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Acoustic trauma slows AMPAR-mediated EPSC decay in the auditory brainstem; reducing GluA4 subunit expression as a mechanism to rescue binaural function

Nadia Pilati[1,6], Deborah M. Linley[7], Haresh Selvaskandan[7], Osvaldo Uchitel[2], Matthias H. Hennig[3,4], Cornelia Kopp-Scheinpflug[1,5] Ian D. Forsythe[1,7]

[1]MRC Toxicology Unit, Hodgkin Bldg, University of Leicester, Leicester LE1 9HN UK. [2]Instituto de Fisiología y Biología Molecular y Neurociencias, Universidad de Buenos Aires-CONICET, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, C1428-Buenos Aires, Argentina. [3]Institute for Adaptive and Neural Computation, School of Informatics, University of Edinburgh, EH8 9AB, UK. [4]SynthSys, C. H. Waddington Building, The Kings Buildings Campus, Edinburgh, UK. [5] Department Biology II, Ludwig-Maximilians-Universität München, D-82152 Planegg-Martinsried, München, Germany. [6]Autifony Srl laboratories, Medicines Research Centre, 37135 Verona, Italy. [7] Department of Neuroscience, Psychology & Behaviour, University of Leicester, Leicester. LE1 9HN UK.

Corresponding author: Ian D. Forsythe, Department of Neuroscience, Psychology & Behaviour
Medical Sciences Building, University of Leicester, LE1 9HN, UK
Telephone: +44 (0) 116 252 5580
Fax: +44 (0) 116 252 5616
E-mail: idf@le.ac.uk

Short title: Acoustic trauma slows EPSCs in the LSO

Pages: 41 pages

Figures and Tables: 8 Figures, 2 Tables

Abstract: 249 words

Key points

1. LSO principal neurons receive AMPAR & NMDAR-mediated EPSCs and glycinergic IPSCs.
2. EPSCs and IPSCs have slow kinetics in prehearing animals, which on maturation accelerate to submillisecond time-course. This correlates with glutamate and glycine receptor subunit mRNA.
3. The NMDAR EPSC also accelerates with maturation to decay time-constants of 2.5ms. This is the fastest NMDAR-mediated EPSC reported.
5. Loud sounds (Acoustic Trauma, AT) slow AMPAR EPSC decay, increasing GluA1 and decreasing GluA4 mRNA.
6. Modelling of Interaural Intensity Difference (IID) suggests that the increased EPSC duration after AT shifts IID to the right and compensates for hearing loss.
7. Two months after AT the EPSC decay times had recovered to control values.
8. Synaptic transmission in the LSO matures by P20, with EPSCs and IPSCs having fast kinetics. AT changes the expression of AMPAR subunits, so that synaptic receptors are slower and changes synaptic integration.

Abstract:

We consider how damaging levels of sound (acoustic trauma, AT) might perturb homeostatic regulation of receptors at synapses in the auditory pathway. Developmental maturation of synaptic function and hearing was characterized in the mouse lateral superior olive (LSO) from P7 to P96 using voltage-clamp and measurement of auditory brainstem responses (ABR). IPSCs and EPSCs undergo rapid acceleration during development, converging to similar sub-millisecond decay kinetics (0.87±0.11ms, 0.77±0.08ms, respectively) in adult mice. This was correlated with mRNA levels for glycinergic and glutamatergic ionotropic receptor subunits, confirming a switch from Glyα2 to Glyα1 for IPSCs and increased expression of GluA3 and GluA4 subunits on maturation. NMDAR EPSC decay time-constants (τ) accelerated from >40ms in prehearing animals, to 2.6±0.4ms in adults, as GluN2C expression increased. AT administered *in vivo* at around P20 disrupted the IPSC and EPSC equilibrium, so that one week later the AMPAR EPSC decay was slowed and mRNA for GluA1 increased while GluA4 decreased. In contrast, GlyR IPSC and NMDAR EPSC decay times were unchanged by AT. Computational modelling confirmed that matched IPSC and EPSC kinetics are required to generate mature interaural level difference (IID) functions. After AT the longer-lasting EPSCs provide a compensatory boost that maintained binaural function with raised auditory thresholds. We conclude that excitatory/inhibitory synaptic drive matures to fast submillisecond kinetics in the LSO. AT disrupts this through a mechanism that favours AMPARs with slow kinetics. We conclude that loud sounds reversibly modify auditory processing in the brain, changing receptor expression for several weeks after exposure.

Introduction

Synapses undergo multiple forms of activity-dependent refinement during development, including synaptic scaling, competition/elimination and adaptation of synaptic current time-course through changes in the subunit composition of channels at the synapse. It is well established that exposure to damaging volumes of sound (acoustic trauma, AT) raises auditory thresholds, injures cochlea hair cells and afferent synapses, and causes hearing loss. The extent to which AT damages or changes aspects of the central auditory pathway is often difficult to assess since effects in the cochlea inevitably propagate into the brain. The objective of this study was to determine the extent to which AT might induce changes at central excitatory and inhibitory synapses of the superior olivary complex (SOC) and to ask whether this affects the interaural level computation of sound localisation.

Neurons in the lateral superior olive (LSO) of the SOC are amongst the first to receive inputs from both ears. Excitatory (glutamatergic) inputs from the ipsilateral cochlear nucleus are integrated with inputs from the contralateral cochlear nucleus via an inhibitory (glycinergic) projection from the medial nucleus of the trapezoid body (MNTB) (Goldberg and Brown, 1968, Glendenning et al., 1985, Tsuchitani, 1988, Wu and Kelly, 1995, Kopp-Scheinpflug et al., 2011). Neuronal, intrinsic properties (Barnes-Davies et al., 2004) and synaptic inputs are tonotopically organized (Rietzel and Friauf, 1998) so that contralateral projections correspond with ipsilateral projections. Hence LSO principal neurons integrate excitatory and inhibitory synaptic responses to the same sound frequencies from opposite ears, and compute interaural intensity differences (IID) to localize sound across the azimuth (Tollin and Yin, 2002, Tollin, 2003).

Both excitatory and inhibitory inputs to the LSO undergo synaptic reorganization before hearing onset at P11/12 (Kandler et al., 2009). Notably, chloride gradients evolve, such that a mixed glycine- and GABA-mediated synaptic response shifts from depolarizing to hyperpolarizing (Kim

and Kandler, 2003, Gillespie et al., 2005, Kim and Kandler, 2010). After hearing onset IPSCs are further refined (Sanes and Friauf, 2000, Kandler and Gillespie, 2005, Kandler et al., 2009): there is a tonotopic reorganization (Kandler et al., 2009) driven by spontaneous activity (Clause et al., 2014), a reduction in the number of terminal arbors (Sanes and Siverls, 1991, Sanes and Takacs, 1993) and an acceleration of IPSC time-courses (Kandler and Friauf, 1995; Walcher et al., 2011). This is accompanied by a shift in neurotransmission of the IPSC from mixed GABA/glycine to predominantly glycinergic receptors by P14 (Nabekura et al., 2004; Sterenberg et al., 2010).

Information transmission along the auditory nerve is compromised and centrally compensated following sound-induced trauma (Kujawa & Liberman, 2009) but we postulate that other activity-dependent modulatory changes in the CNS can further influence auditory processing; for example, by changes in the subunit composition of the synaptic receptors. Such changes would change channel open time, and so be reflected in the decay kinetics of synaptic currents (Magleby and Stevens, 1972, Raman et al., 1994, Koike-Tani et al., 2005) and thereby modifying synaptic integration and neuronal output (Johnston et al., 2009). Thus by examining the kinetics of synaptic currents (rather than merely their amplitude) and comparing this to subunit expression, we can gain insights into activity-dependent mechanisms affecting auditory processing.

Here we characterize the development of both components of the IID computation for the LSO; measuring excitatory (E) and inhibitory (I) synaptic currents across an age-range that spans postnatal maturation in four age-groups (Fig 1a): pre-hearing, hearing onset, young adult and adult mice. Then having determined the developmental time-course of the synaptic currents, we demonstrate that over-excitation in the form of acoustic trauma, changes the expression of receptor subunit mRNA, modifies receptor composition and so adapts the synaptic kinetics. The results show that AT causes activity-dependent changes in synaptic AMPAR subunits, slowing fast excitatory synaptic currents with a time-course of weeks and modifying IID processing in the LSO.

Methods: We have employed Whole-cell patch clamp methods to characterize synaptic currents and quantitative PCR to examine subunit expression over the developmental time-course. Then we have used *in vivo* induction of sound trauma and confirmation of hearing deficits by Auditory Brainstem Responses (ABR), combined with *in vitro* electrophysiological assessment of central synaptic function in the LSO. The impact of these changes on IID was assessed using computational modelling.

Preparation of brain slices. Male and female *CBA/Ca* mice (aged 6-96 days old) were killed by decapitation (using methods approved by the University of Leicester Ethical Committee and the Animals, Scientific Procedures, Act 1986, UK) and brainstem slices containing the superior olivary complex (SOC) were prepared as described previously (Barnes-Davies and Forsythe, 1995, Johnston et al., 2008). Coronal slices (150-200 µm thickness) of SOC containing the LSO were cut in a low-sodium artificial CSF (aCSF) at 0°C. Slices were then incubated in normal aCSF at 37°C for 1 hr and subsequently stored at room temperature. The composition of the normal aCSF was (in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 10 glucose, 1.25 NaH_2PO_4, 2 sodium pyruvate, 3 myo-inositol, 2 $CaCl_2$, 1 $MgCl_2$, 0.5 ascorbic acid. The pH was 7.4 when bubbled with 95%O_2/5%CO_2. The dissection was performed in low-sodium aCSF, 250 mM sucrose was substituted for NaCl, and $CaCl_2$ and $MgCl_2$ concentrations were adjusted to 0.1 and 4 mM, respectively.

Electrophysiology. One slice at a time was transferred to a temperature controlled experimental chamber (Campden Instruments, UK Model 7800) mounted on the stage of an upright microscope. The chamber was perfused at 1ml/min with normal aCSF at 36°C (gassed with 5%CO_2/95%O_2). LSO neurons were visualized using differential interference contrast (DIC) optics (MicroInstruments, UK) and a 40X water-immersion objective (Zeiss). Whole-cell patch-clamp recordings were made from LSO principal neurons using an Axopatch 200B amplifier, Digidata

1440 and pCLAMP10 software (Molecular Devices, Wokingham). Data were sampled at 20-50 kHz and filtered at 5 kHz. Patch pipettes were made using thin-walled borosilicate glass (GC150F7.5, Harvard Apparatus) and a two-stage vertical puller (Narishige, PC-10) and filled with (in mM): Kgluconate 97.5; KCl 32.5; EGTA 5.0; HEPES 40; $MgCl_2$ 1; Na_2phosphocreatine 5 (adjusted to pH 7.2 with KOH). Voltage signals were not corrected for the liquid junction potential (-11 mV). For NMDAR-EPSC recordings, CsCl was substituted for KCl and K Gluconate. Whole-cell capacitance and series resistances were noted from the amplifier and using compensation of 70%. A bipolar platinum electrode was positioned across the MNTB or over the ipsilateral fibres from the cochlear nucleus (Fig 2a) and connected to a voltage stimulator (DS2A, Digitimer Ltd, UK). Stimuli (200-μs duration) were delivered at 0.25 Hz with an amplitude to give maximal postsynaptic responses. Glycinergic IPSCs were recorded in the presence of 10μM bicuculline, 10μM CNQX or NBQX and 50μM D-AP5, whereas AMPA-receptor mediated EPSCs were recorded in the presence of 10μM bicuculline, 0.5-1μM strychnine, and 50μM D-AP5. NMDAR-mediated EPSCs were recorded in the absence of D-AP5 and pharmacologically isolated by perfusion with 10μM bicuculline, 1μM strychnine, and 10μM NBQX. mEPSC and mIPSC were recorded in presence of tetrodotoxin (TTX, 0.5μM) and the respective blockers for inhibitory or excitatory synaptic transmission (as detailed above). All chemicals and drugs were obtained from Sigma (Gillingham, UK), except bicuculline and 2-amino-5-phosphonopentanoic acid (D-AP5), which were from Tocris (Bristol, UK). 'Values of 'n' refer to the number of neurones from which recording were made and sample groups comprised data collected from at least 3 animals. IPSC and EPSC decay times and amplitudes were measured from averaged traces (10-15 records). Miniature IPSC (mIPSC) and mEPSC decay times were measured from averaged traces (20 records). *Recording of auditory brainstem responses (ABR).* CBA/Ca mice (P7-96) were anaesthetized intraperitoneally with a combination of fentanyl (0.15mg/kg), fluanisone (5mg/kg) and hypnovel (2.5 mg/kg). ABRs were evoked by tone pips (at 8/12/16/24/30 kHz, 1-ms rise and fall times, 5-ms duration) or clicks

(broadband between 2-20 kHz, 100 μs), which were produced by a Thurlby Thandar arbitrary waveform generator (TGA 1230, 300 MHz, Tucker Davis, US) and applied at 10 Hz in free field unilaterally using a B&K microphone (B&K 4192). The final ABR response constituted an average of 100-400 individual traces recorded by intradermal electrodes (positive, negative, and ground electrodes were inserted subcutaneously at the vertex, mastoid, and back, respectively) with an input gain of 20μV/div connected to an amplifier (Medelc Sapphire 2A, Oxford Instruments) and sampled at 16 kHz. Hearing thresholds were determined by attenuating the initial stimulus intensity (clipped at 94 dB SPL) by 10 or 3 dB SPL steps (Tucker Davis Technology, USA) until ABR waves I and II could no longer be defined. Each wave represents activity at higher levels of the auditory brainstem, so that Wave IV is thought to represent activity in the LSO. The ABR waveform for mice was defined with respect to the Jackson Lab. phenotyping protocol: (http://phenome.jax.org/db/q?rtn=projects/docstatic&doc=Jaxpheno8/Jaxpheno8_Protocol).   The sample size (n) indicates the number of animals used.

Acoustic trauma. Male or female *CBA/Ca* mice (P17-23) were anesthetized as above and placed for 2h in a custom-made sound-insulated box containing a loudspeaker (Prosound WF09K, freq range 4-40 kHz that delivered a broadband noise (0-30 kHz) at 110 dB (SPL). Animals were exposed once bilaterally as young adults aged P17-P22; the loudspeaker was located above the head at a distance of around 4cm.

RNA extraction and quantitative reverse transcriptase PCR analysis. For developmental studies, male and female CBA/Ca mice were used within the same age-groups as specified in Fig1a. For studies following auditory trauma, male and female CBA/Ca mice aged P23-29 were used (noise exposure occurred 7 days previously). After killing (as above) the brainstem was dissected into ice-cold phosphate-buffered saline (PBS), transferred to OCT medium and frozen using dry ice

and hexane. Transverse cryostat sections (20 μm) were taken from the unfixed tissue and mounted onto Zeiss PEN-Membrane covered slides. Sections were fixed with ethanol and stained with cresyl violet. The LSO were dissected from each section using a Zeiss PALM MicroBeam laser dissection microscope and the RNA was extracted. 10 sections were used from each animal. Total RNA was isolated using QIAGEN RNeasy protocol (Applied Biosystems, Foster City, CA, USA). cDNA synthesis was performed using Superscript III (Invitrogen). PCR primers were designed (Table 1) using the Roche Applied Sciences Primer Design tool (http://www.roche-applied-science.com) and purchased from Invitrogen. Primer sequences (Invitrogen) were optimised with γ-Actin or Rpl-44 as a house keeping genes.

Table 1: PCR Primers

Subunit	*FORWARD PRIMER*	*REVERSE* pRIMER
GluR1	*CCAATTTCCCCAACAATATCC*	*AAAGCTGTCGCTGATGTTCA*
GluR2	*CAGTTTCGCAGTCACCAATG*	*ACCCAAAAATCGCATAGACG*
GluR3	*CCACTTGGATTCCTCCAATAGT*	*GCATACACCCCTCTGGAGAA*
GluR4	*CTGCCAACAGTTTTGCTGTG*	*AAATGGCAAACACCCCTCTA*
GlyR1	*CGATTCTACCTTTGGGAGACC*	*TTCAGCCTCTTTGGAAGCA*
GlyR2	*GACTACACAGAGTTCAGGTTCCAG*	*TCCAGATGTCAATTGCTTTCA*
GlyR3	*GGGAAGCCGCACTGTTACT*	*GAGATCGCGCACTGTTTGT*
GlyR4	*CCGTCACTGAGACCACCAT*	*GCCAGACGTGGGTCATTC*
G-Actin	*CCCTAGCACCTAGCACGATGA*	*GCCACCGATCCAACTGAGTAC*

Primers were verified by a BLAST sequence and gel electrophoresis. qRT-PCR was performed using SYBR Green PCR Master Mix in the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The thermal-cycler protocol was: stage 1, $50°C$ for 2min; stage 2, $95°C$ for 10 min; and stage 3, 40 cycles at $95°C$ for 15 s and $60°C$ for 1 min. Each sample was run in triplicate. Quantification was performed using the Pfaffl Method (Pfaffl, 2001). Data are presented as means ± SEM. ('N' constituted data from one animal).

Computational Modelling: The LSO neuron model used here is based on a previous implementation by Karcz et al. (2011), with parameters set to match the new *in vitro* data obtained in this study at different ages or following acoustic trauma (Table 2). The neuron was implemented as a single-compartment, leaky integrate and fire unit with conductance-based synapses:

$$\tau_m \dot{V}(t) = \sum_i g_i(t)V(t) + \sum_c g_c(t)(V(t) - E_c) - (V(t) - V_r),$$

The membrane time-constant was (τ_m) = 1.5 ms (Wu and Kelly, 1991, Sanes and Takacs, 1993), and the resting potential (V_r) = -60 mV. The neuron received four ipsilateral excitatory and four contralateral inhibitory inputs, with total conductances g_i (t) and g_c (t) and associated reversal potentials E_i = 0 mV and E_c = -75 mV (Balakrishnan et al., 2003). Synaptic conductances are expressed in units of the leak, and are therefore dimensionless, and were modelled as:

$$\tau_{\{i,c\}} \dot{g}_{\{i,c\}}(t) = g_{\{i,c\}}^m \delta(t - t_s) - g_{\{i,c\}}(t),$$

with spike times $\{t_s\}$, time-constants τ_i and τ_c, and peak conductances g_i^m and g_c^m for the ipsi- and contralateral inputs, respectively, which were set to match experimental data in this study (Table 2). The neuron produces a spike when the membrane potential exceeds the threshold V_{th}, after which it is reset to V_{reset} = −75 mV.

Input spike trains were generated, as described by Karcz et al. (2011), to reproduce the level of precision previously reported for sound-evoked responses in VCN and MNTB neurons of mice (Kopp-Scheinpflug et al., 2003). Noise was introduced by shifting each spike, and all successive

spikes, by a random amount drawn from an exponential distribution with mean and standard deviation σ_i = 0.15ms and σ_c = 0.4ms. The mean latency of the contralateral inputs was set to Δ_c = 1.4ms (Karcz et al., 2011). Sound-level dependent latency differences in MNTB neurons were included by increasing the ipsilateral latency by:

$$\delta_i = -(R_c/R_i - 1) \cdot 1.25\text{ms},$$

where R_i and R_c are the ipsi- and contralateral firing rates, respectively (FitzGerald et al., 2001, Kopp-Scheinpflug et al., 2003, Karcz et al., 2011). Tuning curves were simulated by fixing the spike rate at the ipsilateral input, while systematically changing the rate of the contralateral input from 0 to twice that of the ipsilateral input. Note that these tuning curves do not necessarily represent true IID tuning curves since the precise relationship between SPL and VCN response was not taken into account here. In contrast to Karcz et al. (2011), the optimal tuning curve was estimated for each condition, by setting the synaptic parameters to match the *in vitro* data in this study, and adjusting the spike threshold such that the Fisher information estimated from the simulated IID tuning curve was maximized. Fisher information was computed for each IID level *x* as:

$$F(x) = r'(x)^2/s(x)^2$$

where *r'(x)* is the mean response, differentiated with respect to IID, and *s(x)* the standard deviation of the response (Dean et al., 2005). The mean response was estimated by fitting a sigmoidal function to the simulated IID tuning curve, and noise by fitting a Gaussian function to the response standard deviation, both using 40 different realisations of the same IID tuning curve. Only the values of Fisher information within a range of ±25% of equal amplitudes at the ipsi- and contralateral inputs were considered for optimising the spike threshold, since a lack of response variability at the flanks of the tuning curves could lead to inflated values. Fisher information was computed for a range of physiologically plausible firing thresholds, and the threshold which gave, on average, the best performance across a range of ipsilateral input firing frequencies from 100Hz

to 300Hz was selected. This optimization was done for all ages, but not for the simulations following acoustic trauma, here we assume that at least in the short term, thresholds remained at the level of a mature animal.

Table 2 Near Here

Statistics: Values of 'n' are stated in the above protocols and data expressed as mean ± SEM for the number of neurons indicated. Statistical analyses were performed using an unpaired Student's t-test or ANOVA (one way or two way) when comparing more than two groups. For comparisons of cumulative distribution, a Kolmorogov-Smirnov (K-S) test was used. Statistical significance was concluded when $p \leq 0.05$.

Results

We first assessed the time-course of maturation of normal auditory function *in vivo* by measuring ABRs and then related this to synaptic time-course in the LSO, by using whole-cell patch recording from brainstem slices. Developmental changes in LSO mRNA for excitatory and inhibitory receptor ion channel subunits were also assessed by qRT PCR on tissue isolated by laser microdissection. Administration of AT elevated ABR thresholds in young adult mice, changed synaptic current decays and receptor-ion channel mRNA levels, while the impact of these changes was interpreted through modelling of interaural intensity difference.

Maturation of auditory function: A developmental time-course for the ABR hearing threshold was determined in CBA/Ca mice by assessing 4 age groups (Fig. 1a): prehearing (P7-9), hearing-onset (P12-15), young adult (P17-23) and adult (P29-36). Prehearing and hearing-onset mice showed higher ABR thresholds and ill-defined ABR waves (Fig. 1b) compared to the 2 older age groups, which showed normal ABR responses and thresholds (Fig. 1b). There was a minor further improvement in the ABR thresholds at mid frequencies (12kHz) on maturation from young adults to adult.

Acceleration and convergence of EPSC and IPSC kinetics with maturation. The kinetics of excitatory and inhibitory synaptic inputs to the LSO measured *in vitro* using whole-cell patch recording show a characteristic acceleration over a similar developmental time-course to that described for ABR thresholds *in vivo*. Evoked glycinergic IPSCs exhibited a developmental acceleration that stabilized after hearing-onset (at around P18, young adult), with a decay time-constant (τ) accelerating from 2.6±0.2ms (n=10, prehearing) to 0.79±0.1ms (n=7, adult; Fig. 2c, & 3a, red traces). In parallel, the maximal evoked IPSC conductance progressively declined from 25.3±5.3nS (n=8) in pre-hearing mice to 5.4±1.7nS (n=6) in the young adult mice (Fig. 3b red)

consistent with previous reports measuring refinement of inhibitory synaptic inputs to the LSO (Kim & Kandler, 2003). These synaptic parameters were then stable as mice matured in the adult group.

The decay kinetics of EPSCs decreased from 1.97±0.39ms (n=10) in pre-hearing mice to 0.65±0.09ms (n=5) in young adults; while the amplitude of the maximal evoked EPSC conductance remained stable through development from young adult to adult (Fig 3b, green). The EPSC and IPSC kinetics converged to near identical sub-millisecond values in the mature auditory system with decay time-constants of 0.65±0.09ms (n=5) and 0.79±0.11ms (n=7) respectively, for young adults and 0.77±0.08ms (n=6) and 0.87±0.11ms (n=6) respectively, for adults. This convergence is illustrated by superimposed EPSCs and IPSCs for each of the age groups used in this study (Fig. 2c) and consistent with a binaural physiological mechanism of IID detection through integration of excitatory and inhibitory responses from ipsilateral and contralateral ears, respectively.

This acceleration in the synaptic decay kinetics with development is consistent with functional changes in the subunit composition of AMPAR and/or glycine receptors during maturation. We used qRT PCR to test for changes in mRNA levels of glycine receptor (GlyR) and AMPAR subunits in LSO tissue from mice aged P9, P14, P18 and P35 (Fig. 3c, 3d). The mRNA levels of GlyR subunits (relative to the housekeeping gene) showed a dramatic increase in Glyα2 over Glyα1 at hearing onset, which stabilized or declined slightly into adulthood. This well established switch in GlyR subunit expression over development, with an early predominance of GlyR2 in pre-hearing immature animals (Fig. 2e) and a switch to GlyR1 dominance on maturation after hearing onset, has been reported previously in the spinal cord (Takahashi et al., 1992). The switch to dominance of GlyR1 mRNA preceded the acceleration in IPSC decay kinetics by around 1 week (compare Figs. 3a with 3c), suggesting that the turnover of glycine receptor channels and protein may be relatively slow. A general increase in mRNA for the AMPAR subunits was observed after hearing

onset, with the largest increase in the GluA4 subunit, which has the fastest kinetics (Fig. 3d). This was well correlated with the acceleration in the EPSC kinetics (Fig 2c, 3a).

Susceptibility to acoustic trauma increases with maturation. The above results provide a baseline from which experience-dependent plasticity can be explored in the LSO following induction of an acoustic trauma. AT was induced in anaesthetized CBA/Ca mice on exposure to 110 dB SPL broadband noise for 2h. ABRs were measured before, and one week (+/-1 day) after exposure. At hearing-onset (P12-15), when ABR thresholds were relatively high, AT did not affect ABR waveforms or thresholds across a range of sound frequencies (Fig. 4a). By contrast, when young adults (P17-23) were exposed to the same level of sound, ABR thresholds remained elevated by ~40dB, one week after the acoustic trauma (Fig. 4c). The characteristic ABR waveform was also altered, so that wave IV, reflecting activity in the SOC, was reduced in amplitude or absent (Fig. 4d). The physiological changes induced in the brain by exposure to loud sounds were investigated by comparing synaptic responses from naive and AT-exposed mice using patch-clamp recording.

Acoustic trauma selectively prolonged EPSC decays by decreasing GluA4 and increasing GluA1 expression. One week after AT, mice were culled, *in vitro* brainstem slices prepared and the effect on excitatory and inhibitory synaptic responses examined in LSO principal neurons. No significant changes were observed in the amplitude of electrically evoked AMPAR-mediated EPSCs at holding potentials of -70mV following AT (controls: 7.5±4.4nS, n=5; noise exposed: 6.4±0.8nS, n=11). Rise times for AMPAR EPSCs under control (n=11) and following AT (n=11) were also unchanged (values were 0.54±0.06ms and 0.55±0.06ms, respectively). However, EPSC decay times were significantly prolonged after AT (Fig. 5a; controls: 0.69±0.05ms, green traces, n=11; noise exposed: 1.23±0.05ms, blue traces, n=13, P<0.001).

Consistent with this observation, qRT-PCR showed two important changes in AMPAR mRNA expression: GluA1 mRNA expression increased while the GluA4 subunit expression decreased after AT (Fig. 5b). These mRNA levels and EPSC kinetics are consistent with recordings from native neurons having slow kinetics (deactivation decay time-constants around 2-3ms) when expressing a high abundance of GluA1 subunits and fast kinetics (deactivation kinetics of <1 ms) in neurons expressing a high proportion of GluA4; see table 1 of (Geiger et al., 1995, Yang et al., 2011).

Support for a postsynaptic mechanism for the change in EPSC time-course was provided by the analysis of miniature EPSCs (mEPSCs, Fig. 5c). The kinetics of the mEPSCs were also significantly prolonged after induction of AT; decay tau increased from 0.4±0.06ms (green traces, n=8 cells) in control mice to 0.9±0.1ms (blue traces, n=14) in AT-exposed mice. Independently of the above effects on EPSC kinetics, AT significantly decreased mEPSC frequency, implying a separate presynaptic reduction in transmitter release. The spontaneous frequency dropped from 4±1Hz (n=8) in control mice to 1±0.4Hz (n=16) in AT-exposed mice, without affecting amplitude (control mEPSCs 31±3pA, n=8; and 29±2pA, n=15 in AT-exposed mice, Fig. 5c).

Acoustic trauma does not change IPSC kinetics. We and others have previously shown that the role of GABA$_A$ receptors in mediating the IPSC declines with maturation of the LSO, so that there is little or no GABA$_A$ IPSC in adult mice and the IPSC is mediated by glycine receptors. Inhibitory inputs to the LSO exhibit activity-dependent synaptic refinement during development (Sanes and Friauf, 2000, Kandler and Gillespie, 2005, Kandler et al., 2009). Here we show that glycinergic IPSCs in mature animals do not exhibit activity-dependent plasticity of their subunit composition following AT. Rise times of LSO IPSCs for control and following AT were not significantly different (0.39±0.05ms, n=5; 0.36±0.04ms, n=5, respectively). Decay times and conductance magnitudes were also indistinguishable (Fig. 5d; control tau: 0.87±0.10ms, n=6;

noise exposed tau: 0.83±0.06ms, n=9; control conductance: 4.4±1.0nS, n=4 noise exposed conductance: 5.8±2.3nS, n=6). In accordance with these findings there was no change in mRNA for glycine receptor subunits (Fig. 5e) or in mIPSC kinetics or amplitude (Fig. 5f). The frequency of mIPSCs was reduced after AT from 3.6±0.4Hz (control, n=10) to 0.8±0.3Hz (n=7) as shown in fig. 5f. Hence, AT specifically alters EPSC kinetics, but has no effect on IPSC kinetics. The reduction in frequency of miniature excitatory and inhibitory synaptic responses, implies that there may be a presynaptic reduction in release probability, but this was of similar magnitude at both excitatory and inhibitory synapse and should not influence the overall excitatory/inhibitory balance. Further study of this observation will require examination of unitary evoked presynaptic inputs and is a topic for future studies. We conclude that AT induces slowing of the decay kinetics in both evoked and miniature EPSCs, consistent with a reduced dominance of fast-gating GluA4 in the subunit composition of synaptic AMPAR at the excitatory synapse.

NMDAR EPSCs accelerate with development, but their decay is unchanged by AT. It is well established that glutamatergic, excitatory synaptic responses are generally composed of a fast, AMPAR-mediated EPSC and a slower, voltage-dependent EPSC mediated by NMDAR. Similar results have been observed in the immature MNTB (Steinert et al., 2010) and as shown here in the LSO from prehearing mice (Fig. 6a). This NMDAR-mediated EPSC was blocked by 20μM D-AP5 (Fig. 6b) and decayed with a double exponential comprising a tau_{fast} of 44.8±5.4ms and a tau_{slow} of 115±17.1ms (n=8). The tau_{slow} contributed 34% of the total amplitude. The NMDAR EPSC ran down on whole cell patch recording so that peak amplitude reached half initial values in 43.4±1.8s (n=5). On maturation there was an acceleration in NMDAR EPSC decay kinetics. The voltage-dependence of the NMDAR-EPSC was maintained (Fig. 6c) and the acceleration in decay kinetics was dramatic, with time-constants of tau_{fast} of 2.6±0.4ms and tau_{slow} of 28.8±8.7ms (n=6) in animals older than P23. The tau_{slow} was small, contributing 8.7% of the combined current amplitude and was on the limits of detectability. Following acoustic trauma only tau_{fast} decay could

be reliably observed and fit by a single exponential, and was not significantly different from control (tau$_{fast}$ = 2.2±0.3ms; n=11; Fig. 6d and 6e). The rise time for the NMDA EPSC under control was 0.99±0.06ms (n=6) and this accelerated to 0.60±0.07ms (n=8) following acoustic trauma (Fig. 6e). Examination of NMDAR subunit mRNA levels showed one significant change following exposure to AT: there was an increase in GluN1b mRNA, while GluN1a and the GluN2 subunits were unchanged (Fig 6f). The inset (Fig. 6f) shows that the developmental maturation of expression of GluN2 subunits in the LSO is similar to previous reports from the MNTB, where GluN2C dominates on maturation (Steinert et al., 2010). We conclude that NMDAR EPSCs are present in the LSO, and in mature mice have remarkably fast kinetics (similar to that of AMPAR-mediated EPSCs in some brain areas). It seems likely that an NMDAR-mediated conductance with such fast kinetics may have been missed in the past, either because many recordings were made from immature animals, and/or because it merged with the AMPAR-mediated EPSC and was not recognized. We conclude that AT had little influence on the decay of NMDAR-EPSCs, although the induced faster rise-time, which correlated with higher levels of GluN1b expression will be a topic for further study.

Acoustic trauma disrupts LSO integration of excitation and inhibition. Neurons in the LSO are the first to integrate information about interaural intensity differences (IID); for review see (Tollin, 2003). LSO output can be characterized by the slope and half-activation of its IID functions (Karcz et al., 2011), and the effectiveness of this tuning can be quantified with Fisher information (see Methods). Mature IIDs are achieved by integrating ipsilateral excitatory and contralateral inhibitory inputs, which must converge with sub-millisecond precision (Joris and Yin, 1995, Tollin, 2003). Here we show that the kinetics of IPSCs and EPSCs converge to near identical values during development, reaching sub-millisecond time-constants in adult mice (Figs. 2c and 3a). Incorporating the synaptic current data into a computational model of LSO neurons, and optimizing the spiking threshold as a free parameter to produce optimal tuning curves, showed that IID tuning

becomes more effective and precise as development proceeds (see Methods). Poor tuning was characterized by shallow tuning curves, and to a lesser extent by differences in variability (not illustrated). The slow time-course of inhibition at hearing onset essentially renders IID detection impossible during high rates synaptic activity (Fig. 7a, 7b), while at modest stimulus levels, IID tuning is comparable to the adult performance. In contrast, adult tuning is effective over a wide range of input firing rates, with curves shifting progressively towards more negative IIDs, and consistent with experimental observations (Tsai et al., 2010).

Simulated IID functions using synaptic parameters matched to those after AT were shifted to the right, particularly for strong stimuli (Fig 7c, contralateral stimulus more intense) and output spike rates exceeded the input rate. IID discrimination performance for weak stimuli was essentially unchanged by AT, but IID sensitivity for strong stimuli was predicted to be poor due to the imbalance between excitatory and inhibitory inputs; inhibition failed to effectively suppress the stronger excitation caused by longer-lasting EPSCs (EPSCs: 1.23 ± 0.05 ms, n=13; IPSCs: 0.83 ± 0.06ms, n=9, P<0.001).

What are the implications of this result for binaural IID detection? We observed that ABR thresholds are strongly elevated following acoustic trauma (Fig. 4c), suggesting that under normal conditions, average neural activity in the auditory brainstem will be reduced compared to normal adults. The simulations show that IID detection can be fully effective for weaker activity levels. Therefore, we expect IID tuning to be largely intact for the physiological range of sound-evoked activity in noise-exposed animals. In fact, Fisher information was slightly increased at low stimulus frequencies up to about 100Hz in simulations within the acoustic trauma group, compared to normal adults (not illustrated). This improvement, which was caused by a suppressed response variability due to stronger excitatory drive, was however achieved at the expense of performance at higher frequencies. Overall, these results suggest that the slowing of excitatory synaptic

transmission following acoustic trauma has a detrimental effect on IID tuning for higher rates of neural activity.

Recovery of the EPSC time-course and ABR waveforms. Finally we asked whether the effects of acoustic trauma were permanent. We measured ABRs and excitatory synaptic inputs to the LSO two months after trauma in P90-96 mice. We observed a partial recovery of the ABR thresholds (thresholds were elevated by 30 dB compared to control) and a full recovery of the ABR waveforms (Fig. 8a). Control, untreated mice and noise-exposed animals ~3 months old showed similar ABR responses with the characteristic ABR peaks I-V (Fig. 8b). Wave IV of the ABR which was absent (or reduced) one week after AT recovered and the EPSC decay time recovered to control levels, reaching similar values to those found in naïve mice (EPSC$_{tau}$: 0.8 ±0.04 ms, n=5, Fig.8c, 8d). This suggests that slow, delayed excitation in the LSO could be responsible for altered ABR wave IV and the shift in IID functions.

Discussion

We first determined the maturation of evoked EPSC and IPSC kinetics in the LSO during development from prehearing (P7-P9) to adult (P36) mice. The decay kinetics of the synaptic currents both accelerate and converge to precisely matched sub-millisecond values by P23 and are maintained into adulthood. We then asked how exposure to damaging levels of sound (acoustic trauma) might influence the synaptic kinetics in the LSO. We found using adult mice that the evoked and miniature AMPAR-mediated EPSC decay was slowed after the insult; this was mirrored by a rise in GluA1 and a decrease in GluA4 mRNA levels in the LSO. Acoustic trauma had no influence on the glycinergic IPSC kinetics, so there was now a mismatch in the synaptic integration of the E/I inputs which modelling showed undermines the accuracy of the IID computation. Measurement of miniature synaptic responses showed a decrease in the frequency

of both spontaneous excitatory and inhibitory events, implying reduced presynaptic release probability. Evoked NMDAR EPSCs also showed dramatic acceleration with developmental maturation; with a time-constant of over 40 ms in pre-hearing mice, while in mature mice the NMDAR EPSC decayed with a time-constant of around 2 ms. Although acoustic trauma caused an increase in expression of GluN1b, we found no change in the decay of the evoked NMDAR EPSC.

This study set out to investigate whether acoustic trauma caused long-term changes in central auditory processing (IID) by examining changes in the postsynaptic expression of receptor subunits. The convergence in the IPSC/EPSC time-course occurred simultaneously with ABR maturation and was consistent with simulations of IID functions across the physiological range. AT disrupted both the EPSC/IPSC integration and IID function in the LSO and was consistent with a change in the levels of mRNA for glutamate receptor subunits, while IPSC kinetics and GlyR subunit mRNA were unaltered.

Development of fast inhibitory inputs

Developmental changes in the IPSC prior to hearing onset are well documented, as a refinement in the number of inhibitory inputs (Sanes and Friauf, 2000, Kandler et al., 2009) and changes in the extent of axonal arbors (Sanes and Siverls, 1991). In parallel, there is a developmental shift from mixed GABA/glycinergic IPSCs (Lim et al., 2000, Smith et al., 2000) towards glycinergic transmission which becomes dominant in the adult LSO (Kotak et al., 1998, Nabekura et al., 2004, Kim and Kandler, 2010) (Lohrke et al., 2005). After hearing onset there is further acceleration of the IPSC kinetics by P18 (Walcher et al., 2011), which we confirm here, and show that the fast kinetics is maintained to P35. Glycinergic synaptic currents accelerate during development and this follows changes in the expression of glycine receptor subunits (GlyRs) from GlyRα2 to GlyRα1 in mature animals (Becker et al., 1988, Takahashi et al., 1992). GlyRα1 containing

channels display faster kinetics than other GlyR subunits (Lynch, 2009) and expression of GlyRα1 mRNA increases in the LSO after hearing onset (Piechotta et al., 2001). The qRT-PCR confirmed a switch from GlyRα2 to GlyRα1 mRNA from around hearing onset with no significant change in other GlyR subunits.

IPSC peak conductance declined up to hearing onset, consistent with anatomical pruning of MNTB axon terminals in the LSO (Sanes and Siverls, 1991, Sanes et al., 1992, Kandler et al., 2009). Fast glycinergic transmission typically has IPSC decay time-constants of around 1 millisecond (Stuart and Redman, 1990, Jonas et al., 1998, Smith et al., 2000, Legendre, 2001, Awatramani et al., 2004, Magnusson et al., 2005, Bowery and Smart, 2006, Lu et al., 2008). Co-release of GABA with glycine is commonly observed at inhibitory synapses (Jonas et al., 1998, Lim et al., 2000, Kim and Kandler, 2003; Fischl and Burger 2014) and can accelerate each others kinetics (Lu et al., 2008) although in the LSO no evidence for a significant GABAergic component to the IPSC was observed in mice at ages after weaning (Sterenborg et al., 2010). Our observations concur with the general developmental time-course of glycine receptors observed previously in the rat (Friauf et al., 1997). The molecular basis for the transition to fast glycinergic IPSCs is unclear, since the acceleration continues after hearing onset when GlyRα2 is already highly expressed. Our observations show that similar IPSC and EPSC time-courses contribute to effective integration of binaural information, as also suggested from other studies (Reed and Blum, 1990). Although IPSC kinetics are not affected by AT, a decrease in the frequency of spontaneous synaptic events (mIPSCs and mEPSCs) following AT is consistent with an additional presynaptic mechanism, which will require further investigation. Disruption of hearing (through cochlear ablation, age, earplugging or noise exposure) is generally associated with decreased levels of inhibition (Leao et al., 2004, Vale et al., 2004, Kotak et al., 2005) and glycine receptors are down-regulated in the cochlear nucleus after cochlear ablation (Sato et al., 2000, Asako et al., 2005). Earplugging downregulated Glyα1 in the cochlear nucleus and increased GluA3 (Whiting et al.,

2009). In the LSO neither IPSC time-course nor GlyR subunit mRNA were significantly altered by acoustic trauma.

Fast AMPAR-mediated excitatory transmission in the LSO

AMPAR-mediated EPSCs with fast kinetics are widely expressed in the auditory pathway, driving high-fidelity neurotransmission in birds and mammals (Raman and Trussell, 1992, Zhang and Trussell, 1994, Barnes-Davies and Forsythe, 1995, Golding et al., 1995, Otis et al., 1995, Gardner et al., 1999, Trussell, 1999, Gardner et al., 2001, Joshi et al., 2004, Koike-Tani et al., 2005, Koike-Tani et al., 2008, Steinert et al., 2010). Expression studies show that EPSCs exhibiting rapid kinetics are associated with GluR4 containing AMPAR (Mosbacher et al., 1994, Geiger et al., 1995, Lambolez et al., 1996), which are broadly expressed in the auditory brainstem (Rubio and Wenthold, 1999, Yang et al., 2011). Although the EPSC decay kinetics in the MNTB (~0.4ms) (Taschenberger and von Gersdorff, 2000, Futai et al., 2001, Yamashita et al., 2003, Fernandez-Chacon et al., 2004, Postlethwaite et al., 2007) are somewhat faster than the LSO (~0.8ms), the observed prevalence of GluA4 mRNA in the LSO is consistent with the observed fast EPSC decay kinetics. The crucial contribution of GluA4 subunits to fast EPSCs has been demonstrated in the MNTB, where synaptic responses from the calyx of Held were slower and smaller in GluA4 KO mice than in wild-type animals (Yang et al., 2011).

AMPAR-subtype remodelling occurs after repetitive synaptic stimulation, sensory deprivation, drug addiction, and pathological conditions, such as epilepsy (Grooms et al., 2000, Liu and Cull-Candy, 2000, Clem and Barth, 2006, Goel et al., 2011). Here, high levels of acoustic stimulation reduce expression of GluA4 mRNA and slows AMPAR-mediated EPSCs. A similar molecular mechanism of plasticity has been implicated in the classical eye-blink conditioning (Zheng and Keifer, 2009).

Our modelling shows that this increased excitatory charge transfer boosts the damaged excitatory pathway, presumably enabling audible stimuli to provide a stronger excitatory input than in a normal animal. Rather than a 'reversal' to a less mature state, the slower time-course is a homeostatic process to rescue binaural function following acoustic trauma. This hypothesis is consistent with dynamic regulation of glutamate receptors during plasticity at other synapses, where GluA1 upregulation is clearly associated with a persistent decrease in activity (Granger et al., 2011). GluA4 is essential for rapid kinetics (Raman and Trussell, 1992; Yang et al., 2011) and for homeostatic control of excitatory synaptic transmission in the mature brain (Wierenga et al., 2005). Acoustic trauma reduces auditory firing rates (for a given SPL) due to the peripheral damage, and the increased excitatory drive observed in the LSO acts to enhance synaptic function, compensating for this, so that IID detection is still (largely) intact at low firing rates. Overall the modelling shows that increased spontaneous firing following acoustic trauma maintains binaural function in the LSO, in spite of peripheral hearing loss.

NMDAR mediated EPSCs exhibit extreme acceleration on maturation of the LSO

As a consequence of defining the excitatory receptor subtypes of the ipsilateral projection to the LSO in pre-hearing mice, we observed the classic dual component EPSC (Forsythe & Westbrook, 1988) with a fast AMPAR-mediated EPSC and a slow voltage-dependent NMDAR-mediated response. Over the next two weeks of development this D-AP5-sensitive EPSC accelerates to have a decay time-constant of around 2.5ms at +40mV in the presence of $[Mg^{2+}]_o$. This contrasts with NMDAR-EPSCs in immature animals which generally have decay time-constants in the 30-200ms range. Such rapid NMDAR-mediated EPSCs have not been reported previously, although developmental acceleration of NMDAR-EPSCs have been previously characterized in the MNTB (Steinert et al., 2010) where decay time-constants accelerate to around 15ms in mature mice and

rats. Moderate acceleration of NMDAR kinetics during maturation is a common theme in many areas of the brain; hippocampus CA1 synapses have decays of around 100ms (Kirson & Yaari, 1996; Rauner & Kohr, 2011) while at thalamo-cortical synapses NMDAR EPSCs accelerate to around 50ms at P27 (Barth & Malenka, 2001) and in cerebellar granule cells by P21-P40 the dominant fast decay tau is around 30 ms, although a contribution from a slow tau of around 300ms continues (Cathala et al., 2000). This developmental acceleration is not universal and will depend on the particular subunits expressed; for example in the rat nucleus accumbens, decay time-constants of around 200ms are stable from juvenile to adult (Kasanetz and Manzoni, 2009). The acceleration observed here in the LSO is the most extreme yet observed and brings the NMDAR EPSC kinetics to time-courses that are close to those of GluR1A-dominated AMPAR. NMDAR subunit mRNA showed similar changes to those published for the MNTB (Steinert et al., 2010) with adult dominance of GluN2C. The only significant change in NMDAR subunit mRNA following AT was an increase in GluN1b message relative to GluN1a. Although AT had little or no effect on the fast NMDAR-EPSC decay, further investigation is required to understand the contribution of this EPSC to auditory processing in the LSO.

Integration of excitation and inhibition encodes interaural intensity differences

Similar kinetics of excitatory and inhibitory transmission underlies encoding of mature IID functions and detection of a sound source. Mismatched synaptic kinetics shift the IID functions and lower performance (Pollak, 1988, Park et al., 1996, Park et al., 1997, Klug et al., 2000, Tollin, 2003, Park et al., 2004). For example, the IID population code in the LSO is skewed towards the excitatory ear in young (P12-P17) animals (Sanes and Rubel, 1988) and also in animals lacking the potassium channel subunit, Kv1.1 (Karcz et al., 2011). Both of these cases suggest that inhibition is more effective than the excitation. This change in efficacy can be mediated either by differences in thresholds (Sanes and Rubel, 1988, Tsai et al., 2010) or by slower and temporally dispersed

inhibition (Karcz et al., 2011). Using the slow IPSC kinetics (from the young animals), simulation of IID functions supports the notion that this provides tonic inhibition, skewing IIDs to the 'excitatory ear' (Fig. 7).

Our data indicate that the changes in LSO EPSCs after acoustic trauma follow a similar principle, causing IID functions to be skewed to the 'inhibitory ear', since EPSC kinetics are slowed whereas inhibition remains fast. In this scenario excitation outlasts inhibition and is therefore more effective (opposite to the above situation) and IID function is dominated by contralateral auditory space. IID discrimination was also enhanced for low spike rates, preserving function following peripheral damage. For stronger stimuli, shallow IID tuning curves reduce discrimination because excess excitation is not balanced by inhibition, highlighting the importance of carefully balanced timing, kinetics and magnitude of synaptic excitation and inhibition for normal LSO function. Similar conclusions were drawn from *in vivo* patch-clamp recordings in the inferior colliculus of bats (Gittelman and Pollak, 2011).

Hearing deficits caused by acoustic trauma, ageing or genetic susceptibility is primarily considered from a peripheral and cochlea perspective, but changes and adaptations in central auditory processing can mitigate or compound the peripheral auditory deficit (Kujawa & Liberman, 2009; Pilati et al., 2012). We conclude that hearing damage causes changes in the subunit composition of sub-synaptic receptors, with consequences for synaptic integration which outlast the initial 2h trauma by several weeks, are resolved by 2 months and which together with other forms of plasticity, compensate for peripheral auditory damage.

Figure Legends:

Figure 1: Auditory brainstem responses reach maturity about 2 weeks after hearing onset in CBA/Ca mice. a. The studies were conducted in 4 age-groups: pre-hearing (P7-9), hearing-

onset (P12-15), young adult (P17-23) and adults (P29-36). **b**. ABRs were obtained and average response thresholds were plotted for 5 frequencies between 8 to 30 kHz and for click stimuli. The number of samples for each respective group was: pre-hearing, n=3, hearing-onset n=7, young adult n=24, and adults n=8. Right: Averaged ABR waves for each age-group are shown in response to 24 kHz tones at 94 dB SPL; waves I–IV are indicated on ABRs measured from young adult mice. Statistical comparison was performed by a two way Anova (8 to 30 kHz frequencies) and one-way ANOVA (click stimuli). All the values had a level of significant difference of ***P<0.001, except where indicated on the graph: *P<0.05.

Figure 2: EPSCs and IPSCs undergo refinement during development until identical time-courses are generated. a. Diagram showing the location of the LSO, other nuclei and stimulation sites in the superior olivary complex of the *in vitro* brain slice. Ipsilateral excitatory inputs from the aVCN to the LSO are highlighted in green and contralateral inhibitory inputs from the MNTB are highlighted in red. Bipolar stimulating electrodes were placed over the MNTB and over the axons from the ipsilateral cochlear nucleus, as indicated. EPSCs (green, upper right inset) were recorded in the presence of bicuculline (10µM), strychnine (0.5 µM) and D-AP5 (50 µM) and were blocked by the application of CNQX (10µM). IPSCs (red, lower right inset) were recorded in the presence of bicuculline (10µM), CNQX (10µM) and D-AP5 (50 µM) and were blocked by the application of strychnine (0.5 µM). **b.** Representative examples of EPSCs (green) and IPSCs (red) recorded in pre-hearing (P6), hearing-onset (P13), young adult (P22) and adult (P33); average traces (n=20) are superimposed with respect to the rising phase of the response and normalized to the peak amplitude. The decay time-constant (τ) for each trace is indicated. All recordings were from neurons held at a potential of -70 mV.

Figure 3: Mean data showing acceleration in synaptic decay and reduced conductance with changes in the subunit mRNA levels across development. a. Bar charts summarizing EPSC (green) and IPSC (red) decay τ and **b.** conductance in the four age-groups of mice used in this study. Sample size (n) is indicated in the respective bar. EPSCs and IPSCs converge to similar time-course and conductance in young adult mice. Comparing PSC decay showed significance acceleration with age but on maturation no difference in decay between IPSCs and EPSCs. There was a significant reduction in IPSC peak conductance with age, but on maturation there was no difference between EPSC and IPSC conductance. **c.** A plot of the glycine receptor subunit mRNA expression (x=Glyα1-4) relative to γ-actin in the LSO for the four age groups used in this study (n=3 or 4, see qRT PCR methods) – shows the early switch from Glyα2 to Glyα1. **d.** Glutamate receptor subunit mRNA expression (x=GluA1-4) for the four age groups in this study (n=3 or 4). There are significant increases in GluA3 and GluA4 on maturation. Significance was defined as ** P<0.01, (one way ANOVA).

Figure 4: Acoustic trauma (AT) caused little hearing loss in immature mice, but AT raised thresholds in mice after hearing onset. a. Hearing Onset: Plot of mean ABR thresholds for sound frequencies between 8 to 30 kHz and click stimuli for control mice (grey symbols n= 4) and following exposure to acoustic trauma (blue symbols, n=4). Right: Example traces of averaged ABR waves in response to a 24 kHz tone at 94 dB SPL. Noise exposure at hearing-onset mice had little effect on ABR thresholds. **b.** Acoustic trauma in young adult mice elevated ABR thresholds and significantly reduced ABR wave IV. Control (dark grey, n=10) and after acoustic trauma (blue, n=8). Significance was defined as *** P<0.01, ** P<0.01 (unpaired Student t-test for clicks and two way ANOVA for the tone frequencies).

Figure 5: AT prolongs EPSC decay time and increases GluR4 mRNA without affecting IPSC characteristics. EPSCs are colour coded green, IPSCs in red, and AT exposed in blue; n-values

are indicated in the bar graphs. **a.** Evoked LSO EPSC decay time-constants are plotted for control CBA/Ca mice (green) and in those exposed to AT (blue). Right inset shows superimposed average EPSCs (n=10 traces) from a control and AT exposed mouse. **b.** qRT-PCR for GluA1-4 relative to γ-actin, measured from control (green) and AT exposed (blue) mice. AT increased LSO GluA1 and decreased GluA4. **c.** Miniature spontaneous EPSCs (average of 20 events, recorded in the presence of TTX 0.5µM) showed the same slowing of the decay τ to the evoked EPSC. AT caused no change in the mEPSC amplitude distribution, but reduced mEPSC frequency. **d.** Evoked LSO IPSC decay τ are plotted for control (red) mice and from mice exposed to AT (blue). Right inset shows superimposed average IPSCs (n=10 traces) from a control and AT exposed mouse. **e.** qRT-PCR for GlyR1-4 relative to γ-actin, measured from control (red) and AT exposed (blue) mice. AT exposed LSOs exhibited no significant changes in glycine receptor subunits on AT exposure. **f.** Miniatures spontaneous IPSCs (average of 20 events, recorded in the presence of TTX 0.5µM) and mIPSC amplitude were also unaffected by AT, but did reduce mIPSC frequency. Amplitude distributions are shown as cumulative distribution (Kolmogorov-Smirnov test, $P > 0.05$). Synaptic currents were recorded from neurons held at -70 mV. Significance was defined as ** $P < 0.01$,* $P < 0.05$ (unpaired Student's t-test for graphs a, c, d, f and one way ANOVA for graphs b and e).

Figure 6: NMDAR-mediated EPSC decay time accelerates during developmental maturation.
Acoustic trauma in mature mice increased GluN1b mRNA and decreased the NMDAR-EPSC rise time, but had no impact on decay time-course. **a.** Evoked EPSC current-voltage (I/V) relationship from a pre-hearing (P8) LSO principal neuron (in the presence of 10µm bicuculline and 1µm strychnine). Open circles indicate the I/V of the fast AMPAR EPSC, closed circles indicate the latency for the slow NMDAR EPSC. Inset: shows superimposed average EPSC traces for each holding potential (HP) from -80 to +40 mV. Throughout, the stimulus is indicated by a triangle, with artifacts being removed for clarity. **b.** Control EPSCs before (green trace) and after perfusion of 20µm D-AP5 (purple trace) in the same cell, HP = +40 mV, confirming that the slow EPSC

component is mediated by NMDAR. Decay time-constants are indicated by the respective trace. **c.** NMDAR EPSC I/V curve from a mature mouse aged P23, recorded in the presence of 10μm bicuculline, 1μm strychnine and 10μm NBQX. Inset: Average traces from HP +50 mV and -50 mV. **d.** Normalized NMDAR EPSCs from Control (green) and AT (blue) exposed mice (>P23) show similar decay time-course at +40 mV HP. **e.** Bar chart showing mean NMDAR EPSC decay tau and rise-time for control and AT exposed mice, respectively. **f.** Relative expression of GluN1 and GluN2 subunit mRNA in the LSO from control (green) and following AT exposure (blue). GluN1b mRNA at P35 was significantly increased following AT. Inset: Developmental profile of GluN2 subunit mRNA expression shows GluN2C mRNA levels increase and dominate over maturation. Statistical comparison was performed using one-way ANOVA (** $p = <0.0001$).

Figure 7: Modelling shows that convergence of EPSC and IPSC kinetics is required for maturation of IID functions. The relative time-course of excitatory (green) and inhibitory (red) synaptic inputs to the LSO is shown for the three conditions: hearing-onset, adult and following acoustic trauma. In the lower graphs the LSO model output is shown normalized to the input firing rate plotted against the IID across a range of firing frequencies from 100-300Hz (colour coded as indicated on the right: 100Hz, blue, 300Hz, orange). **a.** At hearing onset, inhibitory synaptic currents in the LSO have slow kinetics and large amplitudes. This makes inhibition more effective than excitation and shifts IID functions to the left for higher input frequencies. **b.** Balanced amplitudes and kinetics of EPSCs and IPSCs result in a normal distribution of IID functions in the mature LSO. **c.** Acoustic trauma causes a deceleration of the excitatory synaptic currents, increasing the effectiveness of excitation and leading to a rightward shift of the IID functions.

Figure 8. The AT-induced plasticity in the LSO EPSC is temporary. a. Plot shows ABR thresholds at 8-32kHz and for a click stimulus. Thresholds remained elevated two months after AT (blue) at around P90, compared to similar aged mice which received no AT (grey). b. Mean ABRs (24 kHz, 743 tone pip at 94 dB SPL) for the same data set, show that wave IV is present in control and on recovery from AT at around P90. c. AMPAR EPSC decay time-constants (τ) were measured from the same mice as in a & b. Bar charts shows mean EPSC decay τ from unexposed mice (control, green) and mice that received AT 1 week earlier (blue, n=13) or 2 months earlier (blue, n=5). d. Representative AMPAR-EPSC averages (n=20) for each group; the slowed EPSC decay τ had recovered to control values two months after AT induction. Significance was defined as ***P>0.001, **P<0.01 (Two way ANOVA for tone frequencies in graph a, and unpaired t-test for a clicks in a, and c .

Table 2. Parameters used in the LSO neuron simulations

	τ_i (ms)	τ_c (ms)	g_i^m	g_c^m	V_{th} (mV)
Hearing-onset	0.73	1.55	3.8	14.8	-50.6
Young	0.65	0.79	6.6	5.4	-41.1
Adult	0.76	0.87	7.5	4.4	-37.2
Noise-exposed	1.23	0.83	6.4	5.8	-37.2

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