

Title Developmental hearing loss-induced perceptual deficits are rescued by cortical expression of GABA_B receptors

Running Title GABA_B expression restores perception

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Key Words

hearing loss, auditory cortex, auditory perception, synaptic inhibition, GABA_A, GABA_B

Abbreviations

auditory cortex (AC), gamma-aminobutyric acid A receptor subunit $\alpha 1$ (*Gabra1*), gamma-aminobutyric acid B receptor subunit 1b (*Gabbr1b*), hearing loss (HL), inhibitory postsynaptic potential (IPSP), amplitude modulation (AM), spectral modulation (SM).

Acknowledgements

The work is supported by NIDCD R01DC011284 (DHS and TMM), and F32DC020659 (SM).

Author Contributions

SM, TMM, and DHS designed the experiments and wrote the paper; SM, RF, and TMM performed experiments; SM and DHS designed the viruses.

Conflict of interest

The authors whose names are listed immediately above certify that they have no affiliations with or involvement in any organization or entity with any financial, or non-financial interest in the subject matter or materials discussed in this manuscript.

1 **Abstract**

2 Even transient periods of developmental hearing loss during the developmental critical period
3 have been linked to long-lasting deficits in auditory perception, including temporal and spectral
4 processing, which correlate with speech perception and educational attainment. In gerbils,
5 hearing loss-induced perceptual deficits are correlated with a reduction of both ionotropic GABA_A
6 and metabotropic GABA_B receptor-mediated synaptic inhibition in auditory cortex, but most
7 research on critical period plasticity has focused on GABA_A receptors. We developed viral vectors
8 to express both endogenous GABA_A or GABA_B receptor subunits in auditory cortex and tested
9 their capacity to restore perception of temporal and spectral auditory cues following critical period
10 hearing loss in the Mongolian gerbil. HL significantly impaired perception of both temporal and
11 spectral auditory cues. While both vectors similarly increased IPSCs in auditory cortex, only
12 overexpression of GABA_B receptors improved perceptual thresholds after HL to be similar to those
13 of animals without developmental hearing loss. These findings identify the GABA_B receptor as an
14 important regulator of sensory perception in cortex and point to potential therapeutic targets for
15 developmental sensory disorders.

16 **Significance Statement**

17 Hearing loss in children can induce deficits in aural communication that persevere even after
18 audibility has returned to normal, suggesting permanent changes to the auditory central nervous
19 system. In fact, a reduction in cortical synaptic inhibition has been implicated in a broad range of
20 developmental disorders, including hearing loss. Here, we tested the hypothesis that
21 developmental hearing loss-induced perceptual impairments in gerbils are caused by a
22 permanent reduction of auditory cortical inhibitory synapse strength. We found that virally-
23 mediated expression of a GABA_B receptor subunit in gerbil auditory cortex was able to restore
24 two auditory perceptual skills in juvenile animals reared with hearing loss, suggesting that cortical
25 synaptic inhibition is a plausible therapeutic target for sensory processing disorders.

26 Introduction

27 Reduced cortical inhibition has been implicated in a broad range of developmental disorders
28 including autism, schizophrenia, fragile x syndrome, and impaired sensory processing (Chao et
29 al. 2010; Sanes & Kotak, 2011; Braat & Kooy, 2015; Gainey & Feldman, 2017). For example,
30 visual or auditory deprivation that occurs during developmental sensitive periods leads to weaker
31 inhibitory synapses between GABAergic interneurons and pyramidal cells in primary sensory
32 cortices (Morales et al., 2022; Maffei et al. 2004; Takesian et al. 2012; Mowery et al., 2015). In
33 some cases, these functional effects are correlated with a down-regulation of GABA receptors or
34 loss of GABAergic terminals (Fuchs & Salazar, 1998; Kilman et al. 2002; Jiao et al. 2006; Sarro
35 et al. 2008; Braat et al. 2015). Furthermore, when induced by hearing loss (HL), this reduction of
36 inhibition has been linked to a broad range of perceptual and central processing deficits (Aizawa
37 and Eggermont, 2007, Rosen et al., 2012; Yao and Sanes, 2018; Gay et al., 2014; Polley et al.,
38 2013; Han et al., 2007; Kim and Bao, 2009; Zhang et al., 2001; Mowery et al., 2019). Taken
39 together, these observations lead to the hypothesis that developmental HL induces a reduction
40 of postsynaptic GABA receptor-mediated inhibition in auditory cortex (AC), thereby causing
41 perceptual deficits. Here, we address a prediction that emerges from this hypothesis: increasing
42 GABA_A or GABA_B receptor-dependent inhibition selectively within AC pyramidal neurons after
43 developmental HL should restore performance on auditory perceptual tasks.

44 There is indirect support for the premise that normal perceptual performance is associated with
45 appropriate levels of cortical inhibition in adults. For example, magnetic resonance spectroscopy
46 measurements in humans demonstrate that performance on visual or auditory perceptual tasks
47 are correlated with a higher GABA concentration (Edden et al., 2009; Dobri and Ross, 2021; Ip et
48 al., 2021). Furthermore, a pharmacological manipulation that enhances GABAergic inhibition
49 during behavioral testing leads to improved performance on an auditory temporal perception task
50 in senescent gerbils and improved visual coding in senescent monkeys (Gleich et al., 2003;
51 Leventhal et al. 2003). Consistent with this idea, systemic treatment with a GABA reuptake
52 inhibitor can both restore the strength of inhibitory synapses following developmental HL and
53 rescue an auditory perceptual skill (Kotak et al., 2013; Mowery et al., 2019). Although the
54 relationship between inhibition and mature sensory processing is well established, the relative
55 contribution of ionotropic GABA_A and metabotropic GABA_B postsynaptic receptors is uncertain.
56 Depending on the outcome measure, pharmacological experiments suggest that both types of
57 receptors can be an effective target for restoring normal function or plasticity (Iwai et al. 2003,
58 Möhler et al., 2004; Fagiolini et al., 2004; Kotak et al., 2013; Cai et al., 2017; Zheng et al., 2012).
59 Therefore, selective gain-of-function manipulations are required to determine whether restoring

60 GABA_A or GABA_B receptor-mediated inhibition can remediate a behavioral deficit resulting from
61 a developmental insult.

62 To address this problem, we developed AAV vectors to selectively increase the functional
63 expression of either GABA_A or GABA_B-mediated synaptic inhibition in the AC. One virus was
64 designed to express the $\alpha 1$ subunit of the GABA_A receptor and the second virus was designed to
65 express the 1b subunit of the GABA_B receptor, each under the CaMKII promoter (Perez-Garci et
66 al., 2006; Vigot et al., 2006). Our approach employed a previously validated paradigm in which
67 transient developmental hearing loss (HL) is induced during the AC critical period, causing a
68 reduction in GABA_A and GABA_B-mediated AC inhibition and diminished performance on an
69 amplitude modulation (AM) detection task (Mowery et al., 2015; Caras and Sanes, 2015; Mowery
70 et al., 2016; Mowery et al., 2019). We also introduce a second perceptual task, spectral
71 modulation (SM) detection, with which to assess the effect of HL and the effect of restoring
72 inhibition. The ability to perceive spectral modulation of sound is especially important for speech
73 and speech-in-noise comprehension (Drullman, 1995; Zeng et al. 2005). Furthermore, humans
74 with hearing loss or cochlear implants are impaired in spectral modulation detection, and this is
75 correlated with speech perception (Horn et al. 2017; Ozmeral et al., 2018; Nittrouer et al., 2021).
76 Together, AM and SM cues compose two of the fundamental building blocks of natural sounds
77 (Singh and Theunessin, 2003), and HL-induced deficits have been linked to childhood speech
78 and language acquisition (refs). Our findings suggest that viral expression of a GABA_B receptor
79 subunit, but not a GABA_A receptor subunit, in the AC can remediate the deleterious effects of
80 developmental HL on auditory perception.

81 **Experimental Procedures**

82 Experimental animals: We performed behavioral experiments on 52 normal hearing and 47
83 transient HL gerbils (*Meriones unguiculatus*) in the age range of postnatal days (P) 10-48. For
84 brain slice experiments, we recorded from 34 AC pyramidal neurons, obtained from a total of 8
85 male and female gerbils in the age range P103-169. All animals were born from breeding pairs
86 (Charles River Laboratories) in our colony. All procedures were approved by the Institutional
87 Animal Care and Use Committee at New York University.

88 Induction of transient hearing loss: Reversible HL was induced using earplugs made of molding
89 clay inserted in both ears after ear canal opening, at P10, and sealed with super glue (Mowery et
90 al., 2015; Mowery et al., 2016). Earplugs were checked daily, replaced if needed, and removed
91 at P23. This manipulation produces a threshold shift of 15-50 dB, depending on frequency, as
92 measured with auditory brainstem responses (Caras and Sanes, 2015), and ~25 dB at 4 kHz as
93 measured behaviorally (Mowery et al., 2015).

94 Auditory cortex brain slice recordings: Thalamocortical brain slices were generated as described
95 previously (Kotak et al., 2005; Mowery et al., 2015, 2019). Animals were deeply anesthetized
96 (chloral hydrate, 400 mg/kg, IP) and brains dissected into 4°C oxygenated artificial cerebrospinal
97 fluid (ACSF, in mM: 125 NaCl, 4 KCl, 1.2 KH₂PO₄, 1.3 MgSO₄, 24 NaHCO₃, 15 glucose, 2.4
98 CaCl₂, and 0.4 L-ascorbic acid; and bubbled with 95%O₂-5%CO₂ to a pH=7.4). Brains were
99 vibratome-sectioned to obtain 300-400 µm perihorizontal auditory thalamocortical slices. The AC
100 was identified by extracellular field responses to medial geniculate stimulation.

101 Whole-cell current clamp recordings were obtained (Warner PC-501A) from AC layer 2/3
102 pyramidal neurons at 32°C in oxygenated ACSF. Recording electrodes were fabricated from
103 borosilicate glass (1.5 mm OD; Sutter P-97). The internal recording solution contained (in mM): 5
104 KCl, 127.5 K-gluconate, 10 HEPES, 2 MgCl₂, 0.6 EGTA, 2 ATP, 0.3 GTP, and 5 phosphocreatine
105 (pH 7.2 with KOH). The resistance of patch electrodes filled with internal solution was between 5-
106 10 MΩ. Access resistance was 15-30 MΩ, and was compensated by about 70%. Recordings were
107 digitized at 10 kHz and analyzed offline using custom Igor-based macros (IGOR, WaveMetrics,
108 Lake Oswego, OR). All recorded neurons had a resting potential ≤-50 mV and overshooting action
109 potentials.

110 Inhibitory postsynaptic potentials (IPSPs) were evoked via biphasic stimulation of layer 4 (1-10
111 mV, 10 s interstimulus interval) in the presence of ionotropic glutamate receptor antagonists (6,7-
112 Dinitroquinoxaline-2,3-dione, DNQX, 20 µM; 2-amino-5-phosphonopentanoate, AP-5, 50 µM).
113 The drugs were applied for a minimum of 8 min before recording IPSPs. Peak amplitudes of the
114 short latency hyperpolarization (putative GABA_A component) and long latency hyperpolarization
115 (putative GABA_B component) were measured from each response at a holding potential (V_{hold})

116 of -50 mV. To assess GABA_B receptor mediated IPSPs, the GABA_A receptor antagonist
117 bicuculline (10 μ M) was also added to the bath. We previously verified that short- and long-latency
118 IPSP components represented GABA_A and GABA_B receptor-dependent responses, respectively
119 (see Fig 3D in Mowery et al., 2019).

120 Behavioral training and testing: Amplitude modulation (AM) and spectral modulation (SM) depth
121 detection thresholds were assessed with an aversive conditioning procedure (Heffner & Heffner,
122 1995; Kelly et al., 2006) used previously in our lab (Sarro & Sanes, 2011; Rosen et al., 2012;
123 Buran et al., 2014; Caras and Sanes, 2015, 2017, 2019). The apparatus was controlled by custom
124 Matlab scripts, interfaced with a digital signal processor (TDT). Stimuli were delivered via a
125 calibrated tweeter (KEF electronics) positioned above a test cage which contains a metal water
126 spout and floor plate. Water delivery was initiated by a syringe pump (Yale Apparatus) triggered
127 by infrared detection of spout contact. The speaker and cage were located in a sound attenuation
128 chamber and observed via video monitor. After placement on controlled water access, gerbils
129 learned to drink steadily from the lick spout while in the presence of continuous, unmodulated,
130 band-limited noise (0.1-20 kHz). Separate groups of animals were trained to withdraw from the
131 spout when the sound changed from unmodulated noise (the “safe” cue) to either AM or SM noise
132 (the “warn” cue) by pairing the modulation with a mild shock (0.5-1.0 mA, 300 ms; Lafayette
133 Instruments) delivered through the spout. For the AM task, procedural training was conducted
134 with a warn cue of 0 dB re: 100% modulation depth. For the SM task, procedural training was
135 conducted with a warn cue of 40 dB modulation depth. Repeated pairings of the shock and the
136 warn cue resulted in a rapidly learned association and reliable spout withdrawal, which was used
137 as a behavioral measure of modulation detection. Warn trials were interspersed with 2-6 safe
138 trials (2-6 seconds), during which the unmodulated sound continued unchanged; the
139 unpredictable nature of the warn presentation prevented temporal conditioning.

140 After the initial associative learning, five AM or SM depths, bracketing the threshold (AM task: -3
141 to -27 dB re: 100% depth in 3 dB steps; SM task: +3 to 27 dB), were presented in descending
142 order. Note that AM depth was calculated relative to a completely modulated sinusoidal waveform
143 (dB re: 100% depth), such that larger negative values represent depths that are more difficult to
144 detect. SM depth was calculated relative to unmodulated noise, such that smaller positive values
145 represent depths that are more difficult to detect (dB re: 0% depth). Average stimulus level was
146 held constant at 45 dB SPL to ensure that detection was based on the modulation cue. SM stimuli
147 were generated using 3200 random-phase sinusoidal components spaced between 0.1 and
148 20kHz at a sampling rate of 44.8Khz (code courtesy of Dr. Donal Sinex). Stimuli were 1 second
149 long with a 20 ms on and off ramps. Stimuli were generated at 2 or 10 cycles/octave. For
150 experiments testing the effects of HL and viral vectors, SM density (2 cycles/octave) and AM rate

151 (5 Hz) remained constant. Psychometric testing spanned 7 or 10 consecutive days. On warn trials,
152 the response was scored as a hit when animals withdrew from the spout. On safe trials, the
153 response was scored as a false alarm when animals incorrectly withdrew from the spout. These
154 responses were used to calculate d' as $z(\text{hits})-z(\text{false alarms})$, a signal detection metric that
155 accounts for individual guessing rates (Green, 1966). Values were fit with psychometric functions
156 and used to calculate thresholds (Wichmann & Hill, 2001a, 2001b). Threshold was defined as the
157 smallest stimulus depth at which $d' = 1$.

158 Development of viral vectors: Two viral vectors were developed to express either the postsynaptic
159 GABA_B 1b subunit (Billinton et al., 1999), or the GABA_A α 1 subunit under the CaMKII promoter.
160 The gene sequences for each (*Gabbr1b* and *Gabra1*) were extracted from the gerbil genome
161 using BLAST (Zorio et al., 2019). For the *Gabbr1b* sequence, results were missing the first 141
162 bp of the full gene sequence, which were replaced with a sequence from the mouse to generate
163 a complete gene sequence. These genes were inserted into viral cassettes with fluorescent
164 reporters. At 2532 base pairs, the *Gabbr1b* sequence was too long to be used in the same
165 cassette used for the GABA_A subunit while maintaining high expression levels in an AAV1
166 serotype. We therefore minimized cassette length by using TurboRFP as the fluorescent reporter,
167 WPRE3 as a posttranscriptional regulatory element, and P2A to cleave the fluorescent reporter
168 (Merzlyak et al., 2007; Choi et al., 2014, Zufferey et al., 1999; Szymczak et al., 2005). Genes
169 were synthesized (*Gabra1*: ThermoFisher; *Gabbr1b*: Genewiz), and the full plasmids were
170 generated, cloned, and packaged (Penn Vector Core):
171 AAV1.CaMKII0.4.Gabbr1b.P2A.TurboRFP.WPRE3.rBG (4×10^{12} viral particles/mL), and
172 AAV1.CaMKII0.4.Gabra1.IRES.mCherry.WPRE.rBG (7×10^{12} viral particles/mL).

173 Virus injections: For all animals, bilateral surgical virus injections into the AC were performed on
174 P23 or P24, after earplug removal. AAV1.hSyn.eGFP.WPRE.bG (5×10^{12} vg/mL) was used as a
175 control. Animals were anesthetized with isoflurane, and incisions made just ventral to the temporal
176 ridge. A small burr hole (0.7 mm diameter) was drilled in the skull ~1.4 mm below the temporal
177 ridge, just over the AC (Radtke-Schuller et al., 2016). Virus was loaded into a glass pipette
178 backfilled with mineral oil. The glass pipettes were made sharp enough to penetrate dura without
179 causing damage and were inserted to a depth of 400 μ m. Virus was injected at a rate of 2 nl/s
180 with a Nanoject III (Drummond), followed by a 5 min period to permit diffusion. For the two custom
181 viruses, a volume of 600 μ l was injected, whereas for the control *GFP* virus, a volume of 250 μ l
182 was injected. Incisions were closed with surgical adhesive and the animal permitted to recover
183 for 7 days.

184 Histology: At the end of each experiment, animals were deeply anesthetized with Euthasol and
185 perfused transcardially with ice-cold PBS followed by 4% paraformaldehyde. Brains were

186 removed and fixed in the same fixative overnight at 4°C. Brains were cryoprotected and sectioned
187 (70 µm) on a vibratome and processed for fluorescence imaging. Only animals exhibiting
188 TurboRFP, mCherry or GFP properly targeted to AC were included in the behavioral data.

189 Statistical analysis. When data were normally distributed (as assessed by the Shapiro-Wilk W
190 Test), values were given as mean ± SEM. Statistical analyses were conducted using MatLab. To
191 compare multiple measures obtained from the same animal, a linear mixed effects model,
192 correcting for subject identity (formula: threshold ~ 1 + group * training day + (1 | subject)) was
193 used to verify a main effect of treatment group or the interaction between training day and
194 treatment group. For individual training days, group comparisons of variables were made using a
195 one-way analysis of variance (ANOVA) followed by least significant differences post-hoc. The HL
196 + GFP group was specified as the control group for all post hoc tests. The best threshold achieved
197 by each animal during 7 or 10 days of psychometric testing (depending on the experiment) was
198 used to determine perceptual thresholds. The significance level was set at $\alpha = 5\%$. All data are
199 expressed as mean ± SEM unless otherwise stated.

200 **Results**

201 To investigate the causal relationship between AC synaptic inhibition and HL-induced perceptual
202 deficits, we developed viral vectors to express the gerbil gene sequences for subunit $\alpha 1$ of the
203 GABA_A receptor (*Gabra1*) or subunit 1b of the GABA_B receptor (*Gabbr1b*), each under a CAMKII
204 promoter (see Methods). Our reasoning was that overexpression of a single subunit of either
205 receptor would remove a rate limiting step in the surface expression of the functional multimeric
206 receptor, thereby increasing IPSP amplitude.

207 **Functional assessment of virally expressed GABA_A and GABA_B receptor subunits**

208 To determine whether each viral vector led to functional upregulation of cortical inhibitory
209 postsynaptic potentials (IPSPs), we performed whole cell current clamp recordings from
210 pyramidal neurons in auditory cortex brain slices 21-27 days after virus injection into AC ([Figure](#)
211 [1A](#); see Methods). IPSPs were elicited in response to local electrical stimulation, as described
212 previously (Mowery et al., 2016; Mowery et al., 2019). [Figure 1B](#) shows the vector used to express
213 the *Gabra1* subunit under a CaMKII promoter, and a fluorescence image of the reporter molecule,
214 mCherry, as observed during whole cell recordings. [Figure 1C](#) shows representative IPSPs
215 recorded from a *Gabra1*-infected (orange trace; yellow pipet in panel B) and an uninfected AC
216 neuron (gray trace) at a holding potential (V_{hold}) of -50 mV in the presence of glutamate receptor
217 blockers (20 μM DNQX; 50 μM AP-5). [Figure 1D](#) shows that the peak amplitudes of the short
218 latency IPSP hyperpolarization (putative GABA_A component, labeled “A”) was significantly greater
219 for infected neurons (Mean \pm SEM; infected: 12.8 ± 0.6 mV; uninfected: 6.8 ± 0.6 mV; $q=2.05$,
220 $df=26$, $p<0.0001$). Therefore, the *Gabra1* vector increased GABA_A receptor-mediated IPSP
221 amplitude in AC pyramidal neurons.

222 [Figure 1E](#) shows the vector used to express the postsynaptic *Gabbr1b* subunit (Billinton et al.,
223 1999) under a CamKII promoter, and a fluorescence image of the reporter molecule, turboRFP,
224 as observed during whole cell recordings. [Figure 1F](#) shows representative IPSPs recorded from
225 a *Gabbr1b*-infected (blue trace; yellow pipet in panel E) and an uninfected AC neuron (gray trace)
226 at a holding potential (V_{hold}) of -50 mV in the presence of glutamate receptor antagonists (20 μM
227 DNQX; 50 μM AP-5) and a GABA_A receptor antagonist (10 μM bicuculline). [Figure 1G](#) shows that
228 the peak amplitudes of the long latency IPSP hyperpolarization (putative GABA_B component,
229 labeled “B”) was significantly greater for infected neurons (Mean \pm SEM; infected: 8.7 ± 0.6 mV;
230 uninfected: 5.8 ± 0.4 mV; $q=2.03$, $df=32$, $p=0.0002$). Therefore, the *Gabbr1b* vector increased
231 GABA_B receptor-mediated IPSP amplitude in AC pyramidal neurons.

232 **Assessing perceptual performance on AM and SM tasks**

233 [Figure 2](#) outlines the full experimental protocol. We induced reversible developmental hearing
234 loss, beginning at ear canal opening (P10) and ending after the auditory critical period (P23) by

235 inserting earplugs (Figure 2A, orange shading), as described previously (Caras and Sanes, 2015).
236 We injected separate groups of HL-reared animals with the *Gabra1* (n = 8) or *Gabbr1b*-expressing
237 vector (n = 9), or a GFP control virus (n = 9), bilaterally in AC between P23 and P25, after earplug
238 removal. We also included a normal hearing group (n = 15; Figure 2A, gray shading). Following
239 a 7 day recovery period, animals were water restricted and began behavioral training on P30.
240 Separate groups of animals were tested on AM depth detection (Sarro and Sanes, 2011; Rosen
241 et al., 2012; Caras & Sanes, 2015) or SM depth detection. As described in Methods, control and
242 HL-reared animals were trained to drink from a lick spout during continuous noise (0.1-20 kHz,
243 45 dB SPL). For the AM detection task, animals were initially trained to withdraw from the spout
244 when 5 Hz amplitude modulation at 0 dB re: 100% occurred (Figure 2B, top). For the SM detection
245 task, animals were initially trained to withdraw from the spout when 2 cycles/octave density
246 spectral modulation at 40 dB depth occurred (Figure 2B, bottom). Procedural learning continued
247 until animals achieved a $d' \geq 1.3$ with a 0 dB re: 100% depth AM stimuli or 40 dB depth SM stimuli
248 (4-8 days). We then conducted 7 days of psychometric testing as animals' performance gradually
249 improved on the AM or SM task (Figure 2C). Perceptual thresholds improved as gerbils
250 responded to smaller modulation depths due to perceptual learning (Caras & Sanes, 2017).
251 We first tested the effect of HL and GABA receptor subunit expression on AM depth detection.
252 Figure 3A presents representative psychometric functions for two individual animals and shows
253 that AM detection was superior for the HL-reared gerbil that received bilateral AC injections of a
254 *Gabbr1b*-expressing vector (HL+*Gabbr1b*; blue line) as compared to the HL-reared gerbil that
255 received AC bilateral injections of a *GFP*-expressing vector (HL+*GFP*; green line). As
256 schematized in Figure 2A, top, we obtained thresholds for animals in these two groups, as well
257 as HL-reared animals that received a *Gabra1*-expressing vector (HL+*Gabra1*), and normal
258 hearing (NH) animals.
259 Figure 3B shows thresholds by group over 7 days of psychometric testing. A linear mixed-effects
260 model comparing the effects of training day and virus condition on thresholds, and taking into
261 account individual subject behavior, indicates that viral treatment was a significant factor ($F =$
262 10.83 , $p = 9.83 \times 10^{-7}$). Therefore, expression of the *Gabbr1b* subunit restored normal behavioral
263 performance on the AM detection task in HL-reared animals in a manner that was independent of
264 training day.
265 There was a significant effect of treatment group on day 1 of perceptual testing (one-way ANOVA,
266 $p = 4.02 \times 10^{-5}$, $F = 10.46$, $df = 40$, Figure 3C) and day 7 of perceptual testing (one-way ANOVA,
267 $p = 6.85 \times 10^{-8}$, $F = 20.07$, $df = 40$, Figure 3D). A post hoc comparison revealed that AM detection
268 thresholds were significantly poorer for transient HL-reared animals that received a control virus
269 (HL+*GFP*) as compared to normal hearing animals (NH). This was the case both for day 1

270 (HL+*GFP* = -5.61 ± 0.99 dB, NH = -10.94 ± 0.77 dB, $p = 8.35 \times 10^{-4}$) and day 7 of testing (HL+*GFP*
271 = -8.90 ± 0.91 dB, NH = -14.56 ± 0.70 dB, $p = 1.00 \times 10^{-4}$). This finding confirms the effect of HL
272 reported previously (Caras and Sanes, 2015; Mowery et al., 2019).
273 Post hoc comparisons also revealed that GABA_B subunit expression, but not GABA_A receptor
274 expression could partially restore AM detection thresholds in HL-reared animals. Animals in the
275 HL+*Gabbr1b* group displayed significantly lower AM detection thresholds than the HL+*GFP*
276 group, both at day 1 (HL+*Gabbr1b* = -10.37 ± 0.99 dB, $p = 0.009$, NH = -10.94 ± 0.77 dB, $p = 8.35$
277 $\times 10^{-4}$) and day 7 of perceptual testing (HL+*Gabbr1b* = -12.47 ± 0.91 dB, $p = 0.04$). In contrast,
278 the HL+*Gabra1* group did not differ significantly from the HL+*GFP* group, either at day 1
279 (HL+*GFP* = -5.61 ± 0.99 dB, HL+*Gabra1* = -5.24 ± 1.06 dB, $p = .99$), or day 7 of perceptual testing
280 (HL+*GFP* = -8.90 ± 0.91 dB, HL+*Gabra1* = -6.01 ± 0.96 dB, $p = .15$).

281 **SM detection performance in normal hearing gerbils**

282 To develop a behavioral test of perception of spectral modulation (SM), we modified the aversive
283 conditioning paradigm such that unmodulated white noise transitioned to spectrally modulated
284 noise on “warn” trials. Similar to the AM detection task, SM stimuli were presented at multiple
285 depths in 3 dB increments to determine each animal’s perceptual threshold. Since SM detection
286 has not been assessed previously in gerbils, we first sought to validate two features of this
287 psychometric task. SM is described by a density (cycles/octave) which specifies the peak-to-
288 trough distance of the logarithmic sinusoidal frequency filter used. Humans achieve their best
289 thresholds in the range of 2-4 cycles/octave and thresholds increase at 10 cycles/octave (Eddins
290 and Bero, 2007). We trained gerbils on the same aversive conditioning paradigm used for AM,
291 but with the change cue being a SM stimulus at either 2 or 10 cycles/octave. We found that best
292 thresholds achieved during 10 days of psychometric testing were significantly lower at 2
293 cycles/octave (5.4 ± 1.43 dB, $n = 14$) than 10 (8.3 ± 2.5 dB; $p = 0.363$, $t = -2.148$, $df = 11$, unpaired
294 t-test, $n = 8$; [Figure S1](#)). Therefore, all subsequent SM detection psychometric tests used 2
295 cycles/octave. To confirm that gerbil SM detection is robust to changes in level, as reported for
296 humans (Eddins and Bero, 2007), we alternated between 45 dB SPL and 36 dB SPL during 4
297 additional days of testing with 2 cycles/octave stimuli ($n = 8$). There was no significant difference
298 in thresholds over each pair of testing days (45 dB days: 9.2 ± 4.0 dB, 36 dB days: 10.66 ± 5.03
299 dB, $p = 0.562$, $t = 0.593$, $df = 14$, paired t-test). This indicates that gerbil perception of SM stimuli
300 is similar to that displayed by humans.

301 ***Gabbr1b* expression restores SM detection following developmental hearing loss**

302 We next tested the effect of HL on SM depth detection, and the ability of GABA receptor
303 expression to rescue a HL-induced perceptual deficit. [Figure 4A](#) presents representative
304 psychometric functions for two individual animals and shows that SM detection was superior for

305 the HL-reared gerbil that received bilateral AC injections of a *Gabbr1b*-expressing vector
306 (HL+*Gabbr1b*; blue line) as compared to the HL-reared gerbil that received AC bilateral injections
307 of a *GFP*-expressing vector (HL+*GFP*; green line). As schematized in [Figure 2A](#), bottom, we
308 obtained thresholds for animals in these two groups, as well as HL-reared animals that received
309 a *Gabra1*-expressing vector (HL+*Gabra1*), and normal hearing (NH) animals (HL+*GFP*, n = 8;
310 HL+*Gabra1*, n = 7; HL+*Gabbr1b*, n = 6; NH+*GFP*, n = 7). As observed previously with procedural
311 training on the AM detection task, all 4 groups reached criterion in a similar number of trials and
312 reached similar maximum d' on the SM detection task ([Figure S2](#)).

313 [Figure 4B](#) shows group thresholds over all 7 days of training. A linear mixed effects model shows
314 that viral treatment alone is not a significant factor ($F = 0.634$, $p = 0.594$) but the interaction
315 between group and training day was ($F = 13.257$, $p = 7.64 \times 10^{-8}$). Therefore, expression of the
316 *Gabbr1b* subunit restores normal threshold improvement trends on the SM detection task in HL-
317 reared animals.

318 The SM detection thresholds of all four groups did not differ significantly from one another on day
319 1 of psychometric testing (HL+*GFP*: dB = 20.66 ± 2.11 ; HL+*Gabra1*: dB = 21.38 ± 2.11 ;
320 HL+*Gabbr1b*: dB = 20.83 ± 2.31 ; NH+*GFP*: dB = 20.77 ± 1.96 ; ANOVA, $p = .9951$, $F = 0.02$, $df =$
321 23 , [Figure 4C](#)). However, as shown in [Figure 4D](#), a significant effect of treatment group emerged
322 by day 7 of testing (one-way ANOVA, $p = 1.34 \times 10^{-5}$, $F = 14.5$, $df = 27$). NH+*GFP* and
323 HL+*Gabbr1b* animals both reached low thresholds of 7.8 ± 1.6 dB and 8.0 ± 1.8 dB, respectively.
324 In contrast, thresholds for HL+*GFP* (17.5 ± 1.5 dB) and HL+*Gabra1* (19.6 ± 1.6 dB) animals
325 improved very little over 7 days of testing. There was no significant difference between day 7
326 thresholds for HL+*Gabbr1b* and NH+*GFP* animals and both were significantly better than
327 HL+*GFP* ($p = 0.002$, $p = 0.0011$, respectively).

328 **Discussion**

329 Proper regulation of synaptic inhibition is integral to the development and maintenance of sensory
330 processing. Transient or permanent developmental HL that begins during an AC critical period
331 causes a long-lasting reduction of cortical synaptic inhibition that is attributable to the functional
332 loss of both ionotropic GABA_A and metabotropic GABA_B receptors (Kotak et al., 2005; Takesian
333 et al., 2012; Mowery et al., 2019). This reduction of AC inhibition correlates with impairments in
334 psychometric performance on a range of auditory tasks as well as degraded AC neuron stimulus
335 processing (Rosen et al., 2012; Buran et al., 2014; Caras and Sanes, 2015; Ihlefeld et al., 2016;
336 von Trapp et al., 2017; Yao & Sanes, 2018). To test whether there is a causal relationship between
337 AC inhibition and perceptual skills, we upregulated GABA receptor-mediated inhibition in AC
338 pyramidal neurons through viral expression of the *Gabra1* or *Gabbr1b* subunit genes in animals
339 reared with HL. Our results show that upregulating GABA_B receptor-dependent inhibition through
340 expression of the gerbil *Gabbr1b* subunit gene can rescue two different perceptual deficits, AM
341 and SM detection. In contrast, upregulating GABA_A receptor-mediated inhibition through
342 expression of the gerbil *Gabra1* subunit gene had no effect on perceptual performance. Therefore,
343 our results suggest that the magnitude of AC inhibition is positively correlated with perceptual
344 performance, with postsynaptic GABA_B receptors playing a pivotal role.

345 Amplitude and spectral modulation are discrete features of natural sounds, including speech, and
346 sensitivity to these cues is correlated with speech comprehension (Cazals et al., 1994; Shannon
347 et al., 1995; Singh and Theunissen, 2003; Elliott 2009; Nittrouer et al., 2021). Here we developed
348 a behavioral paradigm to assess SM detection in gerbils, such that the effects of HL could be
349 compared with a previously characterized percept, AM detection (Rosen et al., 2012; Caras &
350 Sanes, 2015). We found that rearing conditions and viral treatment had no effect on procedural
351 training times, in agreement with past results with our AM paradigm, although training times were
352 comparatively longer (Figure 2S). Adult humans display depth detection thresholds of ~2 dB at 2
353 cycles/octave, with poorer performance at higher spectral densities (Eddins and Bero, 2007). In
354 agreement, we found that gerbils have better thresholds at 2 cycles/octave than 10 cycles/octave
355 and that best thresholds are within 3 dB of human performance at 2 cycles/octave (Figure 1S).
356 We also analyzed a concatenated series of hundreds of gerbil vocalizations and found that
357 spectral modulation drops off significantly above 2 cycles/octave (not shown). This validates our
358 use of a new perceptual test with which to assess the impact of hearing loss and the capacity of
359 treatments to restore perception.

360 **Interpreting the pattern of restored AM and SM detection following *Gabbr1b* expression**

361 *Gabbr1b* expression rescued AM and SM detection in HL-reared animals, but with different
362 magnitudes and time courses. For the AM detection task *Gabbr1b*-treatment improved perceptual

363 performance in HL-reared animals from the first day of testing as compared to GFP-treated
364 controls (Figure 3). This improved performance was maintained during the 7 days of testing. This
365 outcome is consistent with a rapid improvement in AM stimulus encoding following *Gabbr1b*
366 expression, but no effect on perceptual learning (i.e., an improvement in detection threshold as a
367 result of practice). In contrast, *Gabbr1b* expression led to a gradual improvement of SM thresholds
368 during the 7 days of testing, identical to NH animals with GFP expression (Figure 4). In principle,
369 these differences in the effect of restoring inhibition could relate to differences in the way that AM
370 and SM stimuli are represented in the AC.

371 Amplitude and spectrally modulated noise are expected to differ in terms of the evoked discharge
372 pattern of AC neurons. AM stimuli are known to produce a strong temporal response that
373 correlates with the AM rate. In contrast, SM stimuli produce a response that is dependent on
374 frequency tuning (e.g., inhibitory sidebands) (Calhoun & Schreiner, 1994, 1998; Atencio &
375 Schreiner, 2010). At the cellular level, one possibility is that feedforward inhibition mediated by
376 Parvalbumin-expressing interneurons tightens the timing of the auditory evoked response in the
377 input Layer 4/5 (Wehr and Zador, 2003; Nocon et al., 2022), which may contribute to perception
378 of amplitude modulation. In contrast, SM stimuli are stationary. Here, intracortical pathways may
379 recruit local interneurons that mediate lateral inhibition, increasing gain in Layer 2/3, thereby
380 improving the detection of energy differences across spectral bands (Kaur et al., 2004; Kaur et
381 al., 2005; Li et. al. 2014).

382 **Relationship of GABA receptor manipulation to the AC critical period**

383 In the current experiments, the manipulations and behavioral assays all occur prior to sexual
384 maturation, a time during which inhibitory functional properties continue to mature (Pinto et al.,
385 2010; Takesian et al., 2012). A large body of research from the developing visual pathway shows
386 that inhibitory synapse development regulates cortical plasticity. Monocular deprivation (MD)
387 leads to reduced cortical activation by the deprived eye during a developmental CP, and
388 experimentally increasing GABAergic transmission can close the critical period prematurely
389 (reviews: Hensch, 2004; Hensch, 2005; Hooks and Chen, 2007). One implication of these
390 observations is that inhibition in adult animals is too strong to permit plasticity. In fact,
391 manipulations that reduce cortical inhibition in adults can induce excitatory synaptic plasticity (He
392 et al., 2006; Sale et al., 2007; Fernandez et al., 2007; Harauzov et al., 2010). Therefore, when
393 inhibitory strength is high, behavioral deficits can be ameliorated by temporarily lowering it. In
394 contrast, our results suggest that when inhibitory strength is low, as occurs after developmental
395 HL, behavioral deficits can be ameliorated by permanently raising it.

396 The gerbil AC displays a well characterized critical period (CP) for the effect of HL that closes at
397 P18 (Mowery et al., 2015). When HL is initiated after P18, there is no reduction to AC inhibitory

398 synapse strength (Mowery et al., 2016). In contrast, when HL is initiated before P18, the reduction
399 of AC inhibitory synapse strength persists to adulthood and can be attributed to the functional loss
400 of both GABA_A and GABA_B receptor-mediated IPSPs (Mowery et al., 2019). Therefore, a core
401 premise of this study is that the loss of one or both forms of postsynaptic inhibition is causally
402 related to perceptual deficits that attend developmental HL. Since the virus was injected into AC
403 on P23, our results suggest that the manipulation need not occur during the cortical CP in order
404 to restore normal neural and behavioral function. This is consistent with our finding that systemic
405 treatment with a GABA reuptake inhibitor (SGRI) from P23-35 also rescues AC inhibition and AM
406 detection thresholds (see Fig 2e in Mowery et al., 2019).

407 Since a reduction of GABA_A receptor mediated inhibition has been implicated in many
408 developmental disorders, it was reasonable to predict that upregulating inhibitory strength through
409 *Gabra1* expression (Figure 1D) would rescue HL-induced deficits on auditory tasks. However, we
410 previously reported that systemic treatment with a GABA_A α 1 receptor agonist, zolpidem, does
411 not restore AM detection thresholds following development HL (see Fig 2f in Mowery et al., 2019).
412 Two lines of evidence may explain why perceptual performance was rescued only by upregulating
413 GABA_B receptor-mediated inhibition. First, GABA_B receptor function may have a direct impact on
414 synaptic plasticity, particularly during development. Postsynaptic GABA_B receptors can induce
415 inhibitory long-term depression (iLTD) at feedforward inhibitory synapses between Parvalbumin-
416 expressing interneurons and Pyramidal neurons in input layers of visual cortex during a
417 developmental CP (Wang and Maffei, 2014). This mechanism has been implicated in auditory
418 map remodeling (Vickers et al., 2018), and GABA_B receptor agonists enhance ocular dominance
419 plasticity (Cai et al., 2017). Second, postsynaptic GABA_B receptors are located extrasynaptically
420 and modulate both the activity of postsynaptic GABA_A receptors and NMDA receptor-driven LTP
421 (Komatsu, 1996; Fritschy et al., 1999; Charara et al., 2005; Booker et al., 2013; Tao et al., 2013,
422 Connelly et al. 2013). GABA_B receptor activation may also induce BDNF release, thereby inducing
423 the addition of perisomatic GABAergic synapses (Fiorentino et al., 2009). Therefore, although
424 both forms of GABAergic inhibition are reduced by HL, dysregulation of GABA_B receptor's
425 modulatory role may have a substantial impact on the acquisition of perceptual skills during
426 development.

427 **Limitations to data interpretations**

428 Expression of AAV vectors begins within one week of infusion, and typically ramps up to a plateau
429 at 2-3 weeks (Reimnsider et al. 2007; Kaplitt et al. 1994; Chamberlin et al. 1998). Therefore, we
430 timed our experiment such that psychometric testing fell within a 2-3 week post-injection window
431 during which time expression should have been maximal. However, it is possible that a continued
432 increase of expression during training was associated with a greater influence on behavioral

433 performance at later testing days. A second consideration is that this study used two specific
434 inhibitory receptors, but the approach did not restrict expression to synapses from any particular
435 type of inhibitory interneuron. Parvalbumin or Somatostatin-expressing interneurons at the
436 thalamorecipient layer or in L2/3, which naturally target GABA_B receptors each synapse onto
437 pyramidal neurons that expressed GABBR1B protein following viral infection (Manz et al. 2019).
438 Additionally, L2/3 Pyramidal neurons extend dendrites to L1 within which Neurogliaform
439 interneurons form synapses that primarily evoke GABA_B receptor-mediated responses and
440 regulate plasticity (Tamás et al., 2003). Therefore, specific interneuronal connections responsible
441 for the reported behavioral effects is unknown. Finally, it is possible that increasing inhibitory
442 transmission could have induced homeostatic upregulation of excitation in AC and a maintenance
443 of excitatory/inhibitory balance (Turrigiano & Nelson, 2004; Le Roux *et al.*, 2006). This potential
444 effect could include a normalization of excitatory cellular properties and may have contributed to
445 the behavioral benefits of our manipulation. In fact, GABA_A receptors have been shown to regulate
446 homeostatic plasticity and this may explain why expressing GABRA1 did not improve
447 performance (Wen et al. 2022; Le Roux et al. 2008; Rannals & Kapur, 2011).

448 **Conclusion**

449 Here we have shown that restoring inhibition in AC alone was sufficient to restore auditory
450 perception after a developmental insult. Restoring cortical synaptic inhibition may be relevant to
451 a range of developmental disorders. For example, GABA levels in visual cortex are reduced in
452 amblyopia and this is correlated with weaker perceptual suppression by the amblyopic eye
453 (Mukerji et al., 2022). By directly comparing the impact of restoring *Gabra1* and *Gabbr1b* protein
454 expression we have shown that this effect was only achieved by upregulating GABA_B receptor-
455 mediated inhibition. This result is surprising given a long focus on GABA_A receptor-mediated
456 inhibition in the field of developmental sensory processing (Fagiolini et al. 2004; Chang et al.,
457 2005). Our findings suggest that regulating GABA_B receptor mediated inhibition remains a
458 plausible target for therapies that seek to prevent or reverse behavioral deficits that attend
459 developmental disorders.

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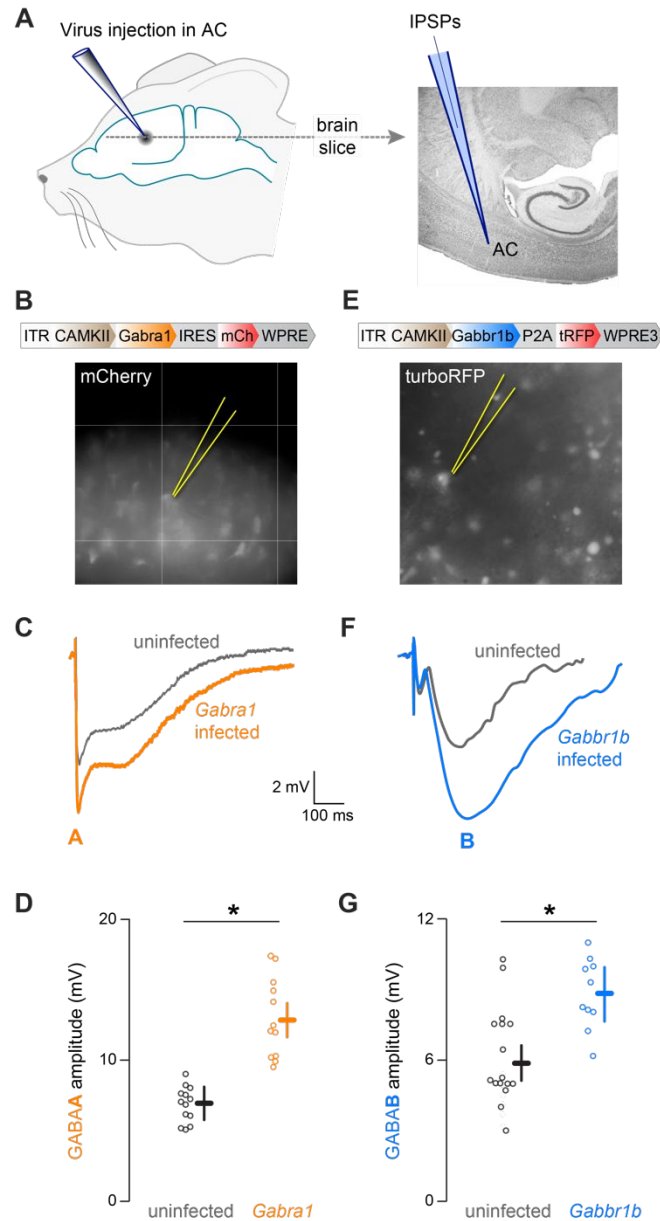
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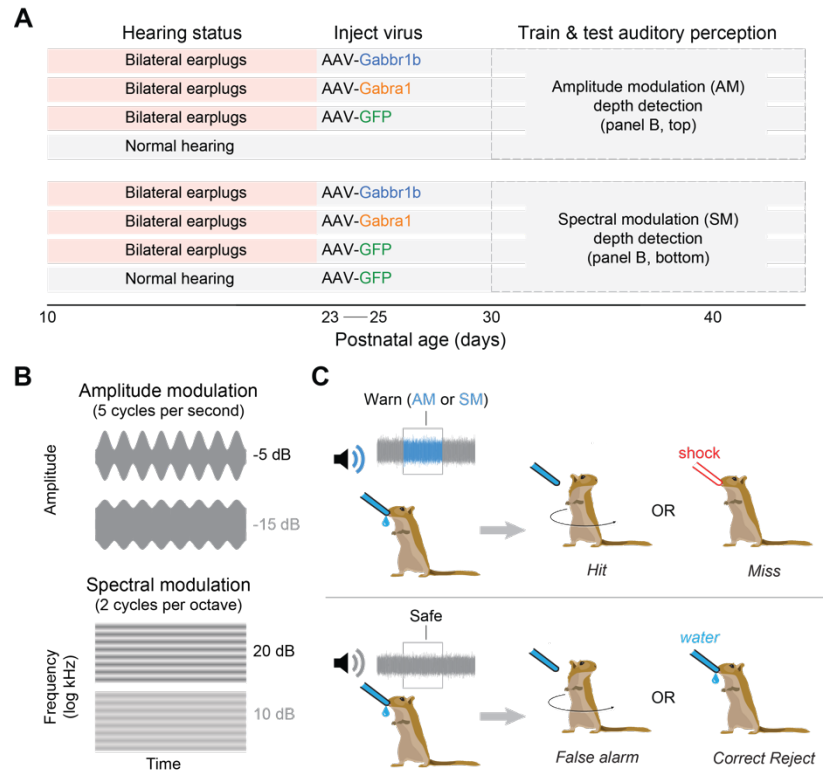
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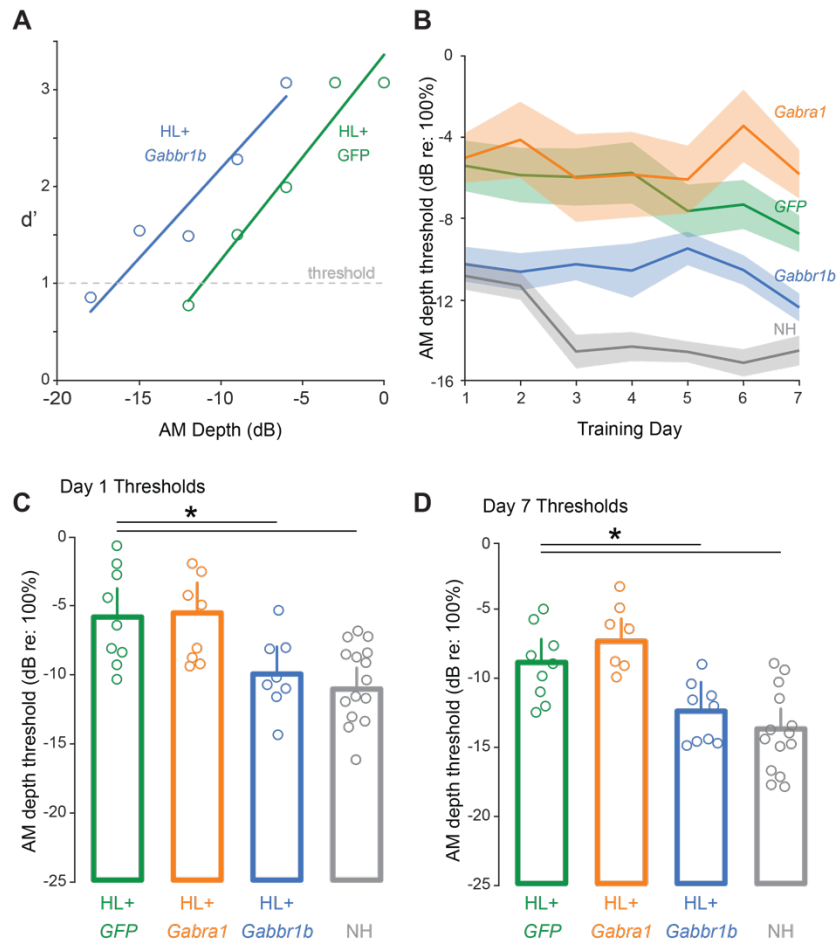
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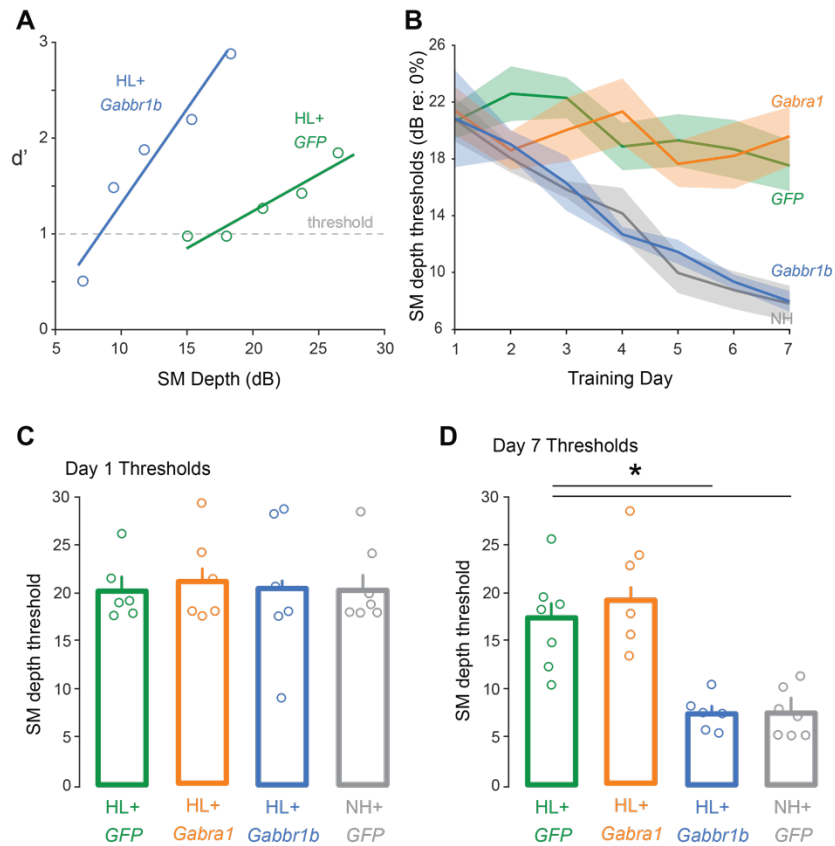
711 **Figure 1: Viral vector design and validation.** (A) For both Gabra1 and Gabbr1b AAVs, primary
 712 auditory cortex layer 2/3 was injected (Nanoject 2; Drummond) with approximately 250 nl of virus.
 713 After three weeks of expression a thalamocortical slice preparation was made and whole cell
 714 recordings (current clamp) from ACx L2/3 pyramidal cells were carried out. (B) Top, Diagram
 715 showing Gabra1 vector. Bottom, micrograph from ACx L2/3 showing Gabra1 infected cells
 716 (fluorescing, mCh) and one patched pyramidal neuron. (C) Representative evoked IPSP showing
 717 the larger GABA_A potential in the Gabra1 infected pyramidal neuron (fluorescing patched cell
 718 from B) vs local uninfected (non-fluorescing) pyramidal neuron from the same slice. (D) Plot
 719 diagram showing the average difference in GABA_A IPSP amplitudes for uninfected versus Gabra1
 720 infected pyramidal neurons. (E) Top, Diagram showing Gabbr1b vector. Bottom, micrograph from
 721 ACx L2/3 showing Gabbr1b infected cells (fluorescent, mCh) and one patched pyramidal neuron.
 722 (F) Representative evoked IPSP showing the larger GABA_B potential in the Gabbr1b infected
 723 pyramidal neuron (fluorescing patched cell from C) vs local uninfected (non-fluorescing) pyramidal
 724 neuron from the same slice. (G) Plot diagram showing the average difference in GABA_B IPSP
 725 amplitudes for uninfected versus Gabbr1b infected pyramidal neurons.



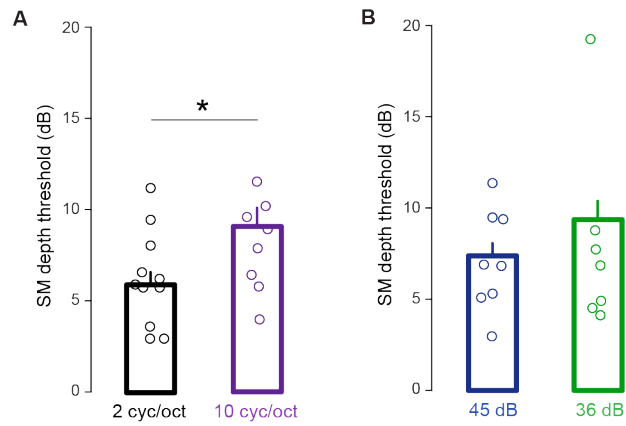
726 **Figure 2: Experimental paradigm.** (A) The experimental timeline, and each of the experimental
727 groups is shown. (B) Example stimulus waveforms are shown for the AM depth detection task
728 (top) and the SM depth detection task (bottom). (C) The Go-Nogo paradigm used for psychometric
729 testing is shown.
730



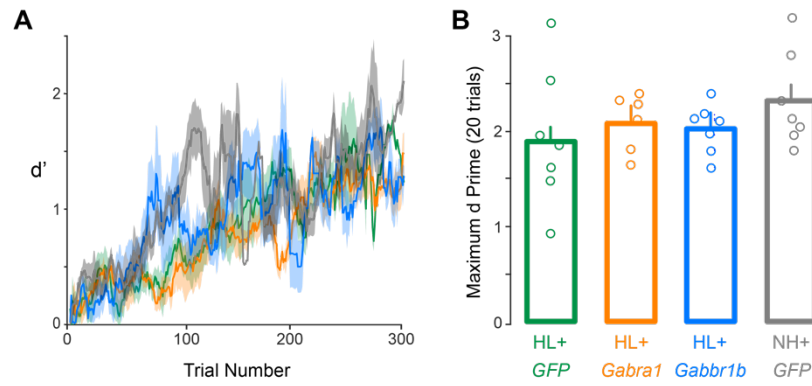
731 **Figure 3: Gabbr1b expression restores AM detection.** (A) Representative behavior for a HL-
 732 reared gerbil expressing *Gabbr1b* (HL+*Gabbr1b*) and a HL-reared gerbil expressing *GFP*
 733 (HL+*GFP*) in AC, both tested after transient hearing loss (HL). (B) AM depth thresholds achieved
 734 by each group over training days. Mean \pm SEM. (C) *Gabbr1b* expression in AC rescued AM
 735 perception relative to *GFP* expression on day 1 of psychometric testing. Bars indicate significant
 736 differences (see text for statistical values). (D) *Gabbr1b* expression in AC rescued AM perception
 737 relative to *GFP* expression on day 7 of psychometric testing. Bars indicate significant
 738 differences (see text for statistical values).



739 **Figure 4: Gabbr1b expression restores SM detection.** (A) Example psychometric curves of
 740 individual gerbils showing d' at each of the 5 modulation depths presented in a single session.
 741 The leftward shift of the HL+*Gabbr1b* function, relative to HL+*GFP* function indicates improved
 742 performance. Bars indicate significant differences (see text for statistical values). (B) Group
 743 performance on each day of psychometric testing. (C) There are no differences in SM modulation
 744 thresholds on the first day of psychometric testing, as calculated by fit crossing $d' = 1$. (D) SM
 745 thresholds on day 7 of psychometric testing. Both HL+*Gabbr1b* and NH+*GFP* groups performed
 746 significantly better than HL+*GFP* animals. Bars indicate significant differences (see text for
 747 statistical values).



748 **Supplemental Figure 1: Spectral modulation detection in normal hearing juvenile gerbils**
749 (A) NH animals display better thresholds for SM at 2 cycles/octave relative to 10. Bar indicates
750 significant difference (see text for statistical value). (B) SM detection thresholds do not change
751 significantly when sound is presented at a lower level of 36 dB SPL ($p = 0.593$).



752 **Supplemental Figure 2: Procedural training for spectral modulation detection** (A) There
753 are no group differences in the number of trials required for procedural training (mean \pm SEM,
754 moving window of 20 trials). (B) There are no significant differences in maximum d' achieved
755 during all procedural training over 20 trial windows (see text for statistical value).