Cerebral Cortex

**Hearing loss prevents the maturation of GABAergic transmission in the auditory cortex**



| | |
|---|---|
| Journal: | *Cerebral Cortex* |
| Manuscript ID: | CerCor-2007-00560.R1 |
| Manuscript Type: | Original Articles |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Kotak, Vibhakar; New York University, Center for Neural Science<br>Takesian, Anne; New York University, Center for Neural Science<br>Sanes, Dan; New York University, Department of Biology; New York University, Center for Neural Science |
| Keywords: | GABAA receptor, alpha1 and beta2/3 subunits, auditory cortex, hearing impairment, development |
| | |



scholarONE™
Manuscript Central

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

**Title** Hearing loss prevents the maturation of GABAergic transmission in the auditory cortex

**Authors** Vibhakar C. Kotak[1], Anne E. Takesian[1], and Dan H. Sanes[1,2]

**Addresses** [1]Center for Neural Science and [2]Department of Biology
4 Washington Place, New York University, NY, NY 10003

**Running Title** Deafness disrupts inhibitory transmission

**Pages** 34
**Figures** 8
**Tables** None

**Words** Abstract: 206
Introduction: 656
Discussion: 1,903

**Corresponding author**
Vibhakar C. Kotak, Ph.D.
Center for Neural Science, 4 Washington Place
New York University, New York, NY 10003

Voice: 212-998-3914 FAX: 212-995-4011
Email kotak@cns.nyu.edu

**Key Words** $GABA_A$ receptor, $\alpha$1 and $\beta$2/3 subunits,
hearing impairment, auditory cortex, development

## Abstract

For Peer Review

Inhibitory neurotransmission is a critical determinant of neuronal network gain and dynamic range, suggesting that network properties are shaped by activity during development. A previous study demonstrated that sensorineural hearing loss (SNHL) in gerbils leads to smaller inhibitory potentials in L2/3 pyramidal neurons in the thalamorecipient auditory cortex, ACx (Kotak *et al*., 2005). Here, we explored the mechanisms that account for proper maturation of GABAergic transmission. SNHL was induced at postnatal day (P) 10 and whole-cell voltage clamp recordings were obtained from layer 2/3 pyramidal neurons in thalamocortical slices at P16-19. SNHL led to an increase in the frequency of GABAzine-sensitive (antagonist) spontaneous (s) and miniature (m) IPSCs, accompanied by diminished amplitudes, and longer durations. Consistent with this, the amplitudes of minimum-evoked (me-) IPSCs were also reduced while their durations were longer. The $\alpha$1 and $\beta$2/3 subunit-specific agonists zolpidem and loreclezole increased control but not SNHL spontaneous IPSC durations. To test whether SNHL affected the maturation of GABAergic transmission, sIPSCs were recorded at P10. These spontaneous IPSCs resembled the long SNHL spontaneous IPSCs. Furthermore, zolpidem and loreclezole were ineffective in increasing their durations. Together, these data strongly suggest that the presynaptic release properties and expression of key postsynaptic $GABA_A$ receptor subunits are co-regulated by hearing.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

**Introduction**

Anatomically, inhibitory neocortical networks are outweighed by their excitatory counterparts by about 1:4, yet, if the integrity of inhibitory circuits is degraded, even a small amount, the properties of an entire network can be profoundly affected (Chagnac-Amitai and Connors, 1989; Caspary *et al*., 1999; Cherubini and Conti, 2001; Korpi *et al*., 2002; Mody and Pearce, 2004; Farrant and Nusser, 2005). Detailed studies on the intrinsic and synaptic properties of the inhibitory interneurons and their influence on the pre as well as postsynaptic gain of the target cells are imperative for the evaluation of their contribution to normal development as well as pathological disorders. In the auditory cortex (ACx), for example, activation of GABAergic circuits leads to sharpening of onset responses and excitatory receptive field properties (Muller and Scheich, 1988; Wang *et* al., 2000; Foeller *et al*., 2001). Such properties and the percepts that they support, such as speech discrimination in background noise, can be compromised by hearing loss, and this, in part, is due to a selective decline in inhibitory synapse function that shapes sound-driven response properties (Caspary *et al*., 2005).

The strength of 2 inhibitory postsynaptic currents represents, in part, the number of synaptic $GABA_A$ receptors, and the specific array of subunits (Otis *et al*., 1994; Nusser *et al*., 1997; Nusser *et al*., 1998). Modulation of the subunit expression and distribution, therefore, has profound consequences on the development and plasticity of inhibitory synapses (Fagiolini and Hensch, 2000; Hensch, 2005) and neural excitability under both physiological and pathophysiological conditions. In the mammalian brain, the most abundant pentameric receptors are comprised of $\alpha$, $\beta$ and $\gamma$ subunits; the most common stoichiometry within the neocortex is $2\alpha$:$2\beta$:$1\gamma$ (Sieghart *et al*., 1999; Mehta and Ticku, 1999; Knight *et al*., 2000).

The developmental expression of specific receptor subunits, and their distribution on a single neocortical or hippocampal neuron, can impart distinct functional properties. For example, experiments in 'knock-in' mice show that,

For Peer Review

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

while α1 subunits may participate in cortical plasticity, α2 subunits regulate neuronal firing (Fagiolini et al., 2004), presumably due to their abundance at synapses on the axon initial segment of pyramidal cells (Nusser *et al*., 1996b; Fritschy *et al*., 1998). The profound changes in developmental expression of specific subunits (Laurie *et al*., 1992) may also underlie the ontogenic changes in IPSC kinetics (Vicini *et al*., 2001; Banks *et al*., 2002; Mody and Pearce, 2004; Bosman *et al*., 2005; Huntsman and Huguenard, 2006; Möhler, 2006; Ing and Poulter, 2007).

Experimental manipulations have shown that inhibitory neurotransmission is a critical determinant of neuronal network gain, suggesting that network properties are shaped by GABAergic transmission (Kilman *et al*., 2002; Burrone *et al*., 2002; Turrigiano, 2007). Prior studies in the developing ventral auditory brainstem and midbrain have shown that the loss of cochlear activity for one to several days *in vivo*, leads to a reduction of inhibitory synaptic gain due to diminished IPSP/IPSC amplitudes and depolarized inhibitory reversal potential/current (Kotak and Sanes, 1996; Vale and Sanes, 2000). In the inferior colliculus neurons, such weakening of IPSCs is characterized by reduced inhibitory conductance and a compromised chloride co-transporter function (Vale and Sanes, 2000; Vale *et al*., 2003). Furthermore, electrophysiological and quantitative EM-immunogold data show that developmental sensorineural hearing loss (SNHL) leads to a significant enhancement in excitatory synapse function in L2/3 pyramidal neurons of the auditory cortex mediated by both AMPA and NMDA receptors (Kotak *et al*., 2005).

The current study addresses the question whether early auditory experience influences the development of GABAergic transmission. Our data show that the amplitudes of IPSCs decline following developmental SNHL. This was accompanied by the inability of the $GABA_A$ receptor subunits, α1 and β2/3, to respond to specific agonists. Moreover, increased frequency of spontaneous IPSCs and mIPSCs indicate parallel alterations at the presynaptic locus. Anatomically, quantitative EM-immunocytochemical assays reveal that presynaptic glutamic acid decarboxylase (GAD) increased and postsynaptic β2/3

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

subunit immunogold counts decreased following SNHL (Sarro et al., accompanying manuscript). Additionally, IPSCs from animals before hearing onset shared characteristics similar to SNHL IPSCs, including insensitivity to the subunit-specific agonists. Thus, SNHL may prevent the normal maturation of GABAergic transmission in the ACx.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

## Material and Methods

*Surgery for sensorineural hearing loss (SNHL)*

All protocols were reviewed and approved by New York University Institutional Animal Care and Use Committee. As described previously (Vale and Sanes, 2002), cochlear ablations were performed on gerbil (*Meriones unguiculatus*) pups at postnatal day 10 (P10), just before the onset of response to airborne sound. Gerbil pups were anesthetized (methoxyflurane), which was assessed by a lack of toe-pinch response Each cochlea was then rapidly removed with a forceps. The wound was sealed, the pups were placed on a heating pad, and then transferred to the breeding pair. Pups were then reared for 6-9 days with their parents under conditions identical to those for control pups. The age of surgery was chosen based on evidence that anteroventral cochlear nucleus cell number is unaffected by cochlear ablation after P9 (Tierney *et al*., 1997). Cochlea removal was confirmed post mortem by opening each cochlea under a dissecting microscope and finding the absence of cochlear tissue but gel foam in its place.

*Thalamocortical brain slice recordings and pharmacology*

Horizontal thalamocortical brain slices that retained the afferent projection from ventral medial geniculate nucleus (MGv) to the auditory cortex (thalamorecipient cortex, ACx; Kotak *et al*., 2005) were generated from P8-20 gerbils. The artificial cerebrospinal fluid (ACSF) contained (in mM): 125 NaCl, 4 KCl, 1.2 $KH_2PO4$, 1.3 MgSO4, 26 $NaHCO_3$, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid (pH 7.3 when bubbled with 95% $O_2$-5% $CO_2$). ACSF was superfused in the recording chamber at 3 ml per min at 32°±1°C. Whole-cell recordings were obtained from layer 2/3 pyramidal neurons that were located in the thalamorecipient cortex. Neurons were visually identified under IR-DIC optics prior to obtaining a GΩ seal. The location of individual neuron cell bodies varied from 125 to 400 µm from the pial surface. The data in this paper were collected

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

from 104 recordings from control (n=43), SNHL (n=45) and pre-hearing (n=16) neurons.

<u>*GABAergic activity and pharmacological manipulations*</u>

To characterize $GABA_A$ receptor-mediated spontaneous and minimum evoked (me-) IPSCs whole-cell voltage-clamp recordings were made at a bandwidth of 10 kHz. The internal patch solution contained (in mM): 100 KCl, 40 K-Gluconate, 10 NaCl, 10 HEPES free acid, 4 MgATP, 0.1 EGTA, 5 QX-314 (pH=7.2 with KOH). Bipolar platinum wire stimulating electrodes were placed in the MGv and in upper cortical layer 4 (L4) approximately 100 µm from the recording site to stimulate the thalamocortical and intracortical pathways, respectively. These stimulating electrodes were fabricated from 0.004" diameter teflon-coated platinum wires (A-M systems) inserted into a ≈2" long double barrel glass electrode. The tips of the wires were bared of the teflon coat (the exposed tip was 0.002" diameter).

To isolate inhibitory GABAergic activity, DNQX (20 µM) and AP-5 (50 µM) were added to the superfusing ACSF to block AMPA and NMDA receptors, respectively. The drugs were applied for a minimum of 8 min before recording inhibitory currents. For acquiring spontaneous and *m*IPSCS, at least ten sweeps of 30 sec duration each were acquired for each neuron at holding potential close to the resting membrane potential; $V_{HOLD}$= -60 mV (Kotak *et al*., 2005). The mIPSC recordings were obtained in the presence of 1 µM TTX.

To examine whether the strength of putative monosynaptic inhibitory connections was altered following SNHL, minimum evoked IPSCs were recorded in normal ACSF in voltage clamp conditions at $V_{HOLD}$= -60 mV. Incremental stimulus intensities were delivered to L4 at 0.1 Hz until an evoked IPSC was discernible (Figure 2A). Stimulation at this intensity produced ≥ 50% failures in eliciting IPSCs. The absolute stimulus intensity varied approximately 5% between preparations and ages. Increasing the minimal intensity by more than 5% decreased the failure rate and increased the IPSC amplitude and possible

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

polysynaptic innervations. Therefore, the minimal stimulation intensity was kept constant throughout each recording. The failure rates were not analyzed.

The function of the two $GABA_A$ receptor subunits was tested by the use of agonists. Zolpidem (100 nM, Tocris) was applied to test for the presence of the $\alpha$1 subunits and loreclezole (10 μM, Tocris) was applied to test for the presence of the $\beta$2/3 subunits. These agents were added to ACSF and bath applied for at least 15 min before recording the effect on spontaneous IPSC durations. These data were separately acquired in 2 second traces; at least 50 traces were recorded per neuron. Thus, they do not constitute a part of the spontaneous IPSC data on amplitudes and frequency recorded before. To validate that the recorded postsynaptic currents (spontaneous IPSCs, mIPSCs and me-IPSCs) were due to $GABA_A$ receptor activity, bicuculline (20 μM, BIC methoiodide, Sigma) *or* GABAzine (500 nM-1μM, Tocris) were applied at the end of some experiments (N=6).

*Data collection and analysis*

Data were collected using a Macintosh G4 running a custom designed IGOR (WaveMetrics, v6.0) macro called SLICE (Kotak *et al*., 2001). Evoked synaptic potentials and currents were analyzed off-line using a second IGOR macro called SLICE ANALYSIS. Analysis was restricted to IPSC amplitudes that were ≥ 6 pA as these events could be reliably identified in baseline noise. Spontaneous IPSC durations were manually analyzed off-line using the SLICE ANALYSIS program, by superimposing 1 ms vertical grids on traces. The time between the onset of the falling phase of the spontaneous IPSC and the return of the decay phase of the IPSC to the original baseline was considered as the total duration (ms). Any spontaneous IPSCs (2 or more) that summated before a single spontaneous IPSC decay returned to baseline, were not considered in this analysis. To ascertain changes of spontaneous IPSC durations following experimental and pharmacological manipulations, analysis was restricted to IPSCs collected separately, and majority (95%) of whose amplitudes varied between 20 and 80 pA (above). Thus, we biased this analysis such that there

For Peer Review

was no statistical difference between the control and SNHL spontaneous IPSC amplitudes (~ 40 pA). This strategy discounted the amplitude-dependent fluctuations in spontaneous IPSC kinetic properties. Statistical tests (ANOVA, students' t-test for normally distributed data, or Wilcoxon-non-parametric test for data that was not normally distributed) were performed using statistical software (JMP, SAS Institute).

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

## Results

### ***Hearing loss alters s & mIPSCs***

To determine whether hearing loss altered the inhibitory network properties, we first recorded spontaneous IPSCs (sIPCS) under voltage clamp conditions at a holding potential of -60 mV after blocking ionotropic glutamate receptor transmission. The results showed that the mean spontaneous IPSC frequency was increased while the mean amplitude was decreased significantly in SNHL neurons. (spontaneous IPSC frequency, mean ± SEM: control, 2 ± 0.2 Hz vs. SNHL, 4.6 ± 0.2 Hz; t test, $t$ = 7.1; df = 22 $p$ = 0.0001; spontaneous IPSC amplitude, mean ± SEM: control, 28 ± 2 pA vs. SNHL, 19 ± 2 Hz; $t$ test, $t$ = 2.6; df = 20, $p$ = 0.01; Figure 1). To further test whether these changes persisted in complete absence of presynaptic action potentials, TTX (500 nM) was added at the end of the recording session in some experiments. These results were comparable to spontaneous IPSCs, in that higher miniature (m)IPSC frequency was accompanied by significantly reduced amplitudes for SNHL neurons (mIPSC frequency, mean ± SEM: control, 2.2 ± 0.4 Hz vs. SNHL, 5.2 ± 0.4 Hz; t test, t = 4.5; df = 9, $p$ = 0.002; mIPSC amplitude, mean ± SEM: control, 35 ± 3 pA vs. SNHL, 19 ± 3 Hz; t test, t = 3.4; df = 9, $p$ = 0.01; recordings not shown).

### ***Hearing loss perturbs spontaneous IPSC kinetics***

Among other factors, spontaneous IPSC kinetic properties reflect underlying function of an array of $GABA_A$ receptor subunits. To measure durations, we used hundreds of control and SNHL spontaneous IPSCs that were separately recorded in 2 second sessions. To exclude the spontaneous IPSC amplitude-based variation in durations, we measured spontaneous IPSC durations of comparable amplitudes with an average of about 40 pA. This analysis showed that spontaneous IPSC durations were significantly longer in the SNHL group. (Spontaneous IPSC duration, mean ms ± SEM: control, 58 ± 4 vs. SNHL, 89 ± 4; $t$ = 5.6; df = 19, $p$ = 0.0004; Figure 2). The effect of hearing loss

For Peer Review

on spontaneous IPSC duration was thus independent of amplitudes because spontaneous IPSC amplitudes did not differ between the two groups; hence, it is discounted as a factor for this analysis (spontaneous IPSC amplitudes, mean pA ± SEM: control 39.6 ± 2, SNHL, 40 ± 3.3, $p$ = 0.9).

***Hearing loss decreases minimum-evoked (me)-IPSCs***

Our previous study showed that hearing loss significantly enhanced putative monosynaptic excitatory input originating from the MGv to L2/3 (Kotak *et al*., 2005). To determine whether the strength of putative monosynaptic inhibitory inputs from GABAergic interneurons to L2/3 pyramidal neurons was affected by SNHL, minimum evoked IPCSs (me-IPSCs) were recorded in the presence of blockers of ionotropic glutamate receptors, DNQX and AP-5. Over 100 intracortically-evoked recordings were obtained from each neurons, of which about 50% responses contained an IPSC. The amplitudes of the 6 smallest me-IPSCs were averaged for each neuron. Comparison of these me-ISPCs revealed that activation of such putative unitary afferents produce significantly reduced IPSC amplitudes in SNHL neurons (minimum intracortically -evoked IPSC amplitudes, mean pA ± SEM: normal, 14 ± 1.9 vs. SNHL, 7.7 ± 0.8; Wilcoxon's test, $X^2$ = 6.4; df = 20, $p$ = 0.01; Figure 3).

Because the duration of spontaneous IPSCs differed, we acquired additional data to compare me-IPSC durations. For this, IPSCs were examined slightly above minimum thresholds for control and SNHL neurons for consistent kinetic measurements. The stimulus intensity was adjusted about 5% above that which produced a 50% failure rate for these experiments. IPSC amplitudes between 15 and 40 pA were recorded and thus, the mean amplitude did not differ between the two groups (near me-IPSC amplitudes, mean pA ± SEM: control, 20.5 ± 8.7 vs. SNHL, 19.1 ± 2.1, df=13, $p$ = 0.7). However, the IPSC durations were significantly longer in SNHL neurons (IPSC duration, mean ms ± SEM: control, 92.5 ± 14.5 vs. SNHL, 156.5 ± 20.4; t = 2.55; df = 13, $p$ = 0.025; Figure 3).

***SNHL may disrupt $\alpha$1 and $\beta$2/3 subunit function***

For Peer Review

For Peer Review

If the hearing loss-mediated increase in spontaneous IPSC duration involves a change in the expression of $GABA_A$ receptor subunits, then the efficacy of specific subunit agonists should be perturbed. To test this idea, agonists for $GABA_A$ receptor subunits known for their high affinity to the benzodiazapine-binding site (zolpidem, α1 agonist) or an anticonvulsant site (loreclezole, β2/3 agonist) were employed. While zolpidem (100 nM) significantly prolonged spontaneous IPSC durations in control neurons, the agonist failed to enhance spontaneous IPSC durations in SNHL neurons (control spontaneous IPSC durations, mean ms ± SEM: before zolpidem, 69 ± 4 vs. after zolpidem, 114 ± 10; $t$ = 5.6; df = 9, $p$ = 0.003; SNHL spontaneous IPSC durations mean ms ± SEM, before zolpidem, 94 ± 6 vs. after zolpidem, 102 ± 7, t = 0.8, df = 9, $p$ = 0.4; Figure 4). Similarly, bath application of the β2/3 subunit agonist, loreclezole (10 μM) significantly lengthened spontaneous IPSC durations in control neurons, but did not significantly affect spontaneous IPSC durations in SNHL neurons (control spontaneous IPSC durations, mean ms ± SEM: before loreclezole, 49 ± 2 vs. after loreclezole, 97 ± 5, Wilcoxon's test, $X^2$ = 6.8; $p$ = 0.009; SNHL spontaneous IPSC durations, mean ms ± SEM: before loreclezole, 83 ± 3 vs. after loreclezole, 94 ± 12, Wilcoxon's test, $X^2$ = 0.01, $p$ = 0.9; Figure 5).

We therefore tested the hypothesis that SNHL may perturb the maturation of processes that underlie shortening of spontaneous IPSC durations following hearing onset. Additional recordings from animals before hearing onset (pre-hearing; P8-10) were therefore performed. The results showed that the spontaneous IPSC durations resembled the prolonged duration spontaneous IPSCs in SNHL neurons, when compared to controls. A three-way comparison of means revealed that there was no significant difference between the spontaneous IPSC durations between SNHL and pre-hearing neurons, while the control spontaneous IPSC duration differed significantly from both groups. (ANOVA, F = 15.2, df = 27; *spontaneous IPSC* durations, mean ms ± SEM: pre-hearing, 90.4 ± 6.5 vs. SNHL, 89 ± 4, t = 0.2, df = 17, $p$ = 0.8; pre-hearing, 90.4 ± 6.5 vs. control, 59 ± 3.8; t = 4.1, df = 17, $p$ = 0.0005, Figure 6). This effect was independent of the amplitudes of spontaneous IPSCs (ANOVA, F = 0.2, df = 20,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

*p* = 8). Recordings of near me-IPSCs from pre-hearing neurons also showed that the durations were similar to SNHL me-IPSCs (IPSC durations, mean ms ± SEM: pre-hearing, 154.8 ± 19.5 vs. SNHL, 156.5 ± 20.4, t = 0.061, df = 13, $p$ = 0.95).

To further test whether the reduced sensitivity of the SNHL spontaneous IPSCs to the $\alpha$1 and $\beta$2/3 subunit agonists reflects an immature state, the agents were tested in pre-hearing neurons. Similar to SNHL, spontaneous IPSCs from pre-hearing animals did not show a significant change in duration after zolpidem or loreclezole applications (pre-hearing *spontaneous IPSC* durations, mean ms ± SEM; before zolpidem, 82.5 ± 4.3 vs. after zolpidem, 84.2 ± 6.0, t test, t = 0.2, df = 7, $p$ = 0.8; before loreclezole, 79.0 ± 3.8 vs. after loreclezole, 90.0 ± 7.4, t test, t = 1.3, df = 7, $p$ = 0.2; Figure 7).

Figure 8 is a schematic diagram summarizing the present findings on GABAergic transmission following SNHL, along with our previous findings on changes depicting heightened intrinsic and excitatory synaptic properties (Kotak *et al*., 2005), and two changes assayed at an EM-immunocytochemical level (Sarro *et al*., accompanying manuscript).

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

## Discussion

### *Deafness and GABAergic properties*

Despite the strides made by behavioral and by *in vivo* physiology experiments, we are just beginning to understand the biophysical and synaptic alterations in the ACx that attend moderate or severe hearing loss. A previous report demonstrated that SNHL elevates excitability in L2/3 pyramidal neurons as reflected by changes in the passive membrane properties, increased thalamocortical excitatory drive, and diminished maximum-evoked IPSPs (Kotak *et al.*, 2005). A fundamental question is whether inhibitory synapse function is altered at both pre- and postsynaptic loci. The present study shows that SNHL prevents the normal maturation of presynaptic release and postsynaptic GABA receptor function. Specifically, while the presynaptic terminals may release GABA more frequently (Figure 1), the diminished amplitudes of unitary afferent-evoked inhibitory currents indicate inadequate postsynaptic $GABA_A$ receptor activation (Figures 1-3, Cherubini and Conti, 2001; Nusser *et al.*, 1997, 1998). Futhermore, prolonged *spontaneous IPSCs* and evoked IPSCs, resembling those recorded in prehearing animals (P10), suggest that SNHL prevents the maturation of inhibitory synaptic properties. These altered IPSC kinetics are well correlated with the diminished efficacy of the $\alpha1$ and $\beta2/3$ subunit-specific agonists in SNHL neurons (Figures 4-5) and pre-hearing neurons (Figure 7). We argue that hearing experience is critical for the emergence of mature pre and postsynaptic properties of GABAergic transmission in the ACX and this involves key subunits integral to the postsynaptic $GABA_A$ receptors.

Complete hearing loss is expected to affect endogenous patterns of activity across the ascending auditory pathway and key integrating elements in the brainstem, midbrain and thalamus. For example, prior to the onset of hearing, gerbil inferior colliculus (IC) neurons display spontaneous activity (Kotak and Sanes, 1995). Maintained discharge at the level of the eighth nerve and ventral cochlear nucleus (VCN) originates within the cochlea of adult and juvenile mammals (Bock and Webster, 1974; Koerber *et al.*, 1966; Tucci *et al.*, 1999;

For Peer Review

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Sheperd *et al*., 1999; Tucci *et al*., 2001; Lee *et al*., 2001; Cook *et al*., 2002., Yu *et al*., 2005), and pre and post-hatched birds (Born and Rubel, 1988; Born *et al*., 1991; Lippe, 1994). An alteration in VCN spontaneous activity is expected to have an impact on IC electrical activity. In the gerbil, 2-deoxyglucose studies demonstrate that the metabolic activity is decreased in the contralateral lobe of the IC following either conductive hearing loss or SNHL, even in relative silence (Tucci *et al*., 1999, 2001). Together, these findings suggest that cochlear ablation, either in infancy or adulthood, results in a significant loss of peripheral action potentials and neurotransmission during the period immediately after ablation, and these factors likely influenced the inhibitory properties we assayed in the cortex.

Partial or total loss of activity in the somatosensory, visual and auditory systems indicate downregulation of GABAergic transmission and our data concur with these findings. For example, blocking action potentials by chronically exposing cultured neurons with tetrodotoxin leads to a significant decline in *m*IPSC amplitudes due to a decrease in the average number of open GABA-gated channels and their inappropriate clustering at the postsynaptic membrane, that includes β subunits (Kilman *et al*., 2002). We observed a similar reduction in the amplitudes of spontaneous and mIPSC in SNHL neurons suggesting decreased $GABA_A$ receptor function is likely associated with a lower number of $GABA_A$ receptors (Nusser et al., 1997, 1998). The diminished spontaneous and mIPSC and putative unitary IPSC amplitudes in SNHL neurons (Figures 1, 2) correspond with a decline in inhibitory conductance in the IC following bilateral SNHL (Vale and Sanes, 2000). It is also possible that decreased inhibitory response amplitudes were due to depolarization of the IPSP reversal potential as shown in the LSO and IC neurons (Kotak and Sanes, 1996; Vale and Sanes, 2000; Vale *et al*., 2003), although we did not test this. Similarly, weakened connections between fast spiking GABAergic interneurons and star pyramidal neurons is observed in L4 of visually deprived animals (Maffei *et al*., 2004). Our observations may additionally explain the reduced amplitude IPSPs when maximum number of afferents are activated (Kotak *et al*., 2005).

For Peer Review

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Our data also suggest that at the presynaptic level, SNHL neurons appear to release more GABA (Figure 1; and increased mIPSCs frequency, results), and this correlates with a significantly enhanced GAD immunoreactivity quantified ultrastructurally at the inhibitory terminals that impinge on L2/3 pyramidal neurons (Sarro et al., accompanying manuscript). In lieu of an enhanced presynaptic GABA release probaility, it is possible that the altered kinetics in spontaneous IPSCs we observe may additionally be caused by postsynaptic $GABA_A$ receptor desensitization (Jones and Westbrook, 1995), although this hypothesis remains to be tested.

***Development and GABAergic properties***

The strong resemblance of spontaneous IPSC characteristics in SNHL neurons with those recorded before hearing onset (Figure 6) implies an arrest in the normal expression of GABAergic transmission at pre and postsynaptic loci. We found that SNHL leads to longer duration spontaneous IPSCs (Figure 2) and near me-IPSCs (Figure 3), which resemble long IPSCs before hearing onset (Figure 6). Developmental shortening of IPSCs is consistent with long mIPSCs recorded during early development from rat cerebellar granule cell and mouse cerebellar stellate neurons (Tia *et al*., 1996; Nusser *et al*., 1997, Vicini *et al*., 2001). In stellate neurons for example, mIPSCs at P11 are long, whereas at P35, they decay five times faster (Vicini *et al*., 2001).

The subunit-specific agonists zolpidem for $\alpha$1 subunits (Pritchett and Seeburg, 1990; Wafford *et al*., 1994; Luddens *et al*., 1995; Rudolph and Möhler, 2004), and loreclezole for $\beta$2/3 subunits (Wingrove *et al*., 1994; Bacci *et al*., 2003) target $GABA_A$ receptor subunits that affect IPSC kinetics. Both $\alpha$1 and $\beta$2/3 subunits are activated by synaptically released GABA (Amin and Weiss, 1993; Connolly et al., 1996; Baumann *et al*., 2003). Functions of these subunits in L2/3 pyramidal neurons can be assayed by the magnitude of effect of these two agents, which act to enhance IPSC durations (Figures 4,5, Bacci *et al*., 2003). Importantly, these agents can be used to assess whether the subunit expression undergoes developmental changes in parallel to the alterations in the amplitudes

For Peer Review

and kinetics of IPSCs. For example, in cerebellar inhibitory synapses, mIPSCs recorded in $\alpha1$ subunit deficient mice at P35 are as slow as those at P11 suggesting that lack of $\alpha1$ subunit insertion in postsynaptic receptors is responsible for the prolonged inhibitory synaptic currents. Also, in recombinant systems, rapid application of GABA to outside-out excised patches generates faster currents with $\alpha1\beta2\gamma2$ than with other subunit combinations (Verdoorn, 1994; Gingrich *et al*., 1995; Lavoie *et al*., 1997; McClellan and Twyman, 1999) which agrees with the predominant stoichiometry ($\alpha1\beta2\gamma2$) of the $GABA_A$ receptor in mammalian cortex (Korpi *et al*., 2002; Möhler, 2006; Huntsman and Huguenard, 2006). Similarly, a progressive shortening of mIPSCs in thalamic relay neurons during the first postnatal month is accompanied by an increased expression of $\alpha1$ subunit transcripts and decrease in the $\alpha2$ transcripts. Further, thalamic organotypic cultured neurons with an overexpressed $\alpha1$ subunit showed faster mIPSCs (Okada et al., 2000). Similar to the $\alpha1$ subunit, the $\beta2/3$ subunits may emerge during postnatal development. For example, IPSC sensitivity to loreclezole increases across postnatal life in rat dentate granule cells (Kapur and MacDonald, 1998), rat medial septum/diagonal band neurons (Hsiao *et al*., 1998), and mouse cerebellar granule cultured neurons (Ortinski *et al*., 2004). In fact, the developmental upregulation of $\beta2/3$ subunit function may depend on $\alpha1$ subunit expression; the effect of loreclezole is compromised in IPSCs from $\alpha1$ subunit -/- mice (Ortinski *et al*., 2004).

Consistent with these studies, our data also suggest that the $\alpha1$ and $\beta2/3$ subunits are upregulated during development. This is shown by the significant augmentation of spontaneous IPSCs in control P17-19 neurons by zolpidem and loreclezole and in contrast, the inability of these agonists to potentiate spontaneous IPSC durations in pre-hearing animals (Figure 6). Similarly, the inability of zolpidem and loreclezole to augment SNHL spontaneous IPSCs strongly implies that SNHL may halt the normal expression of the $\alpha1$ and $\beta2/3$ subunits on L2/3 cells that otherwise would impart shorter IPSC kinetics (Figure 2, Okada *et al*., 2000; Banks *et al*., 2002; Amin and Weiss, 1993; Wafford *et al*.,

For Peer Review

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1994; Gingrich *et al*., 1995; Jones & Westbrook, 1995; Baumann *et al*., 2003). Significantly lower β2/3 immunogold counts observed in SNHL neurons at the normal postsynaptic membraneous site supports this deduction (Sarro *et al*., accompanying manuscript). Importantly, similar to an area-specific expression of the α1 subunits in developing rat neocortex that may be mediated via activity (Paysan *et al*., 1997), the expression and proper distribution of the α1 and β2/3 and subunits appears to be regulated by auditory activity.

The prolonged spontaneous IPSCs observed in prehearing animals and the inability of the subunit-specific agonists to affect their durations implies that α1 and β2/3 are not predominantly expressed in the ACx before hearing onset. This raises an important question: what other subunits may underlie such long IPSCs that may persist in an absence of acoustically driven activity? It is known that the developmental expression of the α1 subunit mRNA in the cerebellum is very low during early life (Bovolin *et al*., 1992; Laurie *et al*., 1992). Other subunits such as α5 (Dunning *et al*., 1999), α2 and α3 are likely to mediate long spontaneous IPSCs similar to those found early in development. As a matter of fact, mRNA for α2 and α3 subunits are observed in early development (Laurie *et al*., 1992).

For Peer Review

***Cortical excitability and GABAergic properties***

The co-adjustments of GABAergic function at pre and postsynaptic loci are in contrast with the amplified strength of the thalamocortical synapses following SNHL. Decreased probability of glutamate release (mEPSC) was accompanied by increased AMPAergic and NMDAergic participation (me-EPSCs) and higher NR2B immunogold counts at the asymmetric synapses (Kotak *et al*., 2005). Coupled with altered membrane properties favoring heightened excitability, the significant decline in postsynaptic GABAergic function may support higher discharge rates and thus alter the network properties of the ACx. Figure 8 shows a diagramatic sketch of a L2/3 pyramidal SNHL neuron summarizing the present and previous findings. Although this model represents synaptic and biophysical properties following SNHL, it may apply to a range of

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

conditions that decrease sound-evoked activity (e.g. the hair cell loss due to ototoxic drugs, aging, or noise-induced trauma, Syka, 2002; Ling *et al*., 2005). Such weakened inhibition may eventually lead to flawed communication among neurons and a disorganized tonotopy (Kaur *et al*., 2004). For example, even mild to moderate forms of conductive hearing loss produces synaptic modifications within the auditory cortex (Xu *et al*., 2007), and reduced GABAergic transmission may contribute. The current findings may also explain, in part, the changes that result from partial hair cell damage including the unmasking of excitatory sidebands and discharge characteristics (Irvine and Rajan, 1997; Rajan, 1998; Salvi *et al*., 2000; Wang *et al*., 2002a,b). Diminished inhibitory transmission may also contribute to temporal jitter to acoustic cues; for example, both the excitatory synaptic and spike precision is compromised at auditory nerve-bushy cell synapses in the cochlear nucleus of early onset hearing impaired mice (Wang and Manis, 2005). The net effects of such pre and postsynaptic modifications upon ACx networks and their input-output functions remain to be identified. The overall downscaling of inhibitory gain shown for SNHL neurons may be associated with various clinical conditions such as epilepsy, tinnitus, and presbycusis that are also characterized by reduced GABAergic gain. In addition to the use of implant-based prostheses, attempts to design targeted drugs with specific affinity toward distinct $GABA_A$ receptor subunits holds potential to alleviate deficits for the hearing impaired.

**Acknowledgements:** Supported by DC006864 (DHS and VCK)

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

## References

Amin J, Weiss DS. 1993. $GABA_A$ receptor needs two homologous domains of the beta-subunit for activation by GABA but not by pentobarbital. Nature. 366:565-569.

Bacci A, Rudolph U, Huguenard JR, Prince D. 2003. Major differences in inhibitory synaptic transmission onto two neocortical interneuron subclasses. J Neurosci. 23:9664-9674.

Banks MI, Hardie JB, Pearce RA. 2002. Development of GABA(A) receptor-mediated inhibitory postsynaptic currents in hippocampus. J Neurophysiol. 88:3097-3107.

Baumann SW, Baur R, Sigel E. 2003. Individual properties of the two functional agonist sites in GABA(A) receptors. J Neurosci. 23:11158-11166.

Bosman LW, Heinen K, Spijker S, Brussaard AB. 2005. Mice lacking the major adult $GABA_A$ receptor subtype have normal number of synapses, but retain juvenile IPSC kinetics until adulthood. J Neurophysiol. 94:338-346.

Bock GR, Webster WR. 1974. Spontaneous activity of single units in the inferior colliculus of anesthetized and unanesthetized cats. Brain Res. 76:150-154.

Born DE, Durham D, Rubel EW. 1991. Afferent influences on brainstem auditory nuclei of the chick: nucleus magnocellularis neuronal activity following cochlea removal. Brain Res. 557:37-47.

Born DE, Rubel EW. 1988. Afferent influences on brain stem auditory nuclei of

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

the chicken: presynaptic action potentials regulate protein synthesis in nucleus magnocellularis neurons. J Neurosci. 8:901-919.

Bovolin P, Santi MR, Puia G, Costa E, Grayson D. 1992. Expression patterns of gamma-aminobutyric acid type A receptor subunit mRNAs in primary cultures of granule neurons and astrocytes from neonatal rat cerebella. Proc Natl Acad Sci. 89:9344-9348.

Burrone J, O'Byrne M, Murthy MN. 2002. Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. Nature. 420:414-418.

Caspary DM, Schatteman TA, Hughes LF. 2005. Age-related changes in the inhibitory response properties of dorsal cochlear nucleus output neurons: role of inhibitory input. J Neurosci 25:10952-10959.

Caspary DM, Holder TM, Hughes LF, Milbrandt JC, McKernan RM, Naritoku DK. 1999. Age-related changes in GABA(A) receptor subunit composition and function in rat auditory system. Neuroscience. 93:307-312.

Chagnac-Amitai Y, Connors BW. 1989. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. J Neurophysiol. 61:747-758.

Cherubini E, Conti F. .2001. Generating diversity at GABAergic synapses. TINS. 24:155-162.

Connolly CN, Krishek BJ, McDonald BJ, Smart TG, Moss SJ. 1996. Assembly and cell surface expression of heteromeric gamma-amobutyric acid type A receptors. J Biol Chem. 271:89-96.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Cook RD, Hung TY, Miller RL, Smith DW, Tucci DL. 2002. Effects of conductive hearing loss on auditory nerve activity in gerbil. Hear Res. 164:127-137.

Dunning DD, Hoover CL, Soltesz I, Smith MA, O'Dowd DK. 1999. GABA(A) receptor-mediated miniature postsynaptic currents and alpha-subunit expression in developing cortical neurons. J Neurophysiol. 82:3286-3297.

Fagiolini M, Hensch TK. 2000. Inhibitory threshold for critical-period activation in primary visual cortex. Nature. 404:183-186.

Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK. 2004. Specific GABAA circuits for visual cortical plasticity. Science. 303:1681-1683.

Farrant M, Nusser Z. 2005. Variations on an inhibitory them: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci. 6:215-229.

Foeller E, Vater M, Kossl M. 2001. Laminar analysis of inhibition in the gerbil primary auditory cortex. J Assoc Res Otolaryngol. 2:279-296.

Fritschy JM, Johnson DK, Mohler H, Rudolph U. 1998. Independent assembly and subcellular targeting of GABA(A)-receptor subtypes demonstrated in mouse hippocampal and olfactory neurons in vivo. Neurosci Lett. 249:99 102.

Gingrich KJ, Roberts WA, Kass RS. 1995. Dependence of the GABAA receptor gating kinetics on the alpha-subunit isoform: implications for structure-function relations and synaptic transmission. J Physiol. 489:529-543.

Hensch TK. 2005. Critical Period Mechanisms in Developing Visual Cortex. Curr Topics Dev Biol. 69:215-237.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Hsiao S, Mahoney JC, West JR, Frye GD. 1998. Development of $GABA_A$ receptors on medial septum/diagonal band (MS/DB) neurons after postnatal ethanol exposure. Brain Res. 810:100-113.

Huntsman MM, Huguenard JR. 2006. Fast IPSCs in rat thalamic reticular nucleus require the $GABA_A$ receptor beta1 subunit. J Physiol. 572:459-475.

Ing T, Poulter MO. 2007. Diversity of GABA(A) receptor synaptic currents on individual pyramidal cortical neurons. Eur J Neurosci. 25:723-734.

Irvine DR, Rajan R. 1997. Injury-induced reorganization of frequency maps in adult auditory cortex: the role of unmasking of normally inhibited inputs. Acta Otolaryngol Suppl. 532:39-45.

Jones MV, Westbrook GL. 1995. Desensitized states prolong GABAA channel responses to brief agonist pulses. Neuron. 15:181-191.

Kapur J, MacDonald RL. 1999. Postnatal development of hippocampal dentate granule cell $\gamma$–aminobuytric acid A receptor pharmacological properties. Mol Pharmacol. 55:444-452.

Kaur S, Lazar R, Metherate R. 2004. Intracortical pathways determine breadth of subthreshold frequency receptive fields in primary auditory cortex. J Neurophysiol. 91:2551-2567.

Kilman V, van Rossum MC, Turrigiano GG. 2002. Activity deprivation reduces miniatures IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. J Neurosci. 22:1328-1337.

For Peer Review

Koerber KC, Pfeiffer RR, Warr WB, Kiang NY. 1966. Spontaneous spike discharges from single units in the cochlear nucleus after destruction of the cochlea. Exp Neurol. 16:119-130.

Korpi ER, Grunder G, Luddens H. 2002. Drug interactions at $GABA_A$ receptors. Prog Neurobiol. 67:113-159.

Kotak VC, Sanes DH. 1995. Synaptically evoked prolonged depolarizations in the developing auditory system. J Neurophysiol. 74:1611-1620.

Kotak VC, Sanes DH. 1996. Developmental influence of glycinergic transmission:regulation of NMDA receptor-mediated EPSPs. J Neurosci. 16:1836-1843.

Kotak VC, Fujisawa S, Lee FA, Karthikeyan O, Aoki C, Sanes DH. 2005. Hearing Loss Raises Excitability in the Auditory Cortex. J Neurosci. 25:3908-3918.

Knight AR, Stephanson FA, Tallman JF, Ramabahdran TV. 2000. Monospecific antibodies as probes for the stoiciometry of recombinant GABA(A) receptors. Recep Chann. 7:213-226.

Laurie DJ, Wisden W, Seeburg PH. 1992. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci. 12:4151-4172.

Lavoie AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE. 1997. Activation and deactivation rates of recombinant GABA(A) receptor channels are dependent on alpha-subunit isoform. Biophys J. 73:2518-256.

Lee DS, Lee JS, Oh SH, Kim SK, Kim JW, Chung JK, Lee MC, Kim CS. 2001.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Cross-modal plasticity and cochlear implants. Nature. 409:149-150.

Ling LL, Hughes LF, Caspary DM. 2005. Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. Neuroscience 132:1103-1113.

Lippe WR. 1994. Rhythmic spontaneous activity in the developing avian auditory system. J Neurosci. 14:1486-1495.

Luddens H, Korpi ER, Seeburg PH. 1995. GABAA/benzodiazepine receptor heterogeneity:neurophysiological implications. Neuropharmacol. 34:245-254.

Maffei A, Nelson SB, Turrigiano GG. 2004. Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. Nat Neurosci. 7:1353-1359.

McClellan AM, Twyman RE. 1999. Receptor system response kinetics reveal functional subtypes of native murine and recombinant human $GABA_A$ receptors. J Physiol. 515:711-727.

Mehta AK, Ticku MK. 1999. An update on $GABA_A$ receptors. Brain Res Rev. 29:196-217.

Mody I, Pearce RA. 2004. Diversity of inhibitory neurotransmission through $GABA_A$ receptors. TINS. 27:569-575.

Möhler H. 2006. $GABA_A$ receptor diversity and pharmacology. Cell and Tiss Res. 326:505-516.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Müller CM, Scheich H. 1988. Contribution of GABAergic inhibition to the response characteristics of auditory units in the avian forebrain. J Neurophysiol. 59:1673-1689.

Nusser Z, Sieghart W, Benke D, Fritschy JM, Somogyi P. 1996. Differential synaptic localization of two major gamma-aminobutyric acid type A receptor subunits on hippocampal pyramidal cells. Proc Natl Acad Sci. USA 93:11939-1144.

Nusser Z, Cull-Candy S, Farrant M. 1997. Differences in synaptic GABA(A) receptor number underlie variation in GABA mini amplitude. Neuron. 19:697-709.

Nusser Z, Hajos N, Somogyi P, Mody I. 1998. Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. Nature. 395:172-177.

Okada M, Onodera K, Van Renterghem C, Sieghart W, Takahashi T. 2000. Functional correlation of GABA(A) receptor alpha subunits expression with the properties of IPSCs in the developing thalamus. J Neurosci. 20:2202-2208.

Ortinski PI, Lu C, Kentaroh T, Zhanyan F, Vicini S. 2004. Expression of distinct $\alpha$ subunits of GABAA receptor regulates inhibitory synaptic strength. J Neurophysiol. 92:1718-1727.

Otis TS, De Koninck Y, Mody I. 1994. Lasting potentiation of inhibition is associated with an increased number of gamma-aminobutyric acid type A receptors activated during miniature inhibitory postsynaptic currents. Proc Natl Acad Sci USA. 91:7698-7702.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Paysan J, Kossel A, Bolz J, Fritschy JM. 1997. Area-specific regulation of gamma-aminobutyric acid type A receptor subtypes by thalamic afferents in developing rat neocortex. Proc Natl Acad Sci. 94:6995-7000.

Pritchett DB, Seeburg PH. 1990. Gamma-aminobutyric acidA receptor alpha 5-subunit creates novel type II benzodiazepine receptor pharmacology. J Neurochem. 54:1802-1804.

Rajan R. 1998. Receptor organ damage causes loss of cortical surround inhibition without topographical map plasticity. Nat Neurosci. 1:138-43

Rudolph U, Möhler H. 2004. Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol. 44:475-498.

Salvi RJ, Wang J, Ding D. 2000. Auditory plasticity and hyperactivity following cochlear damage. Hear Res. 147:261-274.

Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Höger H, Adamiker D. 1999. Structure and subunit composition of GABA(A) receptors. Neurochem Int. 34:379-385.

Syka J. 2002. Plastic changes in the central auditory system after hearing loss, restoration of function, and during learning. Physiol Rev. 82:601-636.

Tia S, Wang JF, Kotchabhakdi N, Vicini S. 1996. Developmental changes of inhibitory synaptic currents in cerebellar granule neurons: role of GABA(A) receptor alpha 6 subunit. J Neurosci. 16:3630-3640.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Tierney TS, Moore DR. 1997. Naturally occurring neuron death during postnatal development of the gerbil ventral cochlear nucleus begins at the onset of hearing. J Comp Neurol. 387:421-429.

Tucci DL, Cant NB, Durham D. 1999. Conductive hearing loss results in a decrease in central auditory system activity in the young gerbil. Laryngoscope. 109:1359-1371.

Tucci DL, Cant NB, Durham D. 2001. Effects of conductive hearing loss on gerbil central auditory system activity in silence. Hear Res. 155:124-132.

Turrigiano G. 2007. Homeostatic signaling: the positive side of negative feedback. Curr Opin Neurobiol. 17:318-324.

Vale C, Sanes DH. 2000. Afferent regulation of inhibitory synaptic transmission in the developing auditory midbrain. J Neurosci. 20:1912-1921.

Vale C, Sanes DH. 2002. The effect of bilateral deafness on excitatory and inhibitory synaptic strength in the inferior colliculus. Eur J Neurosci. 16:2394-2404.

Vale C, Schoorlemmer J, Sanes DH. 2003. Deafness disrupts chloride transporter function and inhibitory synaptic transmission. J Neurosci. 23:7516-7524.

Verdoorn TA. 1994. Formation of heteromeric gamma-aminobutyric acid type A receptors containing two different alpha subunits. Mol Pharmacol. 45:475-480.

Vicini S, Ferguson C, Prybylowski K, Kralic J, Morrow AL, Homanics GE. 2001. GABA(A) receptor alpha1 subunit deletion prevents developmental changes

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

of inhibitory synaptic currents in cerebellar neurons. J Neurosci. 21:3009-3016.

Wafford KA, Bain CJ, Quirk K, McKernan RM, Wingrove PB, Whiting PJ, Kemp JA. 1994. A novel allosteric modulatory site on the $GABA_A$ receptor beta subunit. Neuron. 12:775-782.

Wang J, Caspary D, Salvi RJ. 2000. GABA-A antagonist causes dramatic expansion of tuning in primary auditory cortex. Neuroreport. 285:95-98.

Wang J, Ding D, Salvi RJ. 2002a. Functional reorganization in chinchilla inferior colliculus associated with chronic and acute cochlear damage. Hear Res. 168:238-249.

Wang J, McFadden SL, Caspary D, Salvi R. 2002b. Gamma-aminobutyric acid circuits shape response properties of auditory cortex neurons. Brain Res. 19:219-231.

Wang Y, Manis PB. 2005. Synaptic transmission at the cochlear nucleus end bulb synapse during age-related hearing loss in mice. J Neurophysiol. 94:1814-1824.

Wingrove PB, Wafford KA, Bain C, Whiting PJ. 1994. The modulatory action of loreclezole at the gamma-aminobutyric acid type A receptor is determined by a single amino acid in the beta 2 and beta 3 subunit. Proc Natl Acad. Sci USA. 91:4569-4573.

Xu H, Kotak VC, Sanes DH. 2007. Conductive hearing loss disrupts synaptic and spike adaptation in the developing auditory cortex. J Neurosci. 27:9417-9426.

For Peer Review

Yu X, Wadghiri YZ, Sanes DH, Turnbull DH. 2005. In vivo auditory brain mapping in mice with Mn-enhanced MRI. Nat Neurosci. 8: 961-968.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

***Captions to Figures***

Figure 1. SNHL spontaneous IPSC amplitudes are smaller and frequency is higher. A, B. The top panel (A) shows two sweeps of spontaneous IPSCs recorded in the presence of DNQX and AP-5 for 30 seconds each in a P17 control neuron (A) and an age-matched SNHL neuron (B) at a holding potential of -60 mV. In both cases, each recording was acquired 1 minute apart. Note that amplitudes of spontaneous IPSCs in the SNHL neuron are smaller, whereas the frequency is higher. In both control as well as SNHL recordings, addition of 1 μM GABAzine eliminates spontaneous IPSCs, showing that they were mediated by the activation of $GABA_A$ receptors. C. Bar graphs summarizing the mean amplitude and frequency of spontaneous IPSCs recorded from 11 normal and 12 SNHL neurons. Note that the amplitude of mean spontaneous IPSCs is smaller in SNHL neurons (left panel, filled bar) while the mean frequency of spontaneous IPSCs is greater in SNHL neurons (right panel, filled bar). In this and all subsequent figures, asterisks indicate that the differences are significant (for statistics, see Results).

For Peer Review

Figure 2. SNHL spontaneous IPSC durations are long. A. 2 sweeps of spontaneous IPSCs recorded for 2 seconds each in a P17 control (top, 2 gray traces) and an age-matched SNHL neuron (bottom, black traces) at a holding potential of -60 mV. Each recording was acquired 10 seconds apart. Note that the durations of spontaneous IPSCs in the SNHL neuron are longer. B. Distribution of all spontaneous IPSCs durations, 528 from 10 control neurons and 582 from 10 SNHL neurons, showing that the spontaneous IPSCs durations in SNHL neurons are significantly longer.

Figure 3. SNHL me-IPSC amplitudes are smaller and durations are longer. A. Intracortical me-IPSCs were recorded in voltage clamp at $V_{HOLD}$ of -60mV in the presence of ionotropic glutamate receptor blockers, DNQX and AP-5. me-IPSCs were elicited by stimulating L4 approximately 100 μm away from the L2/3

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

recording site at incremental intensities until the failures were replaced by responses. The intensity at which minimum IPSCs were discernible from failed responses (gray traces) was then chosen for successive recordings (black traces). Such minimal stimulation intensity produced a failure rate of 50% or more. B. With the same method, note that me-IPSCs recorded from an age-matched SNHL neuron are smaller and longer (black traces). C, D. Bar graphs summarizing the mean amplitude and duration of me-IPSCs recorded from 10 normal and 11 SNHL neurons. The amplitude of mean me-IPSCs is smaller in SNHL neurons (top panel, right, filled bar) while the mean duration of near me-IPSCs is longer in SNHL neurons (bottom, right panel, filled bar).

For Peer Review

Figure 4. SNHL disrupts $\alpha$1 subunit function. A. 2 sweeps of spontaneous IPSCs recorded for 2 s each in a P17 control (top, 2 gray traces) at a holding potential of -60 mV. Each recording was acquired 10 sec apart. Note that $GABA_A$ receptor $\alpha$1 subunit-specific agonist, zolpidem, prolongs spontaneous IPSC durations (bottom, 2 black traces). B. Note that in an age-matched SNHL neuron, zolpidem application does not produce an increase in spontaneous IPSC durations. C,D. Distribution of all spontaneous IPSCs durations, 470 from 5 control neurons, and 572 from 5 SNHL neurons, showing that the spontaneous IPSCs durations are significantly prolonged by zolpidem treatment in control neurons but not in SNHL neurons. Horizontal bars indicate mean spontaneous IPSC durations.

Figure 5. SNHL disrupts $\beta$2/3 subunit function. A. 2 sweeps of spontaneous IPSCs recorded for 2 s each in a P17 control (top, 2 gray traces) at a holding potential of -60 mV. Each recording was acquired 10 sec apart. Note that $GABA_A$ receptor $\beta$2/3 subunit-specific agonist, loreclezole, prolongs spontaneous IPSC durations (bottom, 2 black traces). B. Note that in an age-matched SNHL neuron, loreclezole application does not produce an increase in spontaneous IPSC durations. C,D. Distribution of all spontaneous IPSCs durations, 564 from 5 control neurons, and 608 from 5 SNHL neurons, showing that the spontaneous

IPSCs durations are significantly prolonged by loreclezole application in control neurons but not in SNHL neurons. Horizontal bars indicate mean spontaneous IPSC durations.

Figure 6. Pre-hearing IPSC durations are long. A. 2 sweeps of spontaneous IPSCs recorded for 2 seconds each in a P17 SNHL (top, 2 gray traces, taken from Figure 2) and a P10 pre-hearing neuron (bottom, 2 black traces) at a holding potential of -60 mV. Each recording was acquired 10 seconds apart. Note that the durations of spontaneous IPSCs in the pre-hearing neuron are long as in the SNHL neuron. B. Distribution of all spontaneous IPSCs durations, 582 from 10 SNHL neurons, and 469 from 8 pre-hearing neurons, showing the close resemblance between the spontaneous IPSC durations of pre-hearing and SNHL neurons. For comparison, see control spontaneous IPSC durations in Figure 2.

Figure 7. Pre-hearing IPSC durations are insensitive to $\alpha 1$ and $\beta 2/3$ subunit agonists. A. 2 sweeps of spontaneous IPSCs recorded for 2 seconds each in a P10 pre-hearing neuron (top, 2 gray traces) at a holding potential of -60 mV. Each recording was acquired 10 sec apart. Note that the $GABA_A$ receptor $\alpha 1$ subunit-specific agonist, zolpidem, does not prolong spontaneous IPSC durations (2 black traces). B. Note that in another age-matched pre-hearing neuron, the application of the $\beta 2/3$ agonist, loreclezole, does not prolong spontaneous IPSC durations. C,D. Distribution of all spontaneous IPSC durations, from all pre-hearing neurons showing that the spontaneous IPSCs durations are not prolonged by zolpidem (581 spontaneous IPSCs from 4 neurons) or loreclezole (544 spontaneous IPSCs from 4 neurons). This result is similar to that obtained for SNHL neurons (see Figures 4 and 5).

Figure 8. Summary sketch of a L2/3 SNHL pyramidal neuron. This sketch highlights the present findings that hearing loss leads to alterations in inhibitory synaptic properties (top right panel, gray). Further, they accompany co-adjustments in the passive and active intrinsic (bottom panel), and excitatory

For Peer Review

synaptic properties (bottom left panel). An upward arrow indicates increased while a downward arrow indicates decreased function. For example, SNHL caused a rise in resting membrane potential ($V_{REST}$), firing properties, and input resistance ($R_{INPUT}$; bottom panel; Kotak et al. 2005). For thalamocortical synapses, SNHL decreased the release probability (mEPSC frequency), while increasing mEPSC and thalamically-evoked minimum-EPSC amplitudes and enhancing current carried by the NR2B subunits of the NMDA receptor (Left panel).

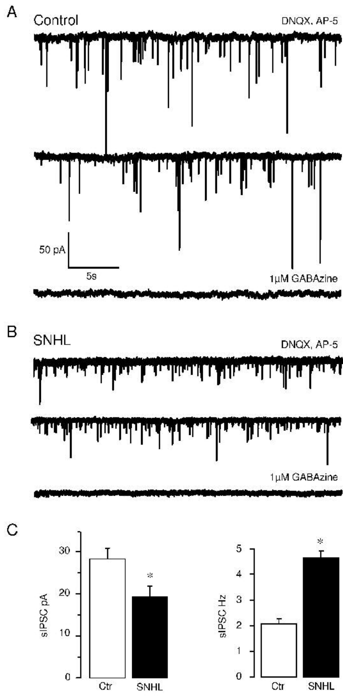
In contrast, the present study shows increased GABA release probability (higher spontaneous and mIPSCs frequency) is accompanied by a decrease in spontaneous IPSC and minimum-evoked IPSC amplitudes. Further, an inability of $\alpha$1 and $\beta$2/3 subunit-specific agonists to alter spontaneous IPSCs recorded from SNHL and pre-hearing neurons sugests an arrest in the maturation of GABAergic transmission.

The bottom right panel (dashed box) displays an observation derived from EM-immunocytochemistry (Sarro et al, accompanying manuscript) that postsynaptic $\beta$2/3 subunit distribution is disrupted. In addition, the presynaptic terminals may synthesize/release more GABA.

The cumulative outcome of such robust homeostatic alterations following hearing loss may adjust the cortical network at a new set point in anticipation that peripheral activity will be restored.

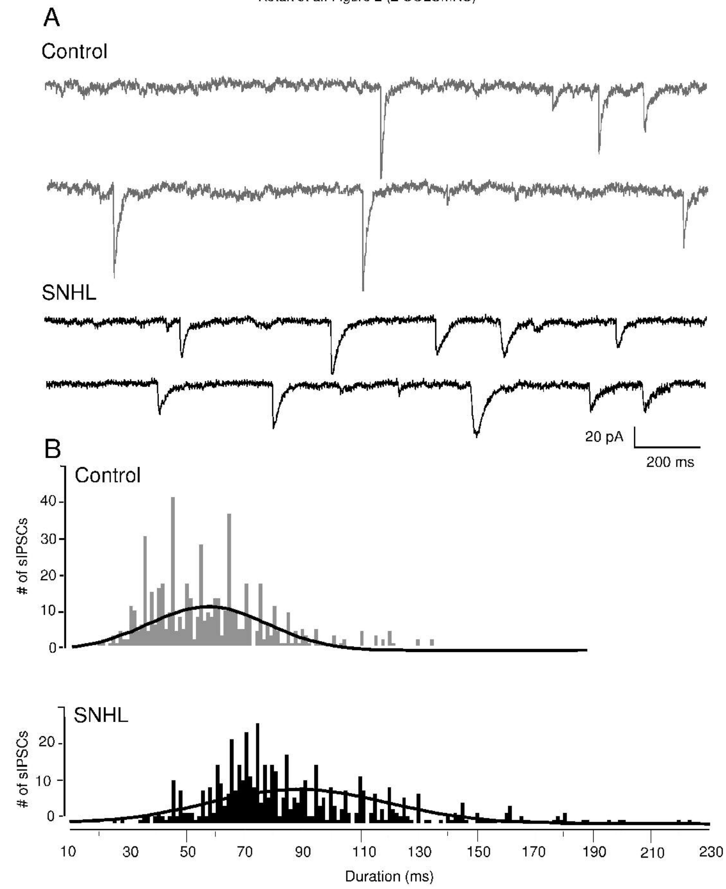
For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



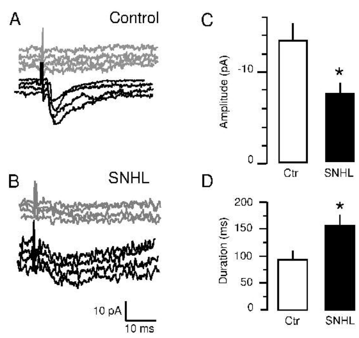

215x279mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



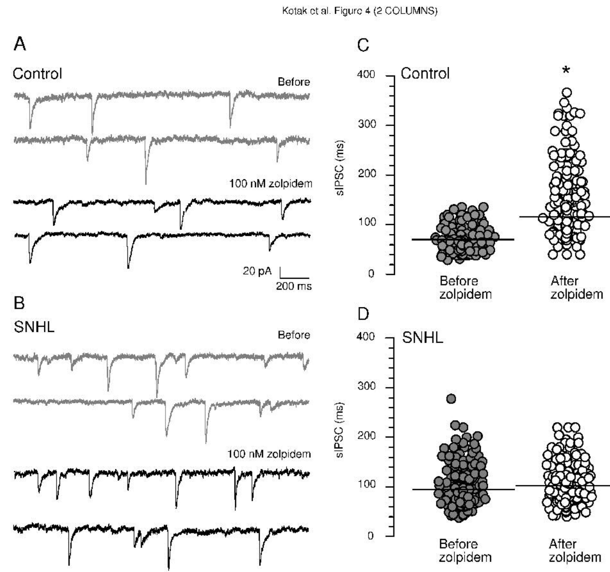

215x279mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60




215x279mm (600 x 600 DPI)

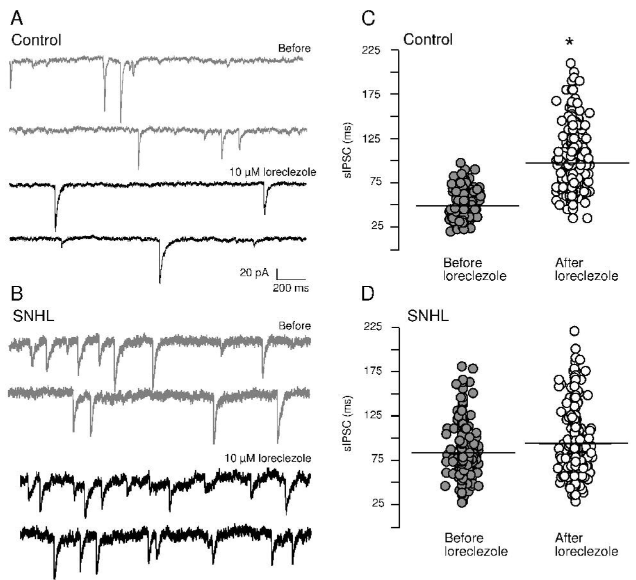
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60




279x215mm (600 x 600 DPI)

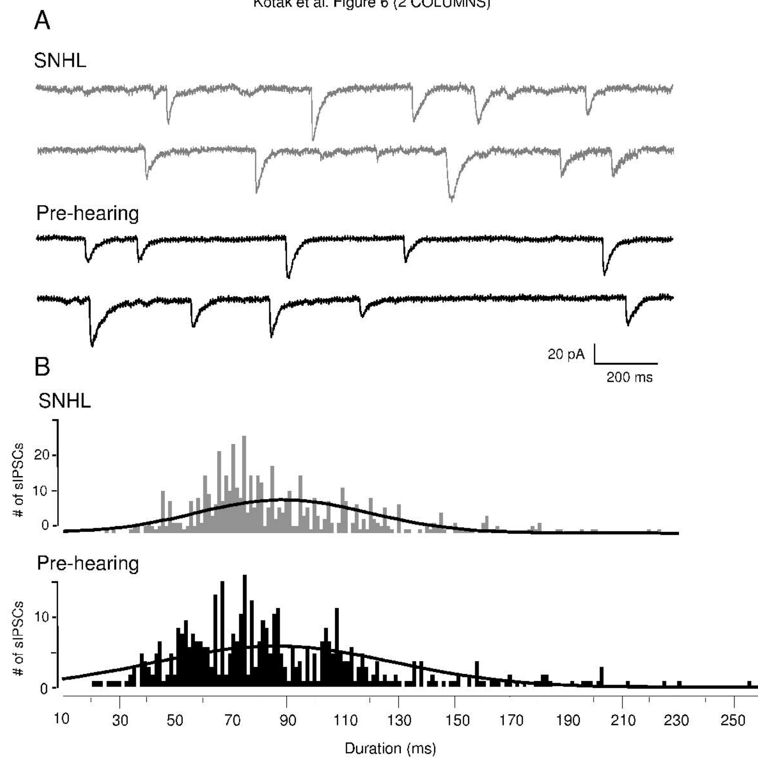
review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



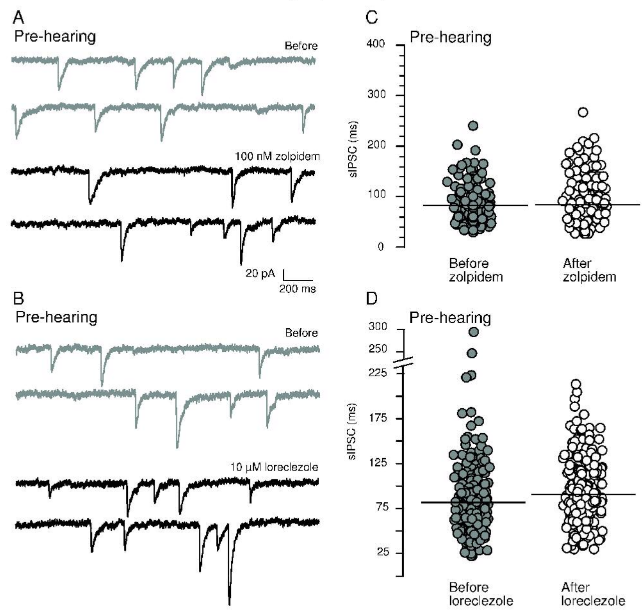

279x215mm (600 x 600 DPI)

review



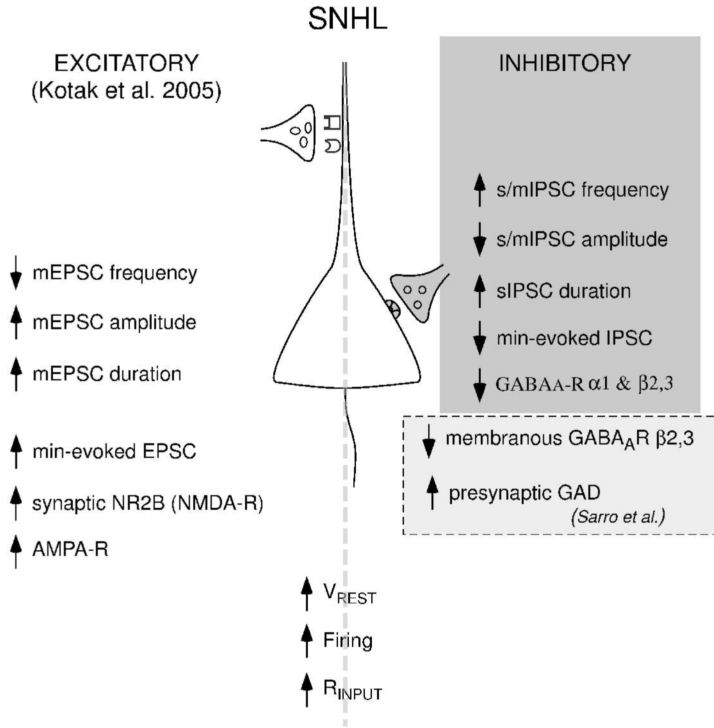

215x279mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60




279x215mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60




215x279mm (600 x 600 DPI)