	100	0
2.5 min	86.6	5.8	40.6	4.0	75.3	8.8	88.9	1.9
5 min	79.2	4.7	28.5	5.1	63.8	8.2	75.4	3.2
7.5 min	69.4	5.2	25.9	7.7	55.8	5.2	65.7	3.0
10 min	63.2	9.9	20.3	4.8	49.4	3.4	59.6	3.4

Supplementary Table 3. The time course of the effect of chloroquine+QX-314 and chloroquine + QX-314 + HC-030031 on relative amplitude of peak sodium current in chloroquine-positive (n=3) and chloroquine-negative (n=3) cells. Note, the current rundown when only vehicle was applied (n=4, both chloroquine-positive and chloroquine-negative cells). The significance of the treatments was obtained by comparison between different treatment groups (see Supplementary table 4).

	Vehicle vs. Chloroquine+ QX-314 (chloroquine-positive neurons)		**Vehicle vs. Chloroquine+ QX-314** (chloroquine-negative neurons)		**Vehicle vs. Chloroquine + QX-314 + HC-030031** (chloroquine-positive neurons)		**Chloroquine + QX-314** (chloroquine-positive neurons) **vs. Chloroquine +QX-314** (chloroquine-negative neurons)		**Chloroquine + QX-314** (chloroquine-positive neurons) **vs. Chloroquine + QX-314 + HC-030031** (chloroquine-positive neurons)		**Chloroquine + QX-314** (chloroquine-negative neurons) **vs. Chloroquine + QX-314 + HC-030031** (chloroquine-positive neurons)	
	p-value	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**	**p-value**	**t**
Before	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0	> 0.05	0
2.5 min	< 0.001	6.2	> 0.05	1.5	> 0.05	0	< 0.001	4.3	< 0.001	6.1	> 0.05	1.7
5 min	< 0.001	6.9	> 0.05	2	> 0.05	0.5	< 0.001	4.5	< 0.001	5.9	> 0.05	1.8
7.5 min	< 0.001	5.9	> 0.05	1.9	> 0.05	0.5	< 0.01	3.8	< 0.001	5	> 0.05	1.3
10 min	< 0.001	5.8	> 0.05	1.9	> 0.05	0.4	< 0.01	3.7	< 0.001	5	> 0.05	1.3

Supplementary Table 4. Comparison of the effects of the application of chloroquine + QX-314 and chloroquine + QX-314 + HC-030031 on the amplitude of sodium currents in chloroquine-positive (n=3) and chloroquine-negative (n=3) TG neurons, calculated using two-way ANOVA non-parametric test with post-hoc Bonferroni test. The difference considered significant if p-value is below 0.05. t – degree of freedom.