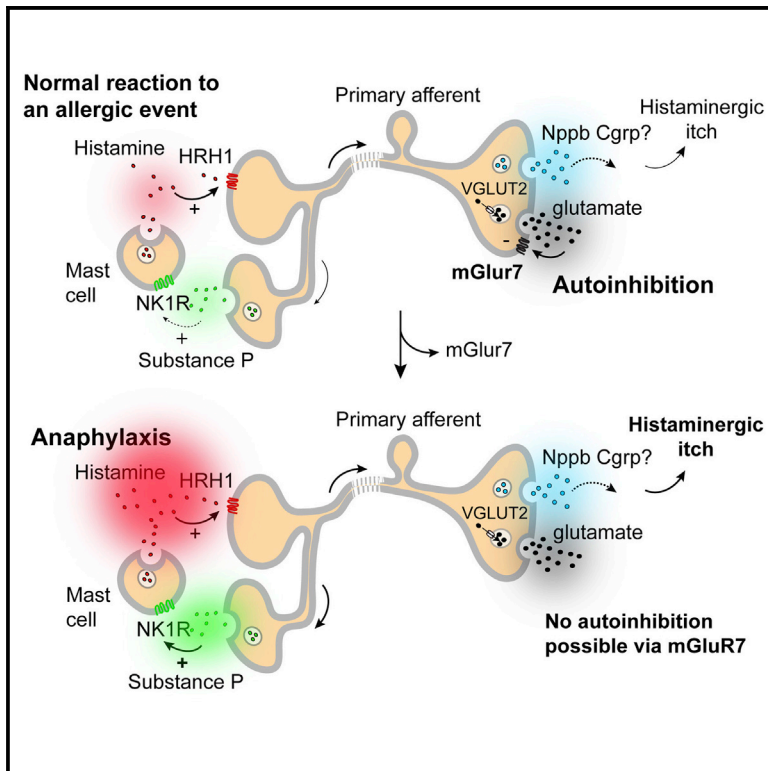


Identification of a Neuronal Receptor Controlling Anaphylaxis

Graphical Abstract



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In Brief

Rogoz et al. use two-photon microscopy, pharmacology, and transgenic mice to show that mGluR7 prevents local allergic events from causing anaphylaxis through presynaptic auto-regulation of peripheral neurons, indicating the role of nervous system control in anaphylaxis.

Highlights

- mGluR7 and glutamate provide autoinhibition to peripheral histaminergic neurons
- mGluR7 ablation and thus faulty regulation of peripheral neurons causes anaphylaxis
- mGluR7 regulates excessive itch via neuronal transmission in central itch pathways
- mGluR7 regulates symptoms of anaphylaxis via communication with the immune system



Identification of a Neuronal Receptor Controlling Anaphylaxis

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SUMMARY

Allergic reactions can in severe cases induce a state of circulatory shock referred to as anaphylaxis. Histamine, the primary mediator of this condition, is released from immune cells, and, therefore, anaphylaxis has so far been considered an immune system disorder. However, we here show that the glutamatergic receptor mGluR7, expressed on a subpopulation of both peripheral and spinal cord neurons, controls histamine-induced communication through calcium-dependent autoinhibition with implications for anaphylaxis. Genetic ablation of mGluR7, and thus altered regulation of histamine-sensing neurons, caused an anaphylaxis-like state in *mGluR7*^{-/-} mice, which could be reversed by antagonizing signaling between neurons and mast cells but not by antagonizing a central itch pathway. Our findings demonstrate the vital role of nervous system control by mGluR7 in anaphylaxis and open up possibilities for preventive strategies for this life-threatening condition.

INTRODUCTION

In 1910, intravenous β -iminazolyethylamine injections were shown to cause a sudden drop in blood pressure and respiratory disturbance (Dale and Laidlaw, 1910). The substance was later named histamine, and the accompanying clinical signs of a severe allergic reaction are now referred to as symptoms of anaphylaxis. Soon after, it was demonstrated that histamine also induces peripheral symptoms such as local itch and flare in the skin upon superficial injection (Lewis, 1927).

Histamine acts on local small diameter primary afferent neurons (Hägermark et al., 1979; Han et al., 2006; Schmelz et al., 1997), which release neurotransmitters from their central and peripheral terminals, to both relay pruritic information to the CNS and to involve the immune system in host defense. Peripheral release of neurotransmitters also leads to additional histamine discharge from nearby mast cells (Alving et al., 1991; Lawrence

et al., 1987), which could potentially escalate an initially modest allergic reaction into a life-threatening systemic anaphylactic shock. Anaphylaxis is, however, an infrequent condition; the lifetime prevalence is estimated to 0.05%–2.0% (Lieberman et al., 2006). This raises the issue whether peripheral neurons are kept under control to prevent local allergic events from turning into anaphylaxis by over-stimulation of the immune system.

Histaminergic itch or pruritus is a dominating local symptom of anaphylactic shock. Pruritic events in the periphery are transmitted to the CNS by different neuropeptides, such as natriuretic polypeptide b (NPPB) (Mishra and Hoon, 2013) and calcitonin gene-related peptide (CGRP) (Rogoz et al., 2014), which are mainly confined within the transient receptor potential vanilloid 1 (TRPV1) lineage neurons (Cavanaugh et al., 2009; Mishra and Hoon, 2013). Peripheral neurons with a possible role in anaphylaxis should therefore be confined within the histamine-sensing NPPB/CGRP/TRPV1 population of primary afferents, and, if so, how is the activity of these neurons regulated?

Glutamate is a fast neurotransmitter used by most, if not all, primary afferents. Genetic ablation studies have shown that glutamate is essential for the transmission of touch and all modalities of pain (Rogoz et al., 2012; Seal et al., 2009), whereas no clear evidence has been found that glutamate released from primary afferents transmits itch. However, we and others have demonstrated that mice with impaired release of glutamate from peripheral neurons (Lagerström et al., 2010; Liu et al., 2010; Rogoz et al., 2012) displayed a profound itch behavior that could be attenuated by antihistamines (Lagerström et al., 2010). Together, these findings suggest that glutamate released from peripheral neurons rather controls and regulates histaminergic itch instead of mediating itch. If true, what could be the possible mechanism and how would that affect other components during a severe allergic reaction? We set out to investigate the neuronal component of the allergic chain to determine whether neurons, via glutamatergic signaling, might prevent a local allergic reaction from escalating into anaphylaxis.

RESULTS

Glutamate released from primary afferents binds and activates both ionotropic and metabotropic receptors. Group II/III metabotropic receptors (mGluRs) were recently shown to exert

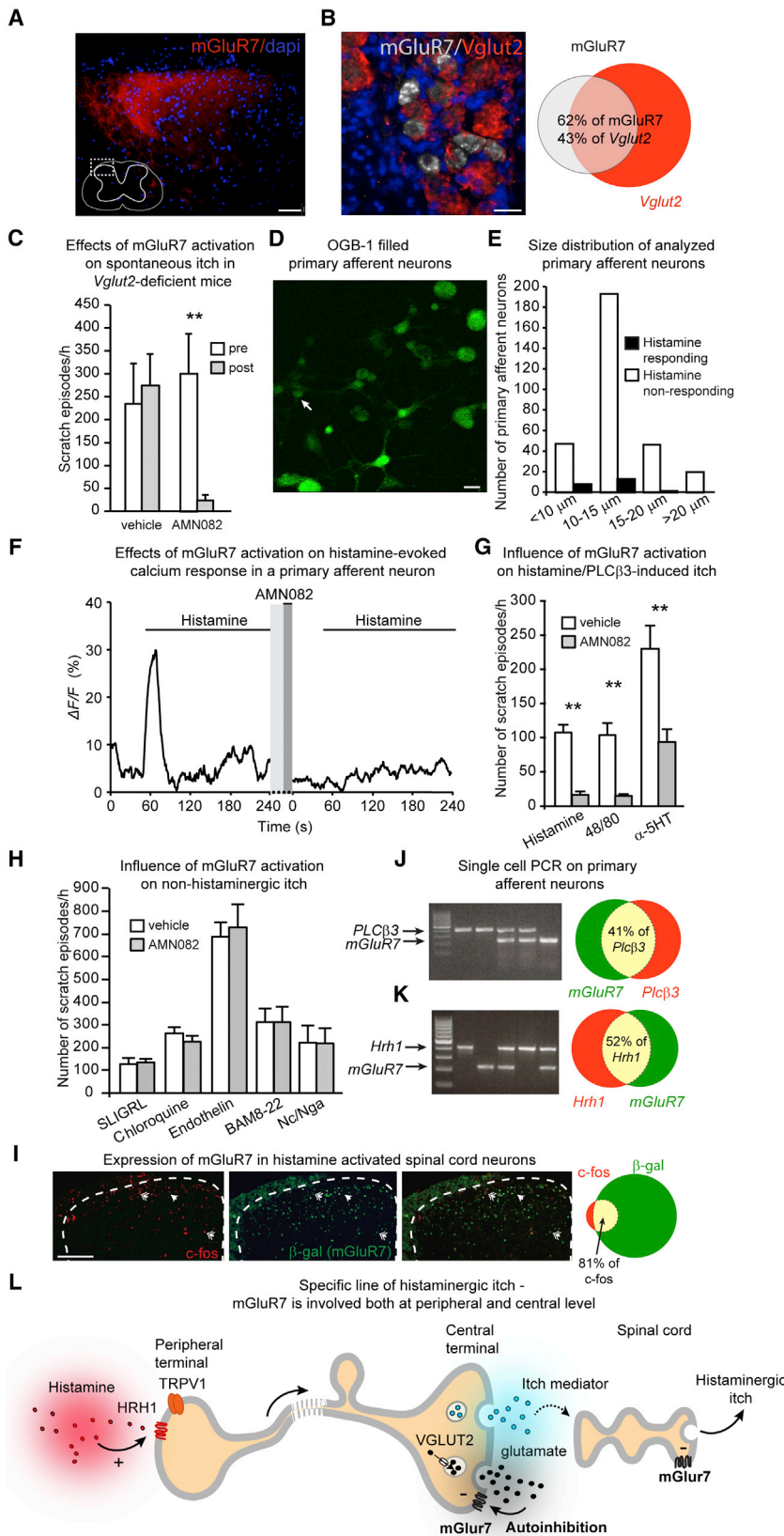


Figure 1. Activation of mGlu7 Regulates Itch, a Local Symptoms of Anaphylaxis, through Presynaptic Inhibition of Histamine-Sensing Primary Afferents

(A) mGlu7-immunoreactive fibers in the dorsal part of the spinal cord.

(B) mGlu7 and *Vglut2* expression in primary afferent neurons (n = 3, 29 sections).

(C) Intrathecal administration of the specific mGlu7 agonist AMN082 (100 μg/animal) in *Vglut2*^{fl/fl};*Trpv1-Cre* mice (n = 8).

(D) Culture of dissociated primary afferent neurons loaded with OGB-1 and imaged using two-photon microscopy. The histamine and AMN082-sensitive neuron shown in (F) is indicated with a white arrow.

(E) Size distribution of OGB-1 loaded dorsal root ganglia (DRG) neurons that responded to 80 μM of histamine (black, n = 22) and non-responding neurons (white, n = 306).

(F) Inhibition of histamine-evoked calcium response by AMN082. Representative trace shows an increase of 30% in the fluorescence signal when histamine (80 μM) was applied to the bath. Pre-treatment with AMN082 (10 μM) for 4 min impaired the histamine-evoked response in the same cell. Light gray bar indicates 15 min washing and dark gray indicates 4 min of AMN082 treatment (a summary of all calcium imaging data, together with the positive controls, can be found in Figure S1B).

(G) Intrathecal injections with vehicle or the mGlu7-specific agonist AMN082 (100 μg/animal) followed by an intradermal injection of histamine (100 μg, n = 6), 48/80 (10 μg, n = 6), or α-methyl serotonin (30 μg, n = 9).

(H) Intrathecal injections with vehicle or the mGlu7-specific agonist AMN082 followed by an intradermal injection of the PAR2 receptor agonist SLIGRL (100 μg, n = 13), chloroquine (10 mM, 50 μl, n = 15), endothelin (3 pM, 50 μl, n = 16), or BAM8-22 (3.5 mM, 50 μl, n = 12). Intrathecal vehicle or AMN082 administration in *Nc/Nga* mice subjected to topical mite allergen treatments (n = 12).

(I) Histamine (100 μg, 5 μl) induced *c-fos* activation in dorsal horn neurons in sedated mGlu7 transgenic mice (n = 4, 33 sections analyzed).

(J) Single-cell PCR analysis of primary afferent neurons (41% of *Plcβ3* mRNA expressing DRGs also expressed mGlu7 and 58% of *mGlu7* mRNA expressing DRGs expressed *Plcβ3* mRNA) (number of cells analyzed = 60; *mGlu7* = 211 bp, *Plcβ3* = 404 bp).

(K) Single-cell PCR analysis of primary afferent neurons (52% of the *Hrh1* mRNA expressing DRGs also expressed mGlu7 and 56% of *mGlu7* mRNA expressing DRGs also expressed *Hrh1* mRNA) (number of cells analyzed = 109; *Hrh1* = 440 bp).

(L) A schematic summarizing the findings in Figure 1. Activation of mGlu7 specifically regulates histaminergic itch. mGlu7 is expressed in a subpopulation of glutamatergic and histamine-sensing primary afferents and in a subpopulation of second-order neurons in the spinal cord. Activation of the mGlu7 receptor in primary afferents blocks histamine-induced calcium currents

(legend continued on next page)

endogenous inhibition on primary afferents expressing TRPV1 in rats (Carlton et al., 2011). One of these is the mGluR7 receptor and to investigate the involvement of mGluR7 in primary afferent regulation we performed real-time qPCR and immunohistochemistry analysis, which showed that mGluR7 was expressed in primary afferents that project to the dorsal part of the spinal cord (Figures 1A and S1A). Interestingly, combined *Vglut2* (Vesicular glutamate transporter 2) in situ hybridization and mGluR7 immunohistochemistry showed that a subpopulation of mGluR7 immunoreactive neurons overlapped with *Vglut2* (62%, Figure 1B), which indicates that the inhibitory group III mGluR, mGluR7, is positioned to execute autocrine inhibition of VGLUT2-positive primary afferent neurons. This finding prompted us to perform intrathecal injections of the mGluR7-specific allosteric agonist AMN082 to activate mGluR7 receptors in *Vglut2*-deficient mice with increased histaminergic itch behavior, which potentially could compensate for the loss of VGLUT2-mediated signaling and thus reverse the phenotype. The intrathecal treatment resulted in a profound attenuation of the scratching displayed by *Vglut2*-deficient mice (Figure 1C). Thus, mGluR7 is co-expressed with *Vglut2* in primary afferent neurons, and can, when activated by an mGluR7 agonist, reverse the histaminergic itch phenotype displayed by *Vglut2*-deficient mice, suggesting that histaminergic itch is regulated by activation of mGluR7 receptors through VGLUT2-mediated glutamatergic transmission.

mGluR7 Selectively Regulates Histaminergic and PLC β 3-Mediated Itch through Ca²⁺-Dependent Presynaptic Autoinhibition

We further characterized the role of mGluR7 in the regulation of histaminergic itch by performing two-photon microscopy imaging of histamine-induced calcium responses in dissociated primary afferents. Our analysis showed that activation of mGluR7 receptors by AMN082 could block histamine-evoked calcium responses in small diameter primary afferent neurons (Figures 1D–1F and S1B), consequently impeding the signaling possibilities of the neurons and providing a mechanistic explanation to how mGluR7 can regulate histaminergic itch.

To analyze the specificity of mGluR7 in itch regulation we combined intrathecal injection of AMN082 with a battery of pruritic substances in wild-type mice. Administration of AMN082 attenuated the pruritic responses evoked by histamine, compound 48/80 (which promotes mast cell degranulation and subsequent histamine release), and α -methyl serotonin (Figure 1G), whereas the pruritic responses evoked by the non-histaminergic agents SLIGRL, the malaria prophylaxis chloroquine, endothelin, or bovine adrenal medulla 8-22 peptide were unaffected by mGluR7 activation (Figure 1H). Furthermore, the pruritic response displayed by inbred Nc/nga mice topically treated with mite antigens (Suto et al., 1999), which is considered non-histaminergic, was also indifferent to AMN082 treatment (Figure 1H). Peripheral activation of mGluR7, through topical

application or intradermal injection of AMN082, did not affect the number of scratch episodes induced by compound 48/80 or histamine, indicating that the auto-feedback regulation of histamine sensing primary afferents take place at the central terminal (Figures S1C and S1D). mGluR7 is also expressed by a subset of spinal cord neurons (Figure 1I), and, to investigate their potential involvement in the selective regulation of histaminergic itch, sedated mice were exposed to an intradermal histamine injection, and the subsequent co-expression of the immediate early gene *c-fos* and mGluR7 in the spinal cord was examined. Histamine evoked *c-fos* expression in neurons positioned in the dorsal horn where 81.4% were found to overlap with mGluR7 expression (β -gal reporter gene) (Figure 1I), indicating that mGluR7 also has the potential of regulating histaminergic itch at the spinal cord level, thereby providing specific regulation at several steps of the histamine-associated line of itch.

Histamine and α -methyl serotonin-evoked itch are both associated with activation of the intracellular enzyme PLC (Phospholipase C) β 3, which is expressed in a subpopulation of peripheral neurons (Han et al., 2006; Imamachi et al., 2009). Single-cell PCR analysis showed that *mGluR7* partially overlaps with *Plc β 3* and *Hrh1* (histamine receptor 1) in primary afferents (Figures 1J and 1K); hence, our combined data strongly suggest that mGluR7 selectively regulates histaminergic/PLC β 3-associated itch through auto-feedback via inhibition of calcium influx at the central terminal of primary afferents with additional regulation potentially provided at the spinal level (Figure 1L).

Loss of *mGluR7* Results in an Anaphylaxis-like Behavior

Next, we used *mGluR7*^{-/-} mice to further investigate the somatosensory functionalities involving mGluR7. Mice lacking *mGluR7* showed no detectable mGluR7 protein expression (Figure 2A), were viable, and displayed normal responses to touch, noxious thermal, and chemical stimuli. However, they showed attenuated responses to noxious mechanical stimuli and increased scratching behavior compared to control littermates (Figure 2B; for absolute values see Figures S2A–S2F). If mGluR7 is regulating histaminergic itch, an intradermal injection of histamine should result in increased itch in *mGluR7*^{-/-} mice. Indeed, intradermal injection of histamine resulted in increased levels of scratching behavior in *mGluR7*^{-/-} mice compared to control mice (Figures 2C and 2G), vehicle injection did not induce an altered behavior (Figures S2G–S2I). In addition, *mGluR7*^{-/-} mice displayed a deviating locomotor behavior that commenced approximately 20 min after the injection. At first, we observed a staggering movement that was followed by almost complete passivity and accompanied by profound hypothermia (Figures 2D, 2E, 2H, and 2I; Movie S1).

Histamine is released in the skin by mast cells (here mimicked by a local intradermal injection) during allergic reactions. In severe allergic reactions, levels of circulating histamine can rise rapidly, which results in anaphylaxis. Notably, plasma levels of

in vitro, and intrathecal administration of an mGluR7 agonist in vivo inhibits histaminergic itch specifically. The location of mGluR7 is presynaptic (Kinoshita et al., 1998).

Scale bar, 74 μ m (A), 30 μ m (B), 15 μ m (E), and 250 μ m (K). ***p* < 0.01. Mann-Whitney two-tailed (D and H); one-way ANOVA, Dunn's multiple comparison post hoc test (C). All Venn diagrams were generated using the online software Venn diagram plotter (PNNL). Data are described as mean \pm SEM.

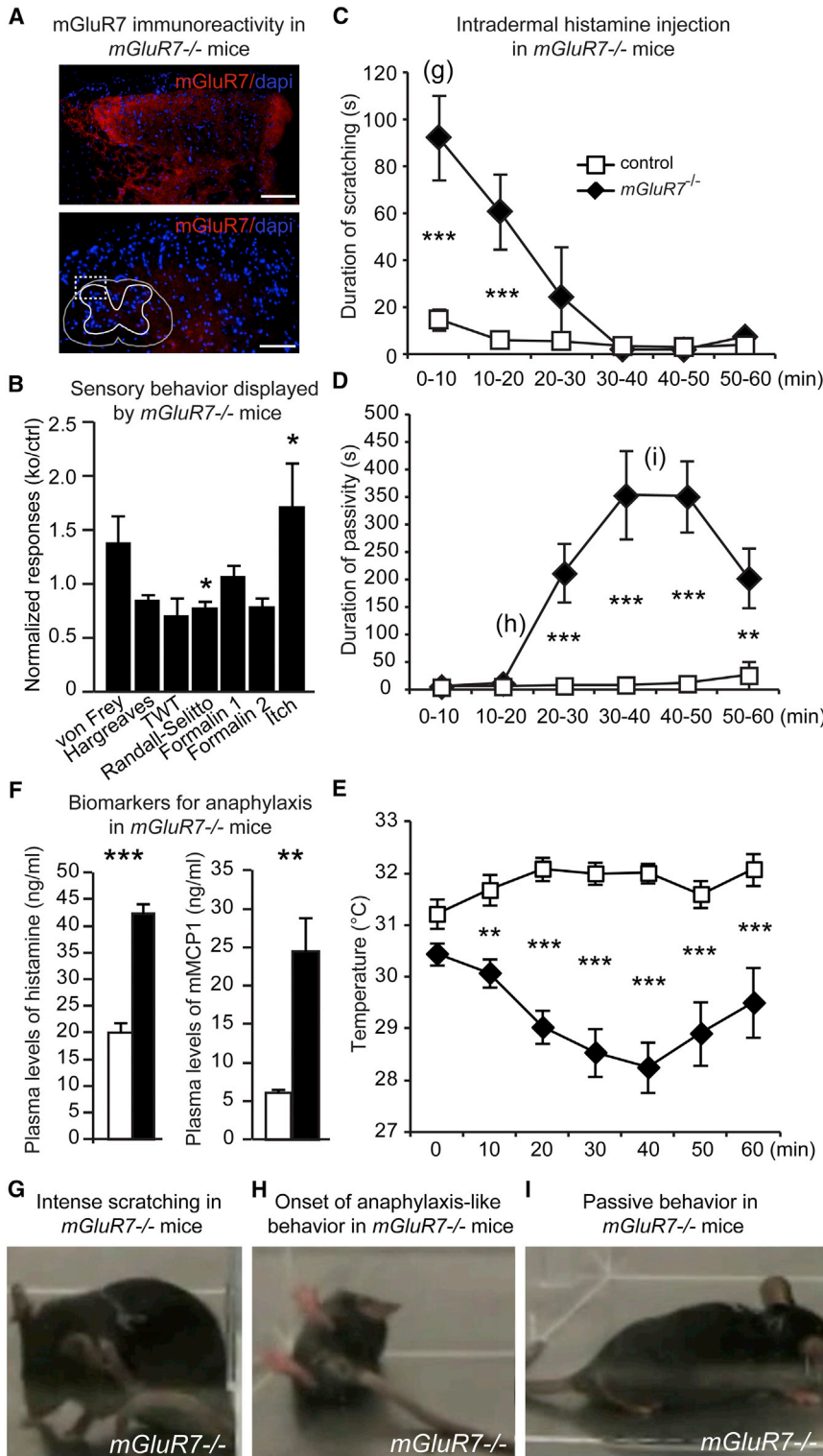


Figure 2. Intradermal Histamine Injection Induced Both Peripheral and Systemic Symptoms of Anaphylaxis in *mGluR7*^{-/-} Mice

(A) mGluR7 immunoreactivity in the dorsal horn of controls and *mGluR7*^{-/-} mice. (B) The sensory phenotype displayed by *mGluR7*^{-/-} mice compared to controls (n = 6–22, see Figures S2A–S2F for statistics and an individual representation of each test). (C) Scratching behavior in *mGluR7*^{-/-} mice and controls after an intradermal histamine injection (100 µg). (D) Passive behavior (no sign of locomotor activity) in *mGluR7*^{-/-} mice and controls after the intradermal histamine injection. (E) Body temperature (measured in the neck region) in *mGluR7*^{-/-} mice and controls after the intradermal histamine injection (n = 10 *mGluR7*^{-/-}, 13 littermate controls, see Figures S2G–S2I for vehicle treatment). (F) Circulating blood levels of histamine (n = 6) and mMCP1 (n = 5) in *mGluR7*^{-/-} mice and controls 60 min after the intradermal histamine injection. (G–I) Snapshots illustrating the different behavioral phases (intense itch, altered locomotor behavior/staggering gate, passivity) in the histamine-evoked anaphylaxis-like behavior in *mGluR7*^{-/-} mice (Movie S1). Scale bars, 74 µm. *p < 0.05, **p < 0.01, ***p < 0.001, Mann-Whitney two-tailed (B, F, and G), repeated measures (RM) ANOVA, Bonferroni post hoc (C–E). Data are described as mean ± SEM.

upon systemic mast cell mediated release and used in the clinic to diagnose anaphylaxis) also were markedly increased (three times higher) in *mGluR7*^{-/-} mice (Figure 2F), whereas the mast cell density and number in the skin was equal between *mGluR7*^{-/-} and control mice (Figures S2J and S2K). The systemic nature of these findings, together with the observed increased itch (4.3 times elevated itch behavior), increased immobility (17.3 times more time spent passive) and hypothermia (maximum mean difference 3.8°C; hypothermia is the most common way to monitor anaphylactic events in mice [Doyle et al., 2013]) and indicate that the histamine injection caused an anaphylaxis-like state, mediated by mast cells (elevated mMCP1 levels), in *mGluR7*^{-/-}

histamine in terminal blood drawn directly after the behavioral experiments were almost twice as high in *mGluR7*^{-/-} mice compared to control mice (Figure 2F). Further analysis showed that mMCP1 levels (mouse mast cell protease 1, elevated

mice but not in control mice. We therefore revisited and expanded our interpretation; mGluR7-mediated regulation prevents both local (histaminergic itch) and systemic symptoms of anaphylaxis.

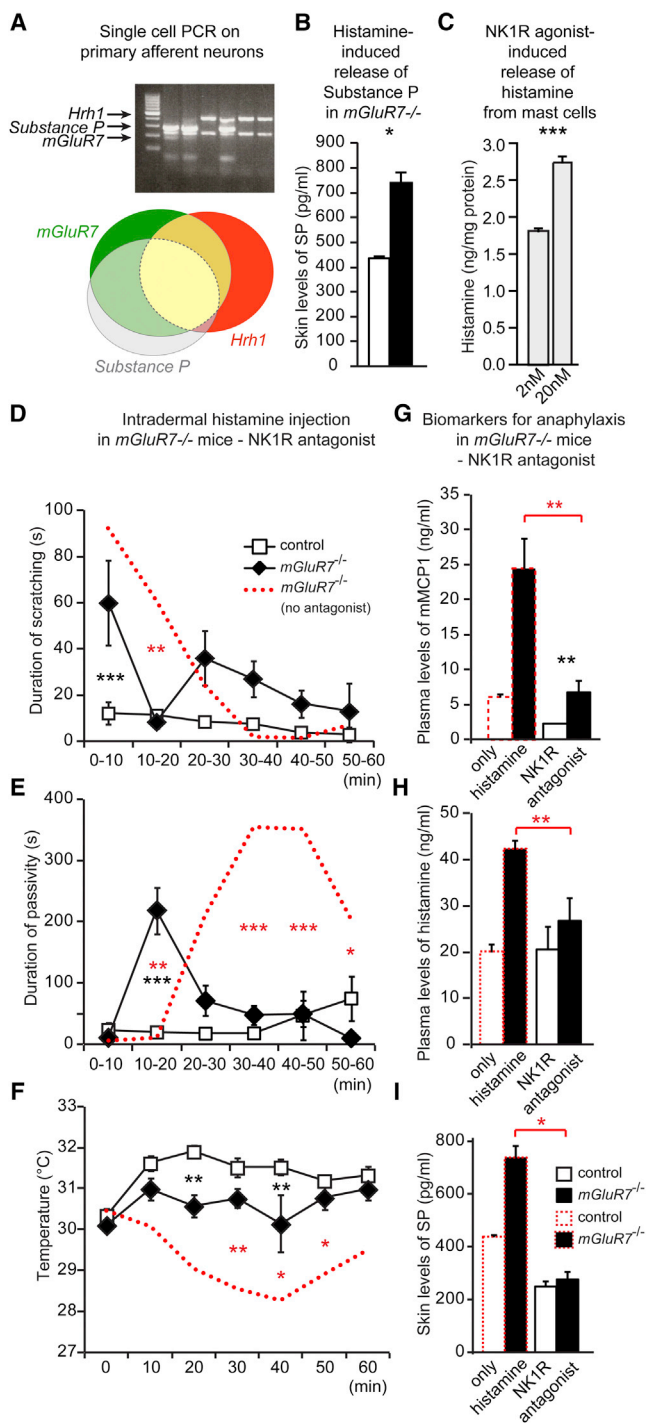


Figure 3. mGlu7 Prevents Peripheral Neurons from Causing Anaphylaxis via Controlled Release of Substance P

(A) Single-cell PCR analysis of primary afferent neurons (58% of the *Hrh1* mRNA/*mGlu7* mRNA expressing DRGs expressed *Substance P* mRNA) (number of cells analyzed = 92; *Substance P* = 266 bp). (B) Skin levels of Substance P in *mGlu7*^{-/-} mice and controls 60 min after intradermal histamine injection (at the site of injection) (n = 4). (C) Release of histamine from mast cells induced by two doses of the NK1R-specific agonist GR73632 (data described as mean ± SD).

mGlu7 Prevents Primary Afferent Caused Anaphylaxis through Control of Peripheral Release of Substance P

How might loss of a presynaptically expressed glutamate receptor in the histamine-associated itch pathway result in a state of anaphylaxis? Upon depolarization by histamine, primary afferents release neurotransmitters in the skin through anti-dromic depolarization of peripheral branches, transmitters that in turn augment inflammation by recruiting and activating immune cells. Hence, we hypothesized that activation of histamine-sensing neurons now lacking mGlu7, which thus presumably lack presynaptic autoinhibition through this receptor, should lead to increased release of peripheral neurotransmitters. Our single-cell PCR analysis showed that a subpopulation of *mGlu7*/*Hrh1* positive neurons expressed the neuropeptide *Substance P* (58%, Figure 3A), connecting mGlu7, histaminergic itch, and a peripherally released neuropeptide. Furthermore, analysis at the histamine injection site showed that tissue levels of Substance P were increased with 69% in *mGlu7*^{-/-} mice compared to control littermates (Figure 3B), corroborating the suggested connection between anaphylaxis-like behavior and altered peripheral neurotransmission due to loss of mGlu7.

Upon release, Substance P can interact with mast cells to promote further histamine release (Hägermark et al., 1978), which augments the inflammatory and pruritic state. We found that mast cells express the receptor for Substance P, NK1R (Figure S3A), and that application of the NK1R-specific agonist GR73632 results in a dose-dependent histamine release from mast cells (Figure 3C). To investigate a mechanism where Substance P may be part of a signal linking mGlu7/HRH1 neurons, mast cells, and anaphylaxis, we treated *mGlu7*^{-/-} mice and controls with the NK1R-specific antagonist Win51708 prior to an intradermal histamine injection (Figures 3D–3F). If this hypothesis is correct, the NK1R antagonist should block Substance P from activating mast cells and thereby prevent further mast cell activation and histamine release and ultimately reduce or abolish the anaphylaxis-like behavior. We found that, after administration of Win51708, *mGlu7*^{-/-} mice displayed attenuated itch levels, recovered faster from the passive state, and did not display hypothermia compared to non-antagonist treated *mGlu7*^{-/-} mice (Figures 3D–3F; for vehicle treatment see

(D) Scratching behavior in *mGlu7*^{-/-} mice (black line) and controls induced by an intradermal histamine injection (100 μg) in the presence of the NK1R antagonist Win51708 (5 mg/kg, given 10 min prior to the histamine injection). The red dotted line indicates the behavior induced by histamine in *mGlu7*^{-/-} mice without prior NK1R antagonist treatment (data shown in detail in Figures 2C–2E, n = 8).

(E) Passive behavior in *mGlu7*^{-/-} mice and controls induced by an intradermal histamine injection in the presence of the NK1R antagonist Win51708 (n = 8; for vehicle treatment, see Figures S3B–S3D).

(G and H) Circulating blood levels of mMCP1 (G) and histamine (H) 60 min after the intradermal histamine injection in *mGlu7*^{-/-} mice and controls pretreated with the NK1R antagonist Win51708 (n = 5/treatment).

(I) Skin levels of Substance P in the injection site 60 min after the intradermal histamine injection in *mGlu7*^{-/-} mice and controls pretreated with the NK1R antagonist Win51708 (n = 5/treatment).

*p < 0.05, **p < 0.01, ***p < 0.001, Mann-Whitney two-tailed (B, C, and G–I); RM ANOVA, Bonferroni post hoc (D–F). Data are shown as mean ± SEM.

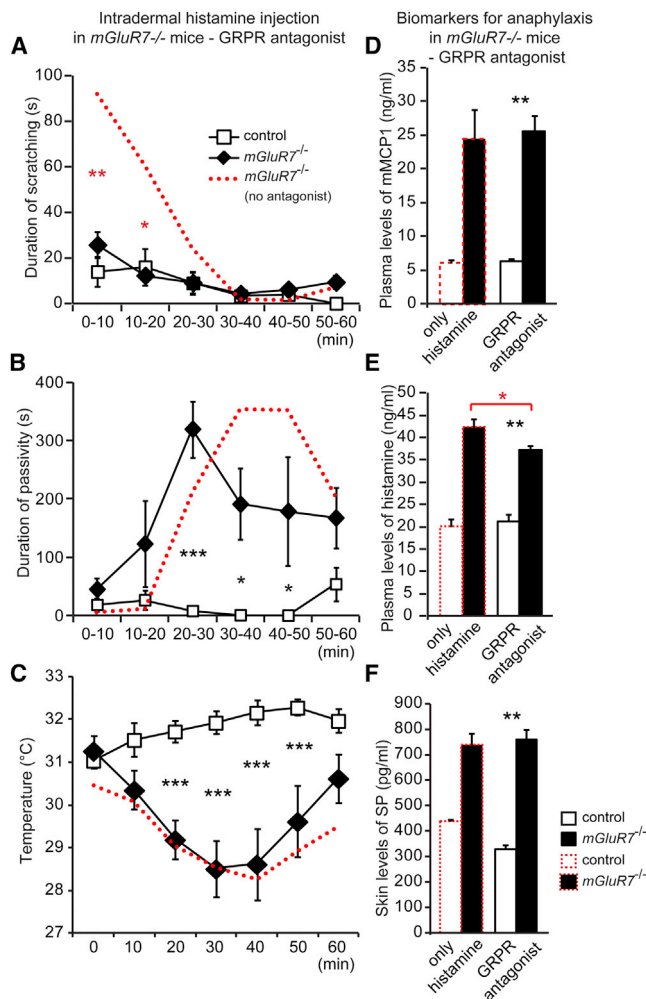


Figure 4. mGluR7 Regulates Itch but Not Systemic Symptoms of Anaphylaxis via GRPR

(A) Scratching behavior in *mGluR7*^{-/-} mice (black line) and controls induced by an intradermal histamine injection (100 μg) in the presence of the GRPR antagonist RC3095 (10 mg/kg) (n = 6). The red dotted line indicates the behavior induced by histamine in *mGluR7*^{-/-} mice without prior GRPR antagonist treatment (data shown in detail in Figures 2C–2E, n = 8).

(B) Passive behavior in *mGluR7*^{-/-} mice and controls induced by an intradermal histamine injection in the presence of the GRPR antagonist RC3095 (10 mg/kg) (n = 6).

(C) Neck temperature in *mGluR7*^{-/-} mice and controls induced by an intradermal histamine injection in the presence of the GRPR antagonist RC3095 (10 mg/kg) (n = 6; for vehicle treatments, see Figure S4).

(D and E) Circulating blood levels of mMCP1 (D) and histamine (E) 60 min after the intradermal histamine injection in *mGluR7*^{-/-} mice and controls pretreated with GRPR antagonist (n = 6).

(F) Skin levels of Substance P in the injection site 60 min after the intradermal histamine injection in *mGluR7*^{-/-} mice and controls pretreated with GRPR antagonist (n = 5).

*p < 0.05, **p < 0.01, ***p < 0.001, Mann-Whitney two-tailed (D–F), RM ANOVA, Bonferroni post hoc (A–C). Data are shown as mean ± SEM.

Figures S3B–S3D). NK1R antagonist treatment also resulted in reduced systemic release of the mast cell mediator mMCP1 and histamine as well as reduced tissue release of Substance

P in *mGluR7*^{-/-} mice compared to non-antagonist controls (Figures 3G–3I). Thus, NK1R antagonists prevent the anaphylaxis-like behavior in *mGluR7*^{-/-} mice evoked by an intradermal histamine injection. As neurons are the only source of Substance P in the skin (Hökfelt et al., 1975), our combined data indicate that, in histamine-evoked processes, mGluR7 controls the peripheral release of Substance P from primary afferents and thereby subsequent activation and release of histamine from mast cells through the NK1R, which prevents peripheral neurons from causing anaphylaxis.

GRPR Signaling Contributes to the Mediation of Peripheral Symptoms, Itch, but Not to Systemic Symptoms of Anaphylaxis

Activation of histamine sensing primary afferent neurons also results in the release of neurotransmitters from central axon terminals that transmit pruritic information to the spinal cord and brain. Although there is some controversy regarding which neurotransmitter is responsible for itch transmission from the periphery to the spinal cord (Liu et al., 2014; Mishra and Hoon, 2013), there is consensus around GRPR (gastrin releasing peptide receptor) as the main downstream mediator for itch transmission in the spinal cord (Mishra and Hoon, 2013; Sun et al., 2009). Therefore, we next tested whether the increase in itch seen in *mGluR7*^{-/-} mice upon histaminergic provocations could be attenuated by antagonizing the centrally expressed GRPR receptor, i.e., does histaminergic mGluR7-controlled itch, also associated with anaphylaxis, depend on GRPR? Intrathecal injection of the GRPR antagonist RC3095 prior to the histamine injection resulted in drastically reduced itch levels in *mGluR7*^{-/-} mice compared to non-antagonist treated *mGluR7*^{-/-} mice (Figure 4G; for vehicle treatments, see Figure S4), adding anaphylaxis-associated itch to the repertoire of pruritic stimuli that GRPR mediate in the CNS. Moreover, in light of the selective regulatory mechanism of histaminergic itch and anaphylaxis presented here, we wondered whether central regulation of itch might also affect systemic symptoms of anaphylaxis. However, blockade of GRPR did not prevent the systemic anaphylaxis-like behavior; RC3095-treated *mGluR7*^{-/-} mice showed similar levels of passivity and hypothermia as non-antagonist treated *mGluR7*^{-/-} mice (Figures 4B and 4C). Furthermore, GRPR antagonist treatment did not prevent the rise of circulating mMCP1 and tissue levels of Substance P (Figures 4D–4F). Thus, we suggest that, whereas mGluR7-regulated anaphylactic itch is dependent on central transmission via GRPR, regulation of systemic symptoms of anaphylaxis is not.

DISCUSSION

In histaminergic itch transmission, primary afferents provide an ascending signaling pathway between the site of release in the periphery and the CNS, which results in the awareness of itch and a targeted scratching behavior. Primary afferents also possess branched peripheral nerve endings, providing histamine-sensitive neurons with the ability to activate the immune system through local neurotransmitter release in the affected tissue (Hägermark et al., 1978). Such release produces the characteristic flare response associated with allergic reactions.

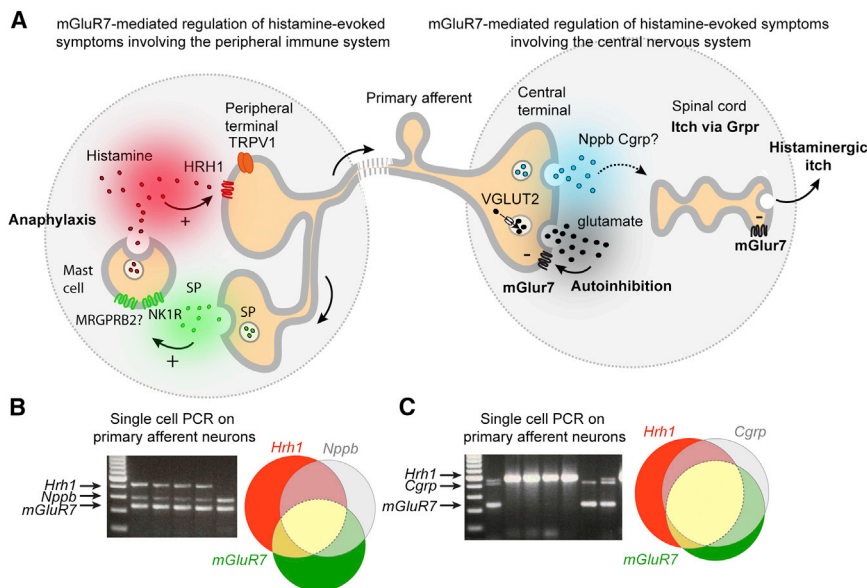


Figure 5. Histamine-Sensing *mGluR7* Neurons Express *Nppb* and *Cgrp*

(A) Single-cell PCR analysis of *Nppb*, *mGluR7*, and *Hrh1* mRNA expression in primary afferent neurons. The analysis showed that 57% of *mGluR7* mRNA/*Hrh1* mRNA expressing neurons also express *Nppb* mRNA (number of cells analyzed = 98; *Nppb* = 280 bp).

(B) Single-cell PCR analysis of *Cgrp*, *mGluR7*, and *Hrh1* mRNA expression in primary afferent neurons. The analysis showed that 85% of *mGluR7* mRNA/*Hrh1* mRNA expressing neurons also express *Cgrp* mRNA (number of cells analyzed = 93, *Cgrp* = 410 bp).

(C) Schematic drawing summarizing our findings, which show that *mGluR7* regulates both peripheral and central aspects of histaminergic-evoked primary afferent neurotransmission, with implications for anaphylaxis. Besides binding to the NK1R, Substance P has recently been shown to interact with MRGPRB2 (McNeil et al., 2015), which also can lead to degranulation of mast cells.

An *mGluR7*-dependent control of primary afferents should thus have two consequences for histamine-induced neurotransmission: altered central transmission, affecting the scratching behavior, and altered peripheral transmission influencing the immune system. We here present data supporting a model for how *mGluR7* might regulate histaminergic transmission, both peripherally and centrally, and how the regulation affects the symptoms of the histamine-involved disorder anaphylaxis.

mGluR7 Regulates the Peripheral Immune System via Substance P and NK1R in Allergic Events

We found that genetic ablation of *mGluR7* altered histamine-induced peripheral transmission from primary afferents as histamine-injected *mGluR7*^{-/-} mice displayed increased levels of Substance P in the innervated tissue; a peptide whose mRNA we showed to be expressed by *mGluR7*/*HRH1* neurons and that can degranulate cultured mast cells via the NK1R. We thus conclude that Substance P constitutes a link between *mGluR7*-regulated primary afferents and mast cells in the immune system that can evoke the systemic symptoms of anaphylaxis. Both mast cells and basophils can release histamine upon binding of Substance P, and therefore an additional influence from basophils cannot be excluded. However, only mast cells release mMCP1, which together with histamine is used as a clinical biomarker for anaphylaxis. We found elevated levels of circulating histamine as well as mMCP1 in blood from *mGluR7*^{-/-} mice, suggesting that the anaphylaxis-like symptoms displayed by *mGluR7*^{-/-} mice are mediated by mast cells (Figure 5A).

Substance P can also activate mast cells through interaction with the MRGPRB2 protein, recently identified as the receptor for basic secretagogue compounds (McNeil et al., 2015), and therefore additional effects from interaction between the MRGPRB2 protein and Substance P cannot be excluded. Our findings, however, suggest that specific interactions with the NK1R, via the use of an NK1R agonist can degranulate mast

cells. Furthermore, the anaphylaxis-like behavior in histamine-provoked *mGluR7*^{-/-} mice could be reversed by administering an NK1R-specific antagonist, which supports that *mGluR7*-regulated primary afferents mainly communicate with mast cells via the NK1R (Figure 5A). In further support, treatment with a Substance P antagonist has been shown to attenuate symptoms of allergy-induced anaphylaxis in the passive cutaneous anaphylaxis model (Siebenhaar et al., 2008).

mGluR7 Regulates the CNS in Allergic Events

The histamine-sensing primary afferents also communicate with the CNS via release of neurotransmitters from their central terminals, which affects our perception of a dominating symptom of anaphylaxis, histaminergic itch. Previous studies have shown that peripheral removal of VGLUT2 results in a profound histaminergic itch phenotype, suggesting that histamine-induced itch is regulated by the neurotransmitter glutamate (Lagerström et al., 2010). Also, group II/III mGluRs, to which *mGluR7* belongs, were recently shown to exert endogenous inhibition on primary afferents expressing TRPV1 in rat (Carlton et al., 2011). Here, we show that presynaptic activation of *mGluR7* receptors on histamine-sensing primary afferents inhibits calcium influx suggesting that the *mGluR7* receptor and glutamate regulate the neuronal activity of these neurons. In support of this hypothesis, activation of *mGluR7* in hippocampal and cortical neurons has been demonstrated to reduce presynaptic Ca⁺² influx, resulting in auto-inhibitory reduction of glutamate release (Martín et al., 2007; Millán et al., 2002). CGRP and NPPB have been identified as potential candidates for transmitting histaminergic itch from the periphery to the spinal cord (Rogoz et al., 2014; Mishra and Hoon, 2013). If glutamate and *mGluR7* regulate histaminergic itch through auto-feedback inhibition, *mGluR7* and *HRH1* should co-localize with these itch-transmitting peptides. Indeed, our single-cell analysis showed that both *Nppb* and *Cgrp* are expressed in the *Hrh1*/*mGluR7* expressing population of primary afferents (Figures 5B and 5C). Thus, similar to the peripheral

regulation of Substance P neurotransmitter release, mGluR7 and glutamate also have the potential to regulate the central release of neurotransmitters (NPPB and/or CGRP) from histamine-sensing primary afferents, thereby exerting a role in the central transmission of histaminergic itch (Figure 5A). Interestingly, *mGluR7*, *Nppb*, *Cgrp*, *Substance P*, and *Hrh1* mRNA transcripts can all be found in a recently identified subpopulation of primary afferents, the NP2 class (Usoskin et al., 2015). Together, these data suggest that NP2 neurons are responsible for the regulation of the peripheral nervous system in anaphylaxis.

Our data suggest that the regulation of primary afferents by mGluR7 can explain both the systemic and local symptoms of anaphylaxis displayed by the *mGluR7*^{-/-} mice. However, other parts of the nervous system could have additional effects in the mGluR7-mediated neuronal regulation of symptoms of anaphylaxis. mGluR7 is also expressed in a subpopulation of second-order neurons in the spinal cord, which could influence the itch phenotype displayed by *mGluR7*^{-/-} mice. Intradermal injection of histamine in sedated mice led to *c-fos* activation in the dorsal spinal cord where 81.4% of the neurons also expressed mGluR7. These data indicate that mGluR7 can regulate the local symptom of anaphylaxis, histaminergic itch, both through presynaptic autoinhibition of primary afferents and spinal cord interneurons.

mGluR7 Regulates the Neuronal Response to Prolonged Sensory Stimuli, Such as Anaphylaxis-Associated Itch, but Not Acute Noxious Heat

Histaminergic itch transmitted by primary afferent neurons depends on the signal transduction pathway involving the noxious heat receptor TRPV1 (Caterina et al., 1997; Imamachi et al., 2009), which shows substantial overlap with *mGluR7* (Figure S5). The overlap and shared signaling pathway would indicate that mGluR7 also regulates acute thermal thresholds. However, this is not the case. Our data show that *mGluR7*^{-/-} mice do not detect noxious heat in a significantly different manner from their littermate controls, which is in agreement with previous findings (Masugi et al., 1999; Stachowicz et al., 2008). These findings could be due to the fact that *mGluR7* and *Trpv1* do not overlap completely and/or that mGluR7 may only regulate more persistent stimuli because of the low affinity for glutamate (Mitsukawa et al., 2005), which requires a prolonged intense release of glutamate to inhibit the presynaptic neuron. Why would we have developed an mGluR7-dependent endogenous mechanism for regulating histamine-sensing neurons, especially since these neurons also partially transmit noxious thermal pain? How could signaling evoked by intense histaminergic stimuli be restrained but not the essential perception of acute noxious heat, which would be devastating for the organism if it were silenced? We speculate that the low affinity of the receptor to glutamate results in a response only after prolonged stimuli. Acute/short term stimuli, such as the initial itch sensation from an infecting parasite or acute noxious heat (Masugi et al., 1999; Stachowicz et al., 2008), is detected. However, prolonged allergic events will cause extended activation of the neuronal population and thus sustained release of glutamate, which in turn initiates the regulatory mechanism to prevent anaphylaxis. In support of this hypothesis, activation of mGluR7 has been shown to regulate states of

prolonged pain such as Carrageenan-induced hypersensitivity to heat (Dolan et al., 2009) and chronic constriction injury-induced allodynia (Osikowicz et al., 2008).

Previously, anaphylaxis has been regarded as a result of an over-active or defective immune system. We now present data suggesting that dysfunctional regulation of primary afferents can cause anaphylaxis. Furthermore, we propose a mechanism for its regulation, through glutamatergic auto-feedback of histamine-associated mGluR7-expressing neurons. Future studies should investigate whether individuals susceptible to anaphylactic shock, or allergens, carry defects in this mechanistic chain of events. Further, the development of mGluR7 agonists may provide possibilities to prevent this life-threatening condition.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice

The *Vglut2*^{fl/fl}; *Trpv1*-Cre mice were generated through a cross between *Trpv1*-Cre mice (C57BL/6Ncrj*DBA/2) and *Vglut2*^{fl/fl} mice (Sv129/R1 * C57BL/6). *mGluR7*^{-/-} mice (129P2/OlaHsd * C57BL/6) were purchased from the MMRRC repository. *Nc/Nga* mice (*Nc/Nga*TndCrj) were purchased from Charles River Laboratories. C57BL/6 mice were bought from Taconic.

Immunohistochemistry

Antibodies were rb anti- β -galactosidase (β -gal) 1:5,000 (8559761, Cappel), rb anti *c-fos* 1:200 (sc-52-G, Santa Cruz Biotechnology), rb anti-mGluR7 1:1,000 (Millipore, 07-239), goat anti-chicken Alexa 488 1:400, donkey anti-rb Alexa 488 1:400, donkey anti-goat RRX 1:400, and goat anti-rb Alexa 647 1:400 (all from Invitrogen).

Calcium Imaging

First, the prepared cultures were loaded with Oregon green Bapta 488 (OGB-1) (Molecular Probes) and then incubated at 37°C for 1 hr in the dark. After the incubation period, each dish was washed two times with HEPES. Two-photon calcium imaging was then performed through a 870-nm excitation wavelength laser (Modelocked Ti:Sapphire laser system [Coherent]), and the signal was recorded using a 40 \times water-immersion objective (Plan Apochromat; 1 numerical aperture [NA]; Zeiss). A LabView custom-made software HelioScan (Langer et al., 2013) was used to acquire the signal in a frame rate of 2 Hz.

Behavior

All animal procedures were approved by the local ethical committee in Uppsala and followed the Directive 2010/63/EU of the European Parliament and of the Council, The Swedish Animal Welfare Act (SFS 1988:534), The Swedish Animal Welfare Ordinance (SFS 1988:539), and the provisions regarding the use of animals for scientific purposes: DFS 2004:15 and SJVFS 2012:26.

For detailed information, see the [Supplemental Information](#).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2015.12.033>.

AUTHOR CONTRIBUTIONS

B.A. was responsible for the *mGluR7* mouse colony. B.A. performed the immunohistochemical analyses. K.R., E.I.M., and M.C.M.F. performed the *c-fos* analysis. K.R. performed the in situ study. H.H.A. and K.R. conducted the qPCR analysis, and K.R. performed the single-cell part. B.A. and K.R. performed the itch behavioral studies, and B.A., L.L.I., H.H.A., E.I.M., and K.R. analyzed the itch data. B.A. performed and analyzed the pain behavior. H.P. and K.R. ran the ELISA assays, and H.P. prepared the mast cells and

performed the mast cell intro experiments. B.A. performed the histochemical analysis of mast cell density in tissue. F.B.F. and C.N. performed the calcium imaging, and K.R. and E.I.M. performed the culture preparation. K.K. contributed to the interpretation of the study and writing of the manuscript. M.C.L. designed the study, analyzed data, and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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