Supporting Information

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SI Materials and Methods

Mice. *ASIC1a*$^{-/-}$ (acid-sensing ion channel) and *ASIC2*$^{-/-}$ mice were described previously (1,2). *ASIC1a*$^{-/-}$, *ASIC2*$^{-/-}$, and wild-type (WT) mice were maintained on a congenic C57BL/6 background. Experimental groups were matched for age (4–6 wk for studies of acute brain slices and 3–4 d for studies of slice cultures). Mice were kept on a standard 12-h light-dark cycle and received standard chow (LM-485; Teklab) and water ad libitum.

Brain Slice Preparation. Mice were anesthetized with isoflurane, and brains were dissected into preoxygenated (5% CO_2 and 95% O_2) ice-cold high-sucrose dissection solution containing (in mM) 205 sucrose, 5 KCl, 1.25 NaH_2PO_4, 5 $MgSO_4$, 26 $NaHCO_3$, 1 $CaCl_2$, and 25 glucose. A vibratome was used to slice brains coronally into 300-μm sections that were maintained in normal artificial cerebrospinal fluid (ACSF) containing (in mM) 115 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 11 glucose, and 25 $NaHCO_3$ bubbled with 95% O_2/5% CO_2 at pH 7.4 at 22–25 °C. Slices were incubated in the solution at least 2 h before recording. For experiments, individual slices were transferred to a submersion-recording chamber and were continuously perfused with the 5% CO_2/95% O_2 solution (~3.0 mL/min) at 22–25 °C.

Patch-Clamp Recording. Slices were visualized with infrared optics, using an upright microscope equipped with differential interference contrast optics (Nikon ECLIPSE FN1). Whole-cell patch-clamp recordings were made from pyramidal neurons in the lateral amygdala. The pipette solution contained (in mM) 135 KSO_3CH_3, 5 NaCl, 10 Hepes, 4 MgATP, 0.3 Na_3GTP, and 0.5 K-EGTA (mOsm = 290, adjusted to pH 7.25 with KOH). Picrotoxin (100 μM) was added to the ACSF throughout the recordings to yield excitatory responses.

Constant current pulses (20–80 μA, 100 μS, 0.05 or 0.1 Hz) were applied through extracellular bipolar electrodes placed at cortical inputs to induce excitatory postsynaptic currents (EPSCs). For whole-cell long-term potentiation (LTP) recordings, high-frequency stimulation (HFS; 100 Hz, 1 s) was used to induce LTP. For injections of acidic ACSF into the slice, the ACSF was buffered with Hepes when pH was >6.0 and was buffered by 10 mM Mes when pH was ≤6.0. Five-second puffs of acidic solution were delivered by a patch-clamp pipette and a Femtojet microinjection system (Eppendorf Inc). For the acid-induced LTP experiments, the test pulse was delivered 50 ms before the end of a 3-s acid injection. The paired acid injection and test pulse were repeated 3 times with a 20-s interval. In other experiments, the pH change in the recording chamber was controlled by the perfusion system. The decay times of EPSCs were fitted with a single exponential.

Brain Slice Culture and Transfection. Mouse amygdala organotypic slice cultures were modified from our earlier hippocampal slice culture study (3). Briefly, whole brain from postnatal 3–4-d-old mice were dissected and cut into 300-μm-thick coronal slices with a Vibratome 1000 plus. Amygdala slices were then dissected and placed in Falcon polyethylene terephthalate-etched membrane culture inserts containing 1-μm pores. Slices were maintained in filter culture medium composed of 25% horse serum, 25% Hanks Balanced Salt Solution, 50% Minimum Essential Medium, 2 mM L-glutamine, 1 mg/mL glucose, 44 mg/mL $NaHCO_3$, and 10 units/mL penicillin/streptomycin. Slices were maintained in a 5% CO_2 humidified incubator. The medium was changed every 3 d.

Synaptic pH Measurement. A syndecan 2-pHluorin fusion was generated by inserting pHluorin sequence into mouse syndecan 2 cDNA (Origene) immediately after the signal peptide sequence to generate a fusion with the pHluorin on the extracellular surface. Briefly, an AfeI site was created in syndecan 2, using QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) with the following primers: forward, 5′-CTGACATCCGATAGCGCTATGTACCTTGAC-3′; reverse, 5′-GTCAAGGTACATAGCGCTATCGGATGTCAG-3′. Cycling conditions were performed according to the manufacturer's protocol. Superecliptic pHluorin was then mutated to introduce 5′ and 3′ flanking AfeI sites, using QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) with the following primers: forward, 5′- AGCGCTATGAGTAAAGGAGAAGAACTTTTCACTGG-3′; reverse, 5′- AGCGCTTGTATAGTTCATCCATGCCATGTGTAAT-3′. The pHluorin containing the flanking AfeI sites and the parent syndecan 2 construct containing the AfeI site were treated with AfeI (NEB) for 2 h at 37 °C. The products were then run on an agarose gel and purified using Qiaquick Gel Extraction (Qiagen). Purified products were ligated together, using Rapid DNA Ligation Kit (Roche), and transformed into MAX Efficiency DH5 Alpha T1 Phage-Resistant Competent Cells (Invitrogen). Colonies were selected and grown overnight in LB Agar containing kanamycin. The next day, DNA was isolated using Wizard Plus SV Minipreps DNA Purification Kit (Promega), and sequence was confirmed by sequencing.

Biolistic transfection of slice cultures was done using a Helios Gene Gun (Bio-Rad) after 1–3 wk culture. Slices were used for synaptic pH measurement 48 h after transfection. In brief, the membrane of the culture insert was cut and transferred to a quick-change imaging chamber (Warner Instruments). Slices were perfused with ACSF at pH 7.4, buffered with 5% CO_2/95% O_2. Experiments were controlled and analyzed through Nikon Elements 4.0 software, with off-line background subtraction. Extracellular bipolar electrodes were placed at cortical inputs to generate stimulation. To assess the relationship between stimulus intensity and pH, we applied electrical stimulation at frequencies from 1 to 100 Hz. The pHluorin signal was detected using a NIKON A1R high-speed confocal microscope. For measurements in HeLa cells, the syndecan 2-pHluorin plasmid was transfected into HeLa cells, using Lipofectamine 2000 (Invitrogen), and the cells were studied 24–48 h later with a series of extracellular pH solutions.

1. Price MP, et al. (2000) The mammalian sodium channel BNC1 is required for normal touch sensation. *Nature* 407(6807):1007–1011.
2. Wemmie JA, et al. (2002) The acid-activated ion channel ASIC contributes to synaptic plasticity, learning, and memory. *Neuron* 34(3):463–477.
3. Zha X-M, Wemmie JA, Welsh MJ (2006) Acid-sensing ion channel 1a is a postsynaptic proton receptor that affects the density of dendritic spines. *Proc Natl Acad Sci USA* 103(44):16556–16561.

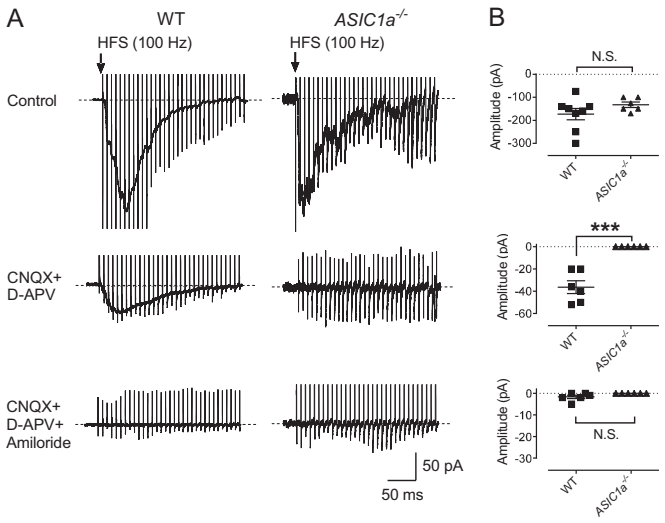

Fig. S1. Comparison of HFS evoked currents with and without GluR blockers and amiloride. (*A*) Representative traces of EPSCs during HFS (100 Hz) in pyramidal neurons in lateral amygdala brain slices of WT and *ASIC1a*$^{-/-}$ mice. HFS-induced EPSCs were first recorded in ACSF under basal conditions (*Upper*), again after 25 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 50 μM (2R)-amino-5-phosphonovaleric acid (D-APV) were added (*Middle*), and then after the addition of 200 μM amiloride (*Lower*). (*B*) Data are mean ± SEM for EPSC amplitudes in conditions in *A*. Each data point is a different cell. ***$P < 0.001$ (Student *t* test; $n = 6$–8 cells in 4 mice). N.S., no significant difference.

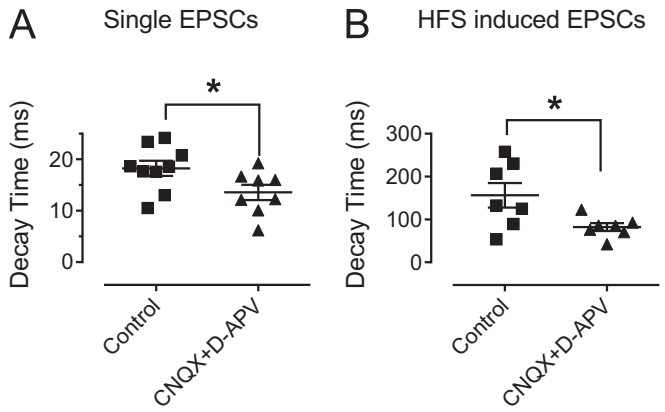

Fig. S2. Comparison of EPSC decay times with and without GluR blockers. (*A*) Data are mean ± SEM of best-fit decay times of EPSCs in pyramidal neurons of lateral amygdala slices under control conditions ($n = 9$ cells in 4 mice) and in the presence of 25 μM CNQX and 50 μM D-APV ($n = 8$ cells in 4 mice). *$P < 0.05$ (Student *t* test). (*B*) Best-fitted decay times of HFS-induced EPSCs without and with 25 μM CNQX and 50 μM D-APV. *$P < 0.05$ (Student *t* test; $n = 7$ cells in 4 mice).

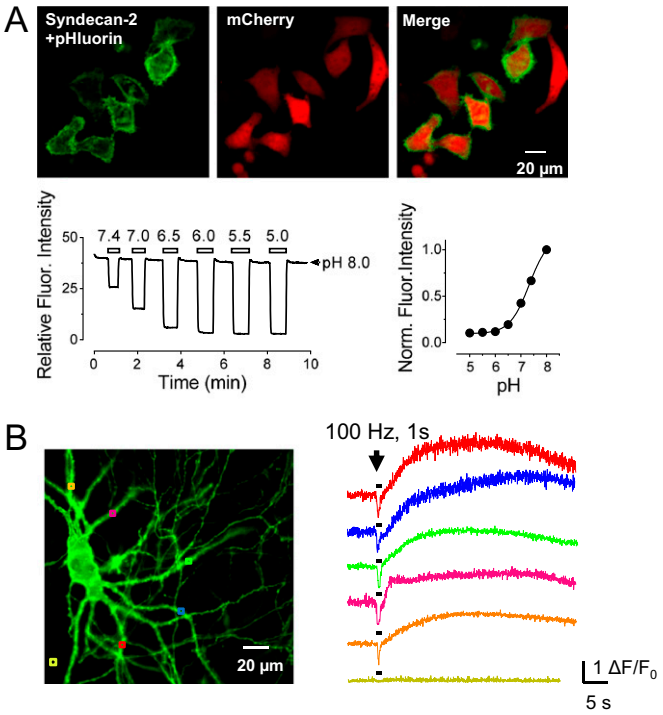

Fig. S3. Presynaptic stimulation reduces extracellular pH. (*A*, *Upper*) Images of HeLa cells expressing syndecan 2-pHlourin, mCherry (as a control), and the merged image. Syndecan 2-pHlourin preferentially localized to cell membranes. (*Bottom, left*) Changes in fluorescence intensity elicited by applications of indicated pH. (*Bottom, right*) Fluorescence intensity at indicated pH values normalized to value at pH 8.0. Best fit of the normalized data yielded $EC_{50} = 7.33 \pm 0.02$ pH ($n = 8$). (*B*, *Left*) Local regions of spines and neighboring dendrites selected for fluorescence measurement. (*Right*) Fluorescence of regions indicated in left before and after HFS.

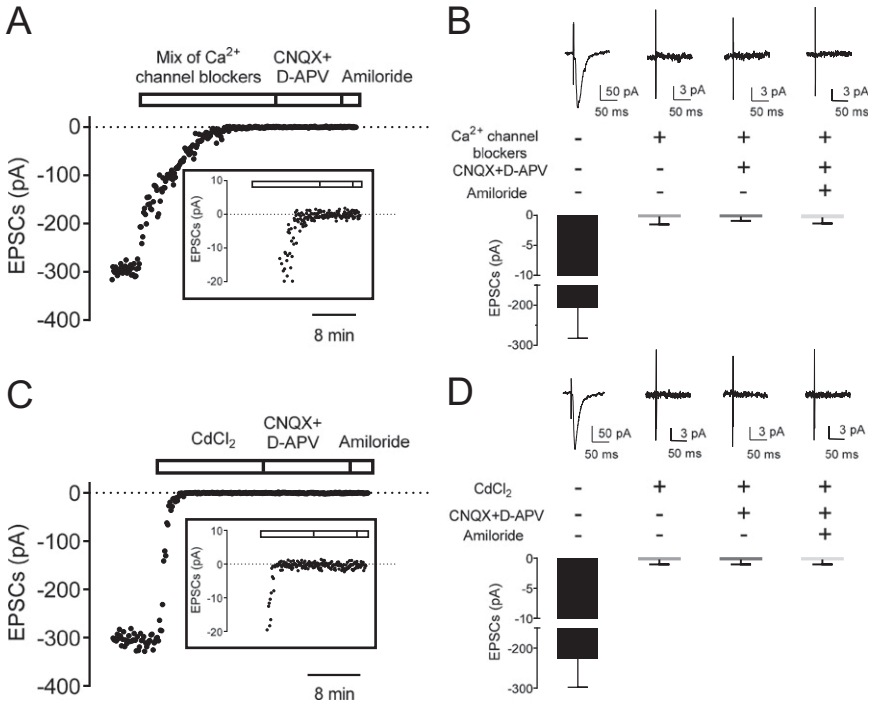

Fig. S4. Blocking Ca^{2+} channels eliminates ASIC-dependent EPSCs. (*A*) Example of EPSCs from a WT lateral amygdala pyramidal neuron shown under basal conditions, after addition of a mixture of Ca^{2+} channel blockers (10 μM nimodipine, 100 nM Agatoxin, 500 nM ω-conotoxin, and 100 nM SNX-842), after addition of GluR blockers (25 μM CNQX and 50 μM D-APV), and then after an ASIC blocker (500 μM amiloride). (*Inset*) EPSCs with an expanded *y* axis. (*B*) Representative traces (*Upper*) and mean ± SEM (*Lower*) for conditions described for *A* ($n = 5$ cells in 3 mice). (*C* and *D*) Similar experiments as in *A* and *B*, except the nonspecific Ca^{2+} channel blocker, 200 μM $CdCl_2$, was applied instead of the mixture of Ca^{2+} channel blockers ($n = 6$ cells in 3 mice).

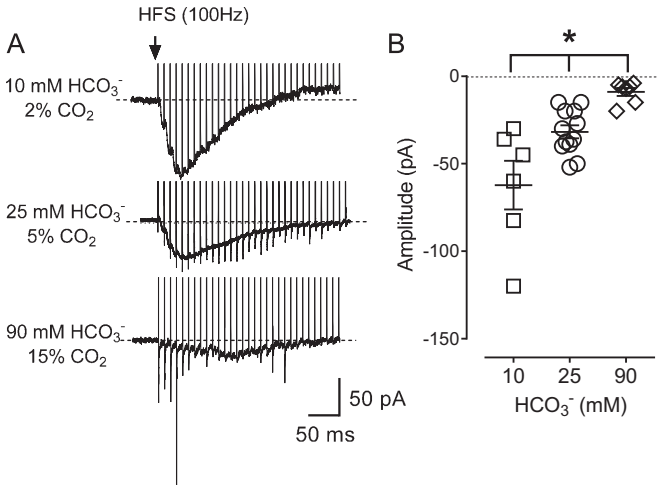

Fig. S5. HFS-induced ASIC-dependent EPSCs under different buffer conditions. (*A*) Representative HFS (100 Hz) induced ASIC-dependent EPSC traces in ACSF with three different buffer conditions: 10 mM HCO_3^-/2% CO_2 at pH 7.4, 25 mM HCO_3^-/5% CO_2 at pH 7.4, and 90 mM HCO_3^-/15% CO_2 at pH 7.4. (*B*) EPSC amplitudes for conditions described for *A*. 10 mM HCO_3^- ($n = 12$ cells in 6 mice), 25 mM HCO_3^- ($n = 6$ cells in 4 mice), and 90 mM HCO_3^- ($n = 7$ cells in 4 mice). One-way ANOVA; Tukey's post hoc multiple comparison. $^*P < 0.05$.

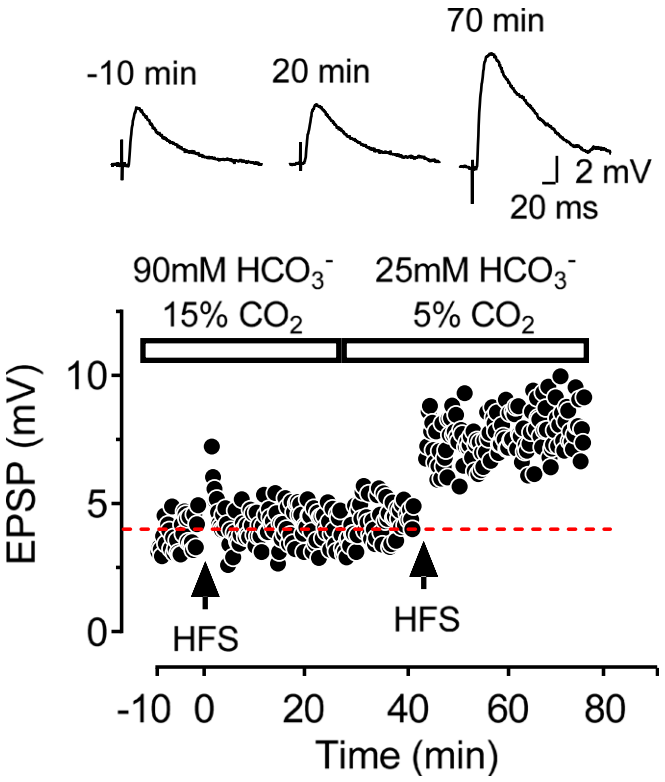

Fig. S6. Attenuation of LTP by increased pH buffer was not a result of inhibition of EPSPs or irreversible changes in brain slices. EPSPs recorded from lateral amygdala pyramidal neurons perfused with 90 mM HCO_3^-/15% CO_2 at pH 7.4 ACSF. HFS (arrow at time 0) did not alter EPSPs (103 ± 11% baseline). At time indicated by bars, the solution was changed to 25 mM HCO_3^-/5% CO_2 at pH 7.4. Then HFS was performed a second time (141 ± 9% baseline; $P < 0.01$; Student *t* test). Representative EPSP traces at top were obtained at times indicated. Data are example from one cell; similar results were obtained in 8 cells from 4 mice.

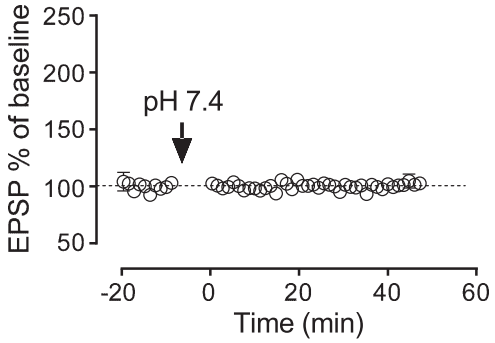

Fig. S7. Extracellular application of pH 7.4 solution did not induce LTP. Application of pH 7.4 ACSF to amygdala pyramidal neurons did not induce LTP (100 ± 1% of baseline; $n = 4$ cells in 3 mice).