Published in final edited form as:

Science. 2017 June 30; 356(6345): 1352–1356. doi:10.1126/science.aaf4612.

Restoring auditory cortex plasticity in adult mice by restricting thalamic adenosine signaling**

Jay A. Blundon^{1,^}, Noah C. Roy^{1,^}, Brett J. W. Teubner¹, Jing Yu¹, Tae-Yeon Eom¹, K. Jake Sample¹, Amar Pani¹, Richard J. Smeyne^{1,#}, Seung Baek Han¹, Ryan A Kerekes², Derek C. Rose², Troy A. Hackett³, Pradeep K. Vuppala^{4,#}, Burgess B. Freeman III⁴, and Stanislav S. Zakharenko^{1,*}

¹Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

²Electrical and Electronics Systems Research Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

³Department of Hearing and Speech Sciences, Vanderbilt University School of Medicine, Nashville, TN, USA

⁴Preclinical Pharmacokinetics Shared Resource, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

Abstract

Circuits in the auditory cortex are highly susceptible to acoustic influences during an early postnatal critical period. The auditory cortex selectively expands neural representations of enriched acoustic stimuli, a process important for human language acquisition. Adults lack this plasticity. Here we show in the murine auditory cortex that juvenile plasticity can be reestablished in adulthood if acoustic stimuli are paired with disruption of ecto-5'-nucleotidase-dependent adenosine production or A₁ adenosine receptor signaling in the auditory thalamus. This plasticity occurs at the level of cortical maps and individual neurons in the auditory cortex of awake adult mice and is associated with long-term improvement of tone-discrimination abilities. We conclude that, in adult mice, disrupting adenosine signaling in the thalamus rejuvenates plasticity in the auditory cortex and improves auditory perception.

**This manuscript has been accepted for publication in Science. This version has not undergone final editing. Please refer to the complete version of record at <https://na01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.sciencemag.org%2F&data=01%7C01%7Cstanislav.zakharenko%40stjude.org%7C0b4da3ee63f045c23fb608d49bc956bc%7C22340fa892264871b677d3b3e377af72%7C0&sdata=20r7ftq%2B6nEU%2FWuDv5ww%2B8UXg4JYpEIZtbAfdnAsI%3D&reserved=0>. The manuscript may not be reproduced or used in any manner that does not fall within the fair use provisions of the Copyright Act without the prior, written permission of AAAS.

*Correspondence to: Stanislav S. Zakharenko (stanislav.zakharenko@stjude.org).

[^]These authors contributed equally to this work

[#]Present address: Thomas Jefferson University, 233 South 10th Street, Bluemle Hall, #206, Philadelphia, PA 19107 (R.J.S.); University of Tennessee Health Science Center, 855 Monroe Avenue, Memphis TN 38163 (A.P.); KinderPharm LLC, 100 Arrandale Blvd. (Suite 101), Exton, PA 19341 (P.K.V.)

One Sentence Summary: The auditory cortex of adult mice acquires juvenile plasticity if adenosine signaling is disrupted.

SUPPLEMENTARY MATERIALS

Materials and Methods

Acoustic experiences change cortical representations in the auditory cortex. These changes are a substrate for auditory cognition (1, 2). During a postnatal critical period in rodents [postnatal day (P) 11–P15], passive exposure to an acoustic tone expands the cortical representation of that frequency in the primary auditory cortex (3–7). In adulthood, passive exposure is insufficient to induce plasticity. Cortical map plasticity during the critical period helps construct a stable, adapted representation of the auditory world. Similar phenomena in humans are likely necessary for language acquisition (8, 9). The mechanism by which cortical plasticity becomes restricted in adults has been extensively debated (2, 3, 10–17). Long-term synaptic plasticity (e.g., long-term potentiation and long-term depression) at excitatory thalamocortical projections is restricted to the same critical period as cortical map plasticity in auditory cortex (18–20). In adults, both auditory cortex map plasticity (11, 12, 21) and thalamocortical long-term potentiation and long-term depression are restored when thalamocortical activation is paired with activation of cholinergic input from the nucleus basalis (18, 19). This cholinergic modulation of thalamocortical synaptic plasticity is mediated by adenosine, which inhibits neurotransmitter release via presynaptic A_1 receptors (A_1Rs) (22, 23). Blocking or deleting A_1Rs removes this inhibition of neurotransmitter release and thus enables the expression of thalamocortical long-term potentiation and long-term depression in the auditory cortex of mice matured beyond the early critical period *in vitro* (18, 19).

Thalamic adenosine signaling restricts auditory cortical map plasticity

We tested whether auditory cortex map plasticity can be reestablished after the early postnatal critical period by restricting A_1R -adenosine signaling *in vivo*. We compared auditory cortex map plasticity in mature (P45–P56) wild-type (WT) mice and mice lacking A_1Rs ($A_1R^{-/-}$) exposed to pure tones for 5 to 14 days (Fig. S1). We mapped auditory cortex by recording sound-evoked responses of neurons situated 300 to 400 μm from the pial surface [corresponding to layer (L) 3/4 thalamorecipient neurons] in anesthetized mice. The characteristic frequency (the frequency that evokes the maximal response at the lowest intensity) of each recording site was determined from responses to a range of tone frequencies and intensities. Auditory cortex maps in mature WT mice were unaffected by tone exposure; however, the percentage of sites with a characteristic frequency equal to the exposure frequency increased in sound-exposed $A_1R^{-/-}$ mice (Fig. 1A, B). This increase was specific to the exposure tone frequency: $A_1R^{-/-}$ mice exposed to 7.9 or 16.4 kHz showed a 2.6-fold or 2.9-fold increase in the percentage of recording sites with characteristic frequencies of 7.9 or 16.4 kHz, respectively, compared to naïve (not exposed to tone) $A_1R^{-/-}$ mice (Fig. 1B). We found no effect of tone exposure on tonotopic maps in the auditory thalamus [(i.e., the ventral division of the medial geniculate body of the thalamus (MGv))] of mature $A_1R^{-/-}$ mice (Fig. S2).

We tested multiple methods of reducing A_1R signaling and found each enabled cortical map plasticity in adult mice when paired with tone exposure. First, we injected lentiviruses containing siRNAs against the *Adora1* gene, which encodes A_1Rs , into MGv or auditory cortex of P45–P56 mice. Knocking down A_1Rs in MGv only (Fig. S3, S4) was sufficient to induce auditory cortex map plasticity in mice exposed to a 9.8-kHz tone (Fig. 1C–E). Neither naïve mice injected with these siRNAs in MGv nor tone-exposed mice injected with

control (scrambled) siRNA showed cortical map plasticity (Fig. 1C–E). In contrast, tone exposure produced no cortical map plasticity in mice injected with the same *Adora1* siRNAs into auditory cortex (Fig. S5), suggesting that plasticity at thalamocortical synapses but not corticocortical synapses is essential for the auditory cortex map plasticity that we observed. Auditory cortical map plasticity could also be induced in aged (7- to 8-month-old) mice after knocking down A₁Rs in MGv (Fig. S6).

Second, we conditionally deleted *Adora1* in thalamic excitatory neurons of mature (10- to 13-week-old) mice by injecting an adeno-associated virus (AAV) encoding *Cre-GFP* (AAV-*CamKIIα-Cre-GFP*) into MGv of newly generated *Adora1^{fl/fl}* mice (Fig. S7). After exposure to 9.8 kHz, the number of sites with a 9.8-kHz characteristic frequency increased in auditory cortex of *Adora1^{fl/fl}* mice injected with *Cre-GFP* (*Adora1^{fl/fl}-Cre* mice), compared to *Adora1^{fl/fl}* mice injected with *GFP* alone (*Adora1^{fl/fl}-GFP* mice) (Fig. 1F). *Adora1^{fl/fl}-Cre* mice exposed to the 9.8-kHz tone were better at distinguishing changes in sound frequencies around 9.8 kHz than were *Adora1^{fl/fl}-GFP* mice (Fig. 1G). This improvement in auditory perception was tone specific, as *Adora1^{fl/fl}-Cre* mice exposed to another tone did not show improved performance around 9.8 kHz (Fig. S8). Exposure to 9.8 kHz did not affect frequency-discrimination performance in WT mice (Fig. S9). Neither exposure to 9.8 kHz nor *in vivo* injection of *Cre-GFP* into MGv affected hearing, as measured by auditory brain stem responses or response to the startle (Fig. S10).

Third, we crossed *Adora1^{fl/fl}* and *Gbx2^{CreER}* mice to produce a line of mice with an inducible *Adora1* deletion in thalamocortical neurons. Tamoxifen injection reduced *Adora1* mRNA in *Gbx2^{CreER};Adora1^{fl/fl}* mice (Fig. S11). In situ hybridization in WT mice showed that *Adora1* is enriched in MGv (Fig. S11), suggesting that thalamocortical neurons are especially sensitive to adenosine. Tone exposure produced cortical map plasticity in *Gbx2^{CreER};Adora1^{fl/fl}* mice. The number of sites with a 9.8-kHz characteristic frequency was increased in these mice but not in control mice (Fig. S12).

Fourth, pharmacologic inhibition of A₁R signaling enabled long-lasting auditory cortex map plasticity. We paired tone exposure with a selective, blood-brain barrier-permeable A₁R antagonist FR194921 (2-(1-methyl-4-piperidinyl)-6-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)-3(2H)-pyridazinone), which after intraperitoneal injection persists in the brain for several hours (Fig. S13). Pairing tone exposure with FR194921 for 5 to 9 days induced auditory cortex map plasticity when measured the following day (Fig. S14A). Replacing FR194921 with vehicle or injecting naïve animals with the drug did not induce auditory cortex map plasticity (Fig. S14A). Auditory cortex map plasticity induced by pairing tone exposure with FR194921 persisted for weeks after pairing (Fig. S14B, C), as did improved auditory perception (Fig. S14D–F). Cortical map plasticity was malleable. Mice exposed to a second week of FR194921 treatment, paired with a different frequency, exhibited an increased number of auditory cortex sites responding to the second frequency and showed no residual increase in cortical sites responding to the first frequency (Fig. S14G).

Nt5e and adenosine are elevated in adult mice

We then tested auditory cortex map plasticity after reducing thalamic adenosine production. Of the ectonucleotidases that produce adenosine in the brain (24), only ecto-5'-nucleotidase (*Nt5e*) mRNA was elevated in mature versus neonatal thalamus (Fig. 2A). *Nt5e* protein expression was higher in the thalamus and, to a lesser degree, in auditory cortex of adults versus neonates (Fig. 2B). Consistent with this developmental *Nt5e* increase, total (intracellular and extracellular) adenosine was higher in the thalamus and auditory cortex of adults than of neonates (Fig. 2C). In *Nt5e*^{-/-} mice, this age-dependent elevation of adenosine was absent in the thalamus but evident in auditory cortex (Fig. 2C). Auditory cortex map plasticity was induced in mature *Nt5e*^{-/-} mice, but not in WT littermates exposed to a pure tone (Fig. 2D, E). Mature tone-exposed *Nt5e*^{-/-} mice distinguished frequencies better than naïve *Nt5e*^{-/-} mice (Fig. 2F), without any effect on the auditory brain stem response or response to the startle (Fig. S15). We also knocked down *Nt5e* in the MGv by injecting two different *Nt5e* siRNAs that reduced thalamic *Nt5e* (Fig. S16) and produced auditory cortex map plasticity after tone exposure (Fig. 2G, H). Metabotropic glutamate receptor (mGluR) antagonists inhibit synaptic plasticity at mature thalamocortical synapses (18, 19). CTEP, an mGluR5 inhibitor that crosses the blood-brain barrier (25), blocked auditory cortex map plasticity in tone-exposed *Nt5e*^{-/-} mice (Fig. S17).

A₁R agonist inhibits cortical map plasticity in juvenile mice

In a reverse experiment, we exposed pups to 9.8 kHz during the critical period (P11–P15), while treating them with the blood-brain barrier-permeable A₁R agonist 2-chloro-N⁶-cyclopentyladenosine (CCPA) or vehicle. We mapped auditory cortex 6 to 10 weeks later (Fig. S18A). Tone exposure in these pups produced auditory cortex map plasticity when paired with vehicle but not when paired with CCPA (Fig. S18B).

Auditory plasticity can be reestablished in individual neurons

To test for auditory cortex plasticity in individual neurons, we used two-photon imaging of sound-evoked calcium transients in thalamorecipient neurons of auditory cortex in mature awake mice before and after tone exposure. We infected L3/4 excitatory neurons in auditory cortex (Fig. S19) with a recombinant AAV expressing genetically encoded calcium indicator GCaMP6f (26) under control of the excitatory neuron-specific promoter *CamKIIα* (AAV-*CamKIIα*-GCaMP6f) (Fig. 3A). We determined each neuron's best frequency (i.e., the frequency that evokes the greatest response across all intensities) from calcium transients (Fig. S20) evoked by a range of tone frequencies and intensities (Fig. 3B, C). We measured tuning changes by repeatedly imaging the same auditory cortex neurons before and after as many as 5 days of exposure to 9.8 kHz (Figs. 3D, S21–S23).

All tested approaches that reduced adenosine signaling or production in MGv enabled plasticity in individual neurons when paired with tone exposure. A₁R knockdown in MGv with *Adora1* siRNA paired with 9.8-kHz exposure shifted best frequencies of individual L3/4 neurons toward 9.8 kHz but not in naïve mice with A₁R knockdown or when control siRNA was paired with 9.8-kHz exposure (Fig. 3D–J). *Nt5e* siRNA injected into MGv also

shifted best frequencies of individual neurons toward 9.8 kHz in mice exposed to this frequency but not in naive mice (Fig. 3K–N). Pairing tone exposure with pharmacologic blockade of A₁Rs also caused L3/4 neurons to shift best frequencies toward the exposure frequency. We measured receptive fields in the same population of neurons at days 0, 1, 3, and 5 during the 9.8-kHz tone exposure combined with FR194921 administration. L3/4 neurons in auditory cortex shifted their best frequencies toward 9.8 kHz starting at day 1 and maintained this shift during all the days of tone exposure (Fig. S24A–F). In contrast, pairing 9.8-kHz exposure with vehicle did not shift best frequencies of L3/4 neurons toward 9.8 kHz on any tested day (Fig. S24G–L).

Similar experiments in *Adora1^{fl/fl};Cre* mice (lacking A₁Rs in MGv neurons) showed that L3/4 neurons in auditory cortex also shift best frequencies toward the exposure frequency. Cortical neurons of *Adora1^{fl/fl}* mice, which received MGv injections of *Cre-tdTomato* (AAV-*CamKIIα-Cre-tdTomato*) (Fig. S25) and were exposed to 9.8 kHz shifted best frequencies toward 9.8 kHz at day 1 and maintained this shift at days 3 and 5 of tone exposure (Fig. S24M–R). In contrast, this best frequency shift was not seen in L3/4 neurons of WT littermates injected with *Cre-tdTomato* into MGv and exposed to 9.8 kHz for the same periods of time (Fig. S24S–X). A switch in best frequency after tone exposure did not depend on a neuron's receptive field properties or GCaMP6f response amplitudes (Fig. S26).

These findings suggest that an Nt5e-mediated, age-dependent increase in adenosine production in the auditory thalamus terminates the juvenile critical period of auditory cortex map plasticity via A₁R activation. A₁R activation inhibits virtually every neurotransmitter in the brain, and its strongest inhibitory action is on excitatory glutamatergic synapses (22). A₁R activation reduces presynaptic Ca²⁺ influx and hyperpolarizes presynaptic membrane potential by activating inwardly rectifying K⁺ channels (22). During the critical period, when adenosine levels are low, sustained activity in auditory thalamocortical synapses triggers enough glutamate release to activate metabotropic glutamate receptors and produce long-term potentiation or long-term depression. In adults, higher Nt5e expression leads to elevated production of adenosine, which limits sustained glutamate release via A₁R activation, blocking thalamocortical synaptic plasticity and thereby preventing cortical map plasticity. Restricting thalamic adenosine signaling is sufficient to restore higher levels of glutamate release and synaptic plasticity at thalamocortical synapses in brain slices from adult mice (18, 19, 27) and reestablish cortical map plasticity in adult mice *in vivo*.

Experimental manipulations in adult mice that were expected to enable thalamocortical synaptic plasticity (i.e., knockdown or deletion of A₁Rs or knockdown of Nt5e in thalamic neurons) also facilitated auditory cortical map plasticity. Manipulations expected to inhibit thalamocortical synaptic plasticity (i.e., mGluR5 antagonist treatment in *Nt5e^{-/-}* adult mice and A₁R agonist treatment during the postnatal critical period) blocked cortical map plasticity. Knockdown of A₁Rs in cortical neurons failed to potentiate cortical map plasticity in adult mice. These results point to thalamocortical long-term plasticity as the necessary step that leads to cortical map plasticity and enhanced auditory perception. However, our results do not exclude the possibility that the improvement in auditory perception may result from additional changes at other synapses in the auditory cortex as a downstream consequence triggered by thalamocortical synaptic plasticity.

Implications for the human context

Pairing tone exposure with administration of the specific A₁R antagonist FR194921 resulted in cortical map plasticity and improved auditory perception in adult mice. These results imply that other A₁R inhibitors, such as caffeine, could be used to improve auditory perception in human adults. However, because caffeine, especially at high concentrations, inhibits other adenosine receptors and affects intracellular cyclic AMP and calcium levels (28), its effectiveness at improving auditory perception could be limited, compared to that of FR194921 or other specific A₁R inhibitors.

Cortical plasticity in adults holds potential as a strategy to restore normal neural activity in patients with neurological diseases, such as tinnitus and stroke (29–32). One current method for inducing therapeutic targeted cortical map plasticity in patients is the pairing of sensory stimulation (i.e., a sound) or motor training with repeated electrical stimulation of the vagus nerve using surgically implanted electrodes. Our work offers an alternative, noninvasive method for inducing lasting cortical map plasticity, namely by pairing a sound with administration of a specific A₁R inhibitor that has a short half-life.

Adult cortical plasticity is associated with improved tone-frequency discrimination. In humans, tone discrimination is required for acquiring language and musical skills. Tonal representation in auditory cortex is larger in musicians than in non-musicians (33). This difference is observed primarily in musicians who began practicing their instruments before 9 years of age (34); it is also associated with improved linguistic skills (35, 36). Here, we show that disruption of thalamic adenosine signaling in the adult mouse that restores auditory cortical map plasticity also improves auditory perception. A similar mechanism may improve learning of linguistic, musical, and other auditory skills in adult humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health grants DC012833, MH097742 (S.S.Z.), DC015388 (T.A.H.) and by ALSAC (S.S.Z). The funding sources had no role in study design, data collection, data analysis, decision to publish, or preparation of the manuscript. We thank the Vector Core Laboratories (St. Jude Children's Research Hospital and University of Tennessee Health Science Center) and Joby Westmoreland for assistance in producing AAVs and lentiviruses, Fei Du for assistance in generating *Adora1^{fl/fl}* mice and pharmacokinetic studies, Jaeki Min and Anand Mayasundari for help with FR194921, Arzu Onar-Thomas for statistical assistance, Alex Uptain and Kara Anderson for assistance with imaging experiments and viral injections *in vivo*, and Vani Shanker and Angela McArthur for editing the manuscript. Supplement contains additional data.

REFERENCES AND NOTES

1. Reed A, et al. *Neuron*. 2011; 70:121. [PubMed: 21482361]
2. Schreiner CE, Polley DB. *Curr Opin Neurobiol*. 2014; 24:143. [PubMed: 24492090]
3. Zhang LI, Bao S, Merzenich MM. *Nat Neurosci*. 2001; 4:1123. [PubMed: 11687817]
4. Chang EF, Merzenich MM. *Science*. 2003; 300:498. [PubMed: 12702879]
5. Hensch TK. *Nat Rev Neurosci*. 2005; 6:877. [PubMed: 16261181]

6. de Villers-Sidani E, Chang EF, Bao S, Merzenich MM. J Neurosci. 2007; 27:180. [PubMed: 17202485]
7. Dorn AL, Yuan K, Barker AJ, Schreiner CE, Froemke RC. Nature. 2010; 465:932. [PubMed: 20559387]
8. Doupe AJ, Kuhl PK. Annu Rev Neurosci. 1999; 22:567. [PubMed: 10202549]
9. Tallal P, Benasich AA. Dev Psychopathol. 2002; 14:559. [PubMed: 12349874]
10. Recanzone GH, Schreiner CE, Merzenich MM. J Neurosci. 1993; 13:87. [PubMed: 8423485]
11. Bakin JS, Weinberger NM. Proc Natl Acad Sci U S A. 1996; 93:11219. [PubMed: 8855336]
12. Kilgard MP, Merzenich MM. Science. 1998; 279:1714. [PubMed: 9497289]
13. Bao S, Chan VT, Merzenich MM. Nature. 2001; 412:79. [PubMed: 11452310]
14. Froemke RC, Schreiner CE. Curr Opin Neurobiol. 2015; 35:185. [PubMed: 26497430]
15. Hubener M, Bonhoeffer T. Cell. 2014; 159:727. [PubMed: 25417151]
16. Oberlaender M, Ramirez A, Bruno RM. Neuron. 2012; 74:648. [PubMed: 22632723]
17. Takesian AE, Hensch TK. Prog Brain Res. 2013; 207:3. [PubMed: 24309249]
18. Blundon JA, Bayazitov IT, Zakharenko SS. J Neurosci. 2011; 31:16012. [PubMed: 22049443]
19. Chun S, Bayazitov IT, Blundon JA, Zakharenko SS. J Neurosci. 2013; 33:7345. [PubMed: 23616541]
20. Crair MC, Malenka RC. Nature. 1995; 375:325. [PubMed: 7753197]
21. Froemke RC, Merzenich MM, Schreiner CE. Nature. 2007; 450:425. [PubMed: 18004384]
22. Dunwiddie TV, Masino SA. Annu Rev Neurosci. 2001; 24:31. [PubMed: 11283304]
23. Mitchell JB, Lupica CR, Dunwiddie TV. J Neurosci. 1993; 13:3439. [PubMed: 8393482]
24. Street SE, et al. J Neurosci. 2013; 33:11314. [PubMed: 23825434]
25. Lindemann L, et al. J Pharmacol Exp Ther. 2011; 339:474. [PubMed: 21849627]
26. Chen TW, et al. Nature. 2013; 499:295. [PubMed: 23868258]
27. Blundon JA, Zakharenko SS. Neuroscientist. 2013; 19:465. [PubMed: 23558179]
28. Fredholm BB, Yang J, Wang Y. Mol Aspects Med. 2016
29. Dawson J, et al. Stroke. 2016; 47:143. [PubMed: 26645257]
30. Engineer ND, et al. Nature. 2011; 470:101. [PubMed: 21228773]
31. Hays SA, Rennaker RL, Kilgard MP. Prog Brain Res. 2013; 207:275. [PubMed: 24309259]
32. De RD, Vanneste S, Engineer ND, Kilgard MP. Neuromodulation. 2014; 17:170. [PubMed: 24255953]
33. Pantev C, et al. Nature. 1998; 392:811. [PubMed: 9572139]
34. Pantev C, Roberts LE, Schulz M, Engelien A, Ross B. Neuroreport. 2001; 12:169. [PubMed: 11201080]
35. Marie C, Delogu F, Lampis G, Belardinelli MO, Besson M. J Cogn Neurosci. 2011; 23:2701. [PubMed: 20946053]
36. Moreno S, Besson M. Psychophysiology. 2006; 43:287. [PubMed: 16805867]
37. Johansson B, et al. Proc Natl Acad Sci U S A. 2001; 98:9407. [PubMed: 11470917]
38. Thompson LF, et al. J Exp Med. 2004; 200:1395. [PubMed: 15583013]
39. Chatterjee M, et al. Development. 2012; 139:4633. [PubMed: 23136391]
40. Concas A, et al. J Pharmacol Exp Ther. 1993; 267:844. [PubMed: 8246158]
41. Florio C, Prezioso A, Papaioannou A, Vertua R. Psychopharmacology (Berl). 1998; 136:311. [PubMed: 9600575]
42. Winkowski DE, Kanold PO. J Neurosci. 2013; 33:1498. [PubMed: 23345224]
43. Hackett TA, Barkat TR, O'Brien BM, Hensch TK, Polley DB. J Neurosci. 2011; 31:2983. [PubMed: 21414920]
44. Stiebler I, Neulist R, Fichtel I, Ehret G. J Comp Physiol. 1997; 181:559. [PubMed: 9449817]
45. Insanally MN, Kover H, Kim H, Bao S. J Neurosci. 2009; 29:5456. [PubMed: 19403813]
46. Polley DB, Read HL, Storace DA, Merzenich MM. J Neurophysiol. 2007; 97:3621. [PubMed: 17376842]

47. Barkat TR, Polley DB, Hensch TK. *Nat Neurosci.* 2011; 14:1189. [PubMed: 21804538]
48. Rueckert D, et al. *IEEE Trans Med Imaging.* 1999; 18:712. [PubMed: 10534053]
49. Chun S, et al. *Science.* 2014; 344:1178. [PubMed: 24904170]
50. Pani AK, Jiao Y, Sample KJ, Smeyne RJ. *PLoS One.* 2014; 9:e92422. [PubMed: 24642754]
51. Kuroda S, et al. *Chem Pharm Bull (Tokyo).* 2001; 49:988. [PubMed: 11515591]
52. Maemoto T, et al. *J Pharmacol Sci.* 2004; 96:42. [PubMed: 15351792]
53. Kalk P, et al. *Br J Pharmacol.* 2007; 151:1025. [PubMed: 17558436]
54. Vollert C, Forkuo GS, Bond RA, Eriksen JL. *Neurosci Lett.* 2013; 548:296. [PubMed: 23748072]
55. Baumgold J, Nikodijevic O, Jacobson KA. *Biochem Pharmacol.* 1992; 43:889. [PubMed: 1540242]
56. Finlayson K, Butcher SP, Sharkey J, Olverman HJ. *J Neurosci Methods.* 1997; 77:135. [PubMed: 9489889]
57. Clause A, Nguyen T, Kandler K. *J Neurosci Methods.* 2011; 200:63. [PubMed: 21672556]
58. Ison JR, McAdam DW, Hammond GR. *Physiol Behav.* 1973; 10:1035. [PubMed: 4717640]
59. Wang F, et al. *J Mol Diagn.* 2012; 14:22. [PubMed: 22166544]
60. Chen L, Guo Q, Li JY. *Development.* 2009; 136:1317. [PubMed: 19279136]
61. Madisen L, et al. *Nat Neurosci.* 2010; 13:133. [PubMed: 20023653]

Fig. 1.

Deletion of A_1 R_s in auditory thalamus is sufficient for passive tone exposure to induce auditory cortex map plasticity. (A) Representative auditory cortex maps from WT or $A_1R^{-/-}$ mice exposed or unexposed (naïve) to a 16.4-kHz tone. Black and gray depict blood vessels. D, dorsal; V, ventral; R, rostral; C, caudal. 0, unresponsive sites; X, sites that do not match the tonotopic criteria of primary auditory cortex. (B) Percentage of recording sites vs. characteristic frequencies for naïve (black circles, 5 mice per genotype) or tone-exposed (7.9 kHz: red open circles, 5 mice per genotype; 16.4 kHz: red filled circles, 5 mice) WT (top) and $A_1R^{-/-}$ (bottom) animals [two-way repeated measures ANOVA, genotype: WT: $F_{1,5}=0.644$, $P=0.446$; $A_1R^{-/-}$ (7.9 kHz): two-tailed t test: $t_8=10.516$, $*P<0.001$; $A_1R^{-/-}$ (16.4 kHz): $t_8=5.452$, $*P<0.001$]. (C-E) Auditory thalamus (MGv) injections of siRNAs

and recordings in auditory cortex: percentage of recording sites vs. characteristic frequencies in auditory cortex of WT mice exposed to 9.8 kHz (red circles) or naïve (black circles) after injections of *Adora1* siRNA1 (123), (naïve: 4 mice; exposed: 6 mice; $t_g=4.839$, $*P=0.001$) (C), *Adora1* siRNA2 (789), (naïve: 4 mice; exposed: 4 mice; $t_g=3.498$, $*P=0.01$) (D), or control siRNA, [naïve: 6 mice; exposed: 4 mice; $t_g=0.802$, $P=0.4$] (E). (F) Percentage of recording sites vs. characteristic frequencies in *Adora1^{fl/fl}* mice exposed to 9.8 kHz and injected with *AAV-CamKII α -GFP* (black squares, 8 mice) or *AAV-CamKII α -Cre-GFP* (red squares, 9 mice) into MGv [$t_{15}=4.612$, $*P=0.0003$]. (G) (Top) Schematic of the prepulse inhibition acoustic startle experiment. Prepulse frequencies are 9.800 kHz, 9.604 kHz, 9.408 kHz, 9.016 kHz, 8.232 kHz, or 6.664 kHz, which are, respectively, 0%, 2%, 4%, 8%, 16%, or 32% different from the frequency of the background tone. (Bottom) Mean inhibition of the acoustic startle response in *Adora1^{fl/fl}* mice exposed to 9.8 kHz after injection of *AAV-CamKII α -GFP* (black squares, 10 mice) or *AAV-CamKII α -Cre-GFP* (red squares, 9 mice) into MGv [$F_{1,5}=42.382$, $*P<0.001$]. CF, characteristic frequency; WN, white noise.

Fig. 2.

Age-dependent elevation of adenosine production underlies cortical map plasticity restrictions in adult mice. (A) Mean *Nt5e*, *Acpp*, and *Tnap* mRNA levels (normalized to *Gapdh*) in the auditory thalamus of neonatal (P5–P12, grey bars, 7 mice) or mature (P45–P56, black bars, 6 mice) WT animals (*Nt5e*: $t_{1f}=2.325$, $*P=0.038$; *Acpp*: $t_{1f}=1.145$, $P=0.276$; *Tnap*: $t_{1f}=0.101$, $P=0.921$). (B) Representative Western blots of MGv extracts (top) and mean Nt5e protein levels in the auditory thalamus ($t_{1f}=7.857$, $*P<0.001$) and auditory cortex ($t_{1f}=3.342$, $*P=0.005$) (bottom) of neonatal (grey bars, 8 mice) or mature (black bars, 8 mice) WT mice. (C) Total adenosine levels in the auditory thalamus and auditory cortex of neonatal (grey bars, 10 mice) or mature (black bars, 10 mice) WT mice (thalamus: $t_{1f}=11.257$, $*P<0.001$; auditory cortex: $t_{1f}=10.036$, $*P<0.001$) and neonatal (pink bars, 9 mice) or mature (red bars, 10 mice) *Nt5e*^{-/-} animals (thalamus: $t_{1f}=0.961$, $P=0.35$; auditory cortex: $t_{1f}=3.218$, $*P=0.005$). (D, E) Percentage of recording sites vs.

characteristic frequencies in auditory cortex of naïve (black circles) or 11.4-kHz tone-exposed (red circles) WT (naïve: 4 mice; exposed: 5 mice; $t_{\text{test}}=0.283$, $P=0.785$) (D) and *Nt5e*^{-/-} animals (naïve: 5 mice; exposed: 6 mice; $t_{\text{test}}=2.148$, $*P=0.03$) (E). (F) Mean prepulse inhibition as a function of prepulse frequency in *Nt5e*^{-/-} mice exposed to 9.8 kHz (red circles, 17 mice) or naïve (black circles, 20 mice) ($F_{1,6}=13.643$, $*P<0.001$). (G, H) Percentage of recording sites vs. characteristic frequencies in auditory cortex of naïve (black circles) or 9.8-kHz exposed (red circles) WT mice after injection of *Nt5e* siRNA1 (1264) (naïve: 3 mice; exposed: 4 mice; $t_{\text{test}}=3.33$, $*P=0.02$) (G) or *Nt5e* siRNA2 (1366) (naïve: 5 mice; exposed: 7 mice; $t_{\text{test}}=4.79$, $*P=0.0007$) (H) into MGv. ACx, auditory cortex; CF, characteristic frequency; PPI, prepulse inhibition.

Fig. 3.

Restricting thalamic adenosine signaling or production is sufficient for passive tone exposure to cause a tuning shift in individual neurons in auditory cortex of awake mice. (A) Representative image of GCaMP6f-expressing L3/4 excitatory neurons in auditory cortex. (B, C) Sound-evoked GCaMP6f fluorescence transients (B) and respective receptive field map (C) of an L3/4 neuron. Gray vertical bars in (B) represent sound stimuli. (D) Examples of receptive field heat maps (normalized to maximum intensity) in the same neurons before (day 0) and after (day 5) three experimental conditions: *Adora1* siRNA1 (123) in MGv with

no tone exposure (2 left panels), *Adora1* siRNA1 (123) in MGv with 9.8-kHz exposure (4 middle panels), and control siRNA in MGv with 9.8-kHz exposure (2 right panels). Vertical dotted lines indicate 9.8 kHz. (E-G) Cumulative histograms of best frequencies of recorded neurons in auditory cortex before (black solid lines) and after 9.8-kHz exposure (green lines) under the same 3 experimental conditions as in D (3 mice per condition) (E: 19 neurons, $P > 0.05$; F: 34 neurons, $*P < 0.05$; G: 25 neurons, $P > 0.05$). (H-J) Heat maps (BF maps) depicting shifts in neuron numbers (normalized to % sampled) as a function of best frequencies between day 0 and day 5, under 3 experimental conditions as in D, from data in E-G (H: $P > 0.05$; I: $*P < 0.001$; J: $P > 0.05$). Diagonal lines represent no change in best frequencies. Vertical lines represent 9.8 kHz. (K-N) As described above for E-J, under 2 experimental conditions (3 mice per condition): *Nt5e* siRNA1 (1264) in MGv with no tone exposure (K, M: 24 neurons, $P > 0.05$) or *Nt5e* siRNA1 (1264) in MGv with 9.8-kHz exposure (L, N: 23 neurons, L: $*P < 0.05$; N: $*P < 0.001$). BF, best frequency.