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Research paper

Transient gain adjustment in the inferior colliculus is serotonin- and calcium-dependent

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ABSTRACT

In the inferior colliculus (IC), a brief period of acoustic conditioning can transiently enhance evoked discharge rate. The cellular basis of this phenomenon was assessed with whole cell current-clamp recordings in a gerbil IC brain slice preparation. The current needed to elicit a single action potential was first established for each neuron. A 5s synaptic stimulus train was delivered to the lateral lemniscus (LL), and followed immediately by the initial current pulse to assess a change in postsynaptic gain. The majority of IC neurons (66%) displayed an increase in current-evoked action potentials (Positive Gain). Despite the blockade of ionotropic glutamate receptors, this effect was correlated with membrane depolarization that occurred during the synaptic train. The postsynaptic mechanism for positive gain was examined by selective blockade of specific neurotransmitter receptors. Gain in action potentials was enhanced by antagonists of metabotropic glutamate, acetylcholine, $GABA_A$ and glycine receptors. In contrast, the gain was blocked or reduced by an antagonist to ionotropic serotonin receptors (5-HT_3R). Blocking voltage-activated calcium channels with verapamil also reduced the effect. These results suggest that 5-HT_3R activation, coupled with increased intracellular calcium, can transiently alter postsynaptic excitability in IC neurons.

1. Introduction

The modification of neural coding properties can last for periods of milliseconds to days. An example of auditory coding plasticity over a short interval (~10 ms) is the precedence effect (Blauert, 1972; Litovsky et al., 1999; Tollin and Yin, 2002). Neural correlates of this phenomenon, which involve a neuron's rapid change in sensitivity to an acoustic cue, have been identified in the IC (Burger and Pollak, 2001; Litovsky and Yin, 1998). There are also many studies that demonstrate an altered response to acoustic signals over longer intervals (⩾100 ms). Examples that are relevant to the present study include the increased neural responses observed following frequency modulation, or changes in phase or level that simulate acoustic motion (Sanes et al., 1998; Wilson and O'Neill, 1998; Malone and Semple, 2001). The altered discharge rate persists for hundreds of milliseconds to seconds, and suggests that a form of short-term plasticity exists in the IC (Finlayson and Adam, 1997; Kvale and Schreiner, 2004; Sanes et al., 1998; Spitzer and Semple, 1998; Thornton et al., 1999). It is not yet known whether this form of plasticity arises from intrinsic membrane properties, inputs from primary auditory nuclei, or inputs from neuromodulatory systems (Adams, 1979; Coleman and Clerici, 1987; Faingold, 1991; Sivaramakrishnan and Oliver, 2001). Therefore, the present study was designed to identify cellular mechanisms that could support these forms of short-term plasticity.

The central nucleus of the IC is a locus of synaptic integration in the central auditory system. Both monaural and binaural projections from the brainstem nuclei converge there, and their terminals release the excitatory and inhibitory neurotransmitters, glutamate, GABA and glycine (Glendenning and Baker, 1988; Saint Marie et al., 1999; Glendenning et al., 1992; Gonzalez-Hernandez et al., 1996; Zhang et al., 1998). Furthermore, the balance of excitation and inhibition in the IC is influenced by modulator neurotransmitters. Both acetylcholine and serotonin are present in the IC and their effects on auditory processing have been examined (Contreras and Bachelard, 1979; Hurley et al., 2002). Frequency tuning properties can be altered either by stimulation of ascending cholinergic afferents, or focal application of serotonin (Ji et al., 2001; Ma and Suga, 2003; Hurley and Pollak, 1999; Hurley and Thompson, 2001; Hurley, 2006; Hurley, 2007; Hurley et al., 2008). Indeed, activation of serotonin can have a variety of effects on the timing and magnitude of frequency tuning, due to the diversity of receptor subtypes present in the IC (Hall and Hurley, 2007; Hurley, 2007; Hurley et al., 2008). The recruitment of postsynaptic calcium currents in IC neurons may also support response enhancement. Rebound

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calcium-activated depolarizations can last for several hundred milliseconds in IC neurons (Sivaramakrishnan and Oliver, 2001), while other calcium currents have been shown to last for seconds (N'Gouemo and Morad, 2003).

Whereas much attention has been devoted to the cellular basis of short and long-term plasticity (e.g., paired-pulse depression or facilitation, long-term potentiation), little is known about intermediate forms of plasticity (Buonomano and Merzenich, 1998; Shin et al., 2001). In this paper, we describe a novel form of short-term plasticity in IC neurons, elicited by a brief period of synaptic stimulation in a brain slice preparation. Here we identify a serotonergic mechanism involving ionotropic serotonin receptors (5-HT_3) that causes a transient gain in current-evoked action potentials. We propose that transient states of increased excitability in IC neurons may underlie the short-term increases in discharge rates observed *in vivo*. Interestingly, an independent report (Bohorquez and Hurley, 2009) shows that a 5-HT_3 receptor-dependent mechanism operates during auditory processing in the mouse inferior colliculus.

2. Materials and methods

2.1. Brain slice

Gerbils (*Meriones unguiculatus*) aged postnatal day (P) 10–20 were anaesthetized with chloral hydrate (350 mg/kg), decapitated, and used to make 300–350 μm coronal brain slices through the IC. Slices were obtained at 4 °C for animals aged P10-12, and at room temperature (25 °C) for animals aged P13-20. A broad age range covering periods before and after hearing onset (~P12) were chosen to track developmental or use-dependent differences. All slices contained visible fibers of the lateral lemniscus (LL) ventral to the IC. The artificial cerebrospinal fluid (ACSF) contained (in mM): 125 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 24 $NaHCO_3$, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid (pH 7.3 when bubbled with 95% O_2/5% CO_2). Low Ca^{2+} ACSF contained 0.24 mM $CaCl_2$, and all other ingredients remained the same. The slices were continuously superfused in the recording chamber with oxygenated ACSF at 4 ml/min.

2.2. Electrophysiology

Whole-cell current-clamp recordings (Warner Instruments PC-501A) were obtained at room temperature (≈23 °C), with electrodes fabricated from 1.5 mm outer diameter borosylicate glass microcapillaries (10–15 MΩ). The internal pipet solution contained (in mM): 130 potassium gluconate, 0.6 EGTA, 10 Hepes, 2 $MgCl_2$, 5 KCl, 2 ATP, 0.3 GTP, 5 phosphocreatine (pH 7.2). All neuronal recordings were made in the position of the IC slice known to contain the heaviest SOC innervation (Beyerl, 1978; Brunso-Bechtold et al., 1981; Nordeen et al., 1983) and greatest neuronal density, defined as the central nucleus by Nissl, Golgi, and cell-myelin method (Faye-Lund and Osen, 1985). A GΩ seal was obtained, the membrane was ruptured, and electrode and access resistance were then compensated to about 70% (Kotak et al., 1998).

Each neuron was activated with 500 ms depolarizing current steps in 10 pA increments, to determine the threshold for a single action potential, and the suprathreshold firing pattern. We restricted our analyses to neurons that displayed a repetitive action potential pattern in response to suprathreshold current pulses, either with an initial firing burst, or without (Fig. 2A and B). Spike adaptation was often observed during the current pulse, similar to rat IC neurons (Sivaramakrishnan and Oliver, 2001). Neurons with onset and transient discharge patterns were excluded because their intrinsic membrane properties do not permit an increase in the number of action potentials following a synaptic train stimulus.

After a 5–10 min rest, each neuron was also characterized with 500 ms, 10 pA steps of hyperpolarizing current. The presence or absence of rebound-spiking and delayed rectifier currents (sag current, or I_h) was recorded for subsequent analyses.

2.3. Synaptic stimulation paradigm

To assess the influence of afferent activity on postsynaptic gain, a paradigm was designed which tested the excitability of a neuron after discrete periods of synaptic stimulation. A 500 ms depolarizing current pulse was first injected into each IC neuron, and the pulse amplitude was adjusted to elicit a single action potential. A train of 200 μs stimulation pulses was then delivered to the LL at 100 Hz for 5 s, a form of tetanic synaptic stimulation. Immediately after the synaptic stimulation, the original intracellular current pulse was again injected into the IC neuron. To follow the time course of changes in current-evoked action potentials, the current pulse was redelivered at 30 s intervals, until the response returned to the pre-tetanic level (Fig. 1A). No action potentials were elicited during the train of synaptic stimuli.

To facilitate comparison with earlier *in vivo* results (Sanes et al., 1998; Malone and Semple, 2001), the synaptic stimulation was designed to approximate acoustic conditioning stimuli, which lasted one or more seconds. In the slice model, the stimulation rate of 100 Hz approximates acoustically driven rates of afferent fibers to the IC. A five-second duration was chosen based on preliminary findings that shorter stimulus durations were occasionally ineffective.

Although a range of current magnitudes could elicit a single action potential, we chose the minimum current necessary. By defining the current magnitude at this lower end of spike threshold, estimates of gain remained conservative. Any increase in the action potential number after the synaptic stimulation period would be less likely to fall within a range of response variability, and therefore more likely to reflect a true postsynaptic change. The stimulation paradigm was also designed to minimize response rundown during the course of the experiment. At the first sign of rundown, indicated by either depolarized resting potential or action potential broadening, data acquisition from that neuron was terminated. Thus, after each tetanic stimulation paradigm and subsequent recovery series of test pulses, the neuron was left unstimulated for 10–20 min prior to retesting with a pharmacological agent.

2.4. Pharmacological manipulations

Brain slices were bathed in 4 mM kynurenic acid (KYN) (Sigma) to block ionotropic glutamatergic receptors (Moore et al., 1998) and prevent over-stimulation of the neurons. Specific pharmacological agents were delivered in ACSF at the following concentrations: bicuculline (BIC), 30 μM (Sigma); strychnine (SN), 1 μM (Sigma); DNQX, 0.5 μM (Tocris); AP5, 1 μM (Tocris); a-methyl-4-carboxyphenylglycine (MCPG), 1 μm (Tocris); SCH-50911, 20 μM (Tocris); atropine, 1 μM (Sigma); mecamylamine (Mec), 5 μM (Sigma); LY-278,584, 1 μm (Sigma); verapamil, 20 μM (Sigma); 2-methyl-5-hydroxytryptamine maleate (2-M-5HT), 30 μM (Sigma).

2.5. Data analysis

The responses during synaptic stimulation were measured and analyzed offline with the Igor Slice Analysis Macro (http://www.cns.nyu.edu/~sanes/slice_software/). As a neuron's voltage response during afferent stimulation was obscured by the stimulation artifacts, these artifacts were removed (Fig. 3). The artifacts at 1–3 ms after each stimulus onset were deleted, and the adjacent

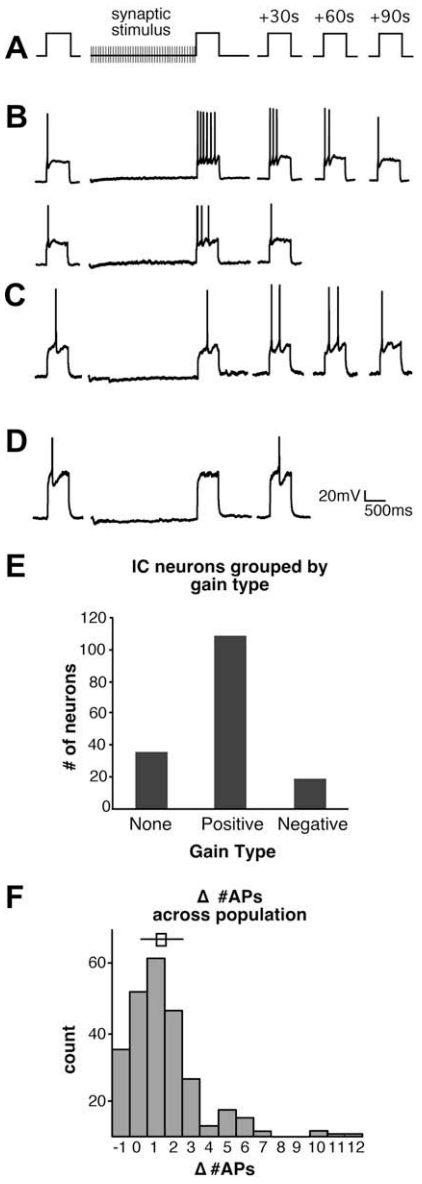

Fig. 1. The gain phenomenon and the distribution of gain types. Afferent stimulation can transiently alter the response of IC neurons. (A) The stimulation paradigm tested action potential response before and after a seconds-long synaptic train, in the presence of Kynurenic Acid (KYN). A control intracellular current pulse is set to evoke one action potential (left). When preceded by a train of synaptic stimuli to the LL (synaptic stimulation period; 5 s, 100 Hz), the same current pulse is redelivered to assess gain change. The current pulse is repeated every 30 s until the response returns to control level. (B–D) Examples of gain change after the LL stimulation. (B) Positive gain change is an increase in the # of current-evoked action potentials. The time to recover can last minutes (top row), or seconds (bottom row, a different neuron). (C) Positive gain can also be delayed until the second current pulse after the synaptic train. (D) The loss of action potential in the post-train current pulse is termed Negative Gain. (E) Summary of the different types of gain effect, among all neurons. Sixty-six percent (109/164) showed an increase in current-evoked action potentials (Positive Gain). The remainder showed a decrease (Negative Gain, 12%), or no change in action potentials (None, 22%). The synaptic stimulation artifact is removed in this and subsequent figures, for better viewing of the membrane potential during the synaptic stimulation period. Artifact removal is explained in Fig. 3. (F) The gain in number of action potentials (Δ # APs) across the neuronal population. In this distribution, the mean gain following a synaptic train was 1.8 ± SE 0.13 APs, denoted by —□—. Increases in APs (Positive Gain) could be large, up to 12 APs. Negative gain count is shown in the −1 bin. This distribution reflects a bias towards Positive Gain among IC neurons recorded in this study.

segments of the trace were joined. Changes in membrane potential (V_m) and the duration of these changes were measured directly from traces in Igor. Statistical comparisons were performed in JMP 5.0, using multivariate ANOVA, and Student's *t*-test (alpha = 0.05). Unless otherwise noted, all values are reported as means ± standard error of the mean (SE).

All protocols were reviewed and approved by the New York University Institutional Animal Care and Use Committee.

3. Results

3.1. Synaptic stimulation influences postsynaptic gain

The stimulus paradigm was designed to assess a postsynaptic change in the response to current pulses, following short periods of synaptic activity. The initial design of the present *in vitro* experiments was motivated by the hypothesis that short-term changes in gain of IC neurons was dependent on synaptic inhibition intrinsic to the IC Sanes et al., 1998). Therefore, all recordings were performed in the presence of KYN, which blocks ionotropic glutamatergic neurotransmission.

As illustrated in Fig. 1A, a control current pulse was delivered at spike threshold, and was repeated three times to establish fidelity of the response (repetitions not shown). A train of stimuli was then delivered to the LL afferent pathway, followed by a series of current pulses to reassess postsynaptic gain. An elevated response was termed Positive Gain (Fig. 1B). A subset of neurons displayed an elevated response only after a 30 s delay (Fig. 1C). A minority of neurons tested (12%) exhibited a transient suppression of the current-evoked response (loss of action potential), which recovered within 30–250 s ($n = 32$), and was termed Negative Gain (Fig. 1D). Approximately 20% of tested neurons displayed no change in the current-evoked response after the synaptic stimulation train (No Gain, $n = 36$).

In 66% of IC neurons studied ($n = 164$), the number of current-evoked action potentials increased following the synaptic train. This value includes neurons that displayed an increased gain immediately or after a 30 s delay. Thus, a majority displayed Positive Gain (Fig. 1E). This elevated response returned to baseline (i.e., one spike) within 30–250 s. For all neurons tested, the average change in number of current-evoked action potentials (Δ) was +1.8 ± 0.13 action potentials (Fig. 1F), including Negative and No Gain effects.

Similar distributions of each gain effect were observed whether or not the neuron displayed an initial burst of action potentials (Fig. 2A). To establish the discharge pattern of each neuron, the response to a range of 500 ms depolarizing current steps was obtained. No correlation was found between suprathreshold discharge pattern and gain type (chi-square likelihood ratio = 13.1, $p > 0.05$) (Fig. 2B).

Neurons were also classified by their response to 10 pA, 500 ms hyperpolarizing current steps. While both rebound-spiking and delayed rectifier currents (sag current, or I_h) were present in the population of recorded IC neurons, no correlation was observed between these intrinsic membrane properties and gain category (chi-square, $p > 0.05$) (Fig. 2C and D).

Recordings were made from IC neurons in a range of postnatal ages, to determine whether gain effects emerged during development. Interestingly, all types of gain were present around the time of hearing onset (P10-13), and the distribution of gain effects did not differ significantly from P14-20 neurons (pairwise *t*-test, $p > 0.05$).

3.2. Effect of synaptic stimulation on membrane potential

Afferent stimulation artifacts were removed from each trace prior to analysis, as described in Section 2 (Fig. 3A). During the period of synaptic stimulation, membrane potential was typically

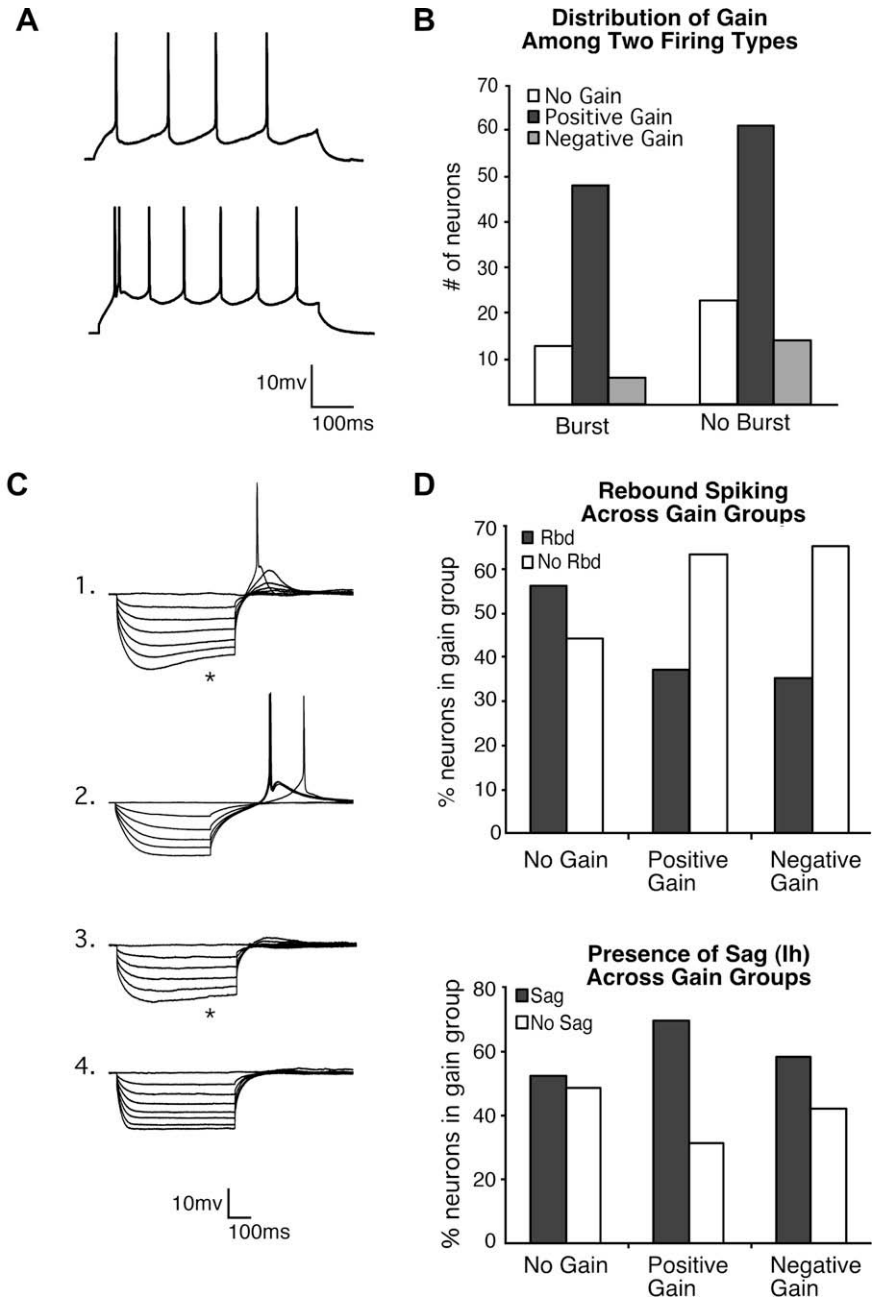

Fig. 2. (A) Examples of two different suprathreshold firing patterns: burst (top) and no burst (bottom). (B) Distribution of gain categories within each suprathreshold firing type. Similar distributions of each gain effect were observed in each firing type. No correlation was found between suprathreshold discharge pattern and gain type (chi-square likelihood ratio = 13.1, $p > 0.05$). (C) Four types of responses to hyperpolarizing steps of current are shown. Included in the dataset were neurons that showed rebound spikes to steps of hyperpolarizing current (C1 and C2), and those that did not (C3 and C4). In both rebound (Rbd) and no rebound (No Rbd) cells, a hyperpolarization-activated depolarizing current (I_h) was observed, noted by a "sag" shape at the largest hyperpolarization current steps (C1 and C3, *). Many cells exhibited neither rebound spikes nor sag current (C4). (D) The distribution of cell characteristics described in C, across gain types. No correlation was found between these intrinsic membrane properties and gain type.

displaced from rest. The majority of recorded neurons displayed a hyperpolarizing component (61%), whereas about one third (35%) displayed both hyperpolarizing and depolarizing components. The hyperpolarizing component appeared during at the outset of the stimulus train, and typically returned to the resting membrane potential before the end of the train.

To determine whether the magnitude and duration of hyperpolarizing and depolarizing segments were correlated with gain effect, analyses of deflections from V_{rest} (resting membrane potential) during the synaptic stimulation train were performed (Fig. 3B). Both the maximum amplitude and duration of each component were measured and compared between four gain categories: No Gain, Negative Gain, Positive Gain of 1–2 action potentials (1–2 APs), or Positive Gain of >2 action potentials (>2 APs). The magnitude and duration of membrane depolarization were largest in the Positive Gain group (Fig. 3C, grey bars). In contrast, the duration of hyperpolarization was longest for neurons that displayed Negative Gain (Fig. 3C, black bars).

To determine whether the gain effect was dependent, on a depolarizing shift specific to synaptic stimulation, a tonic 5 pA current was injected during the 5 s pre-test pulse period, in place of synaptic stimulation. Positive gain neurons ($n = 3$) were allowed

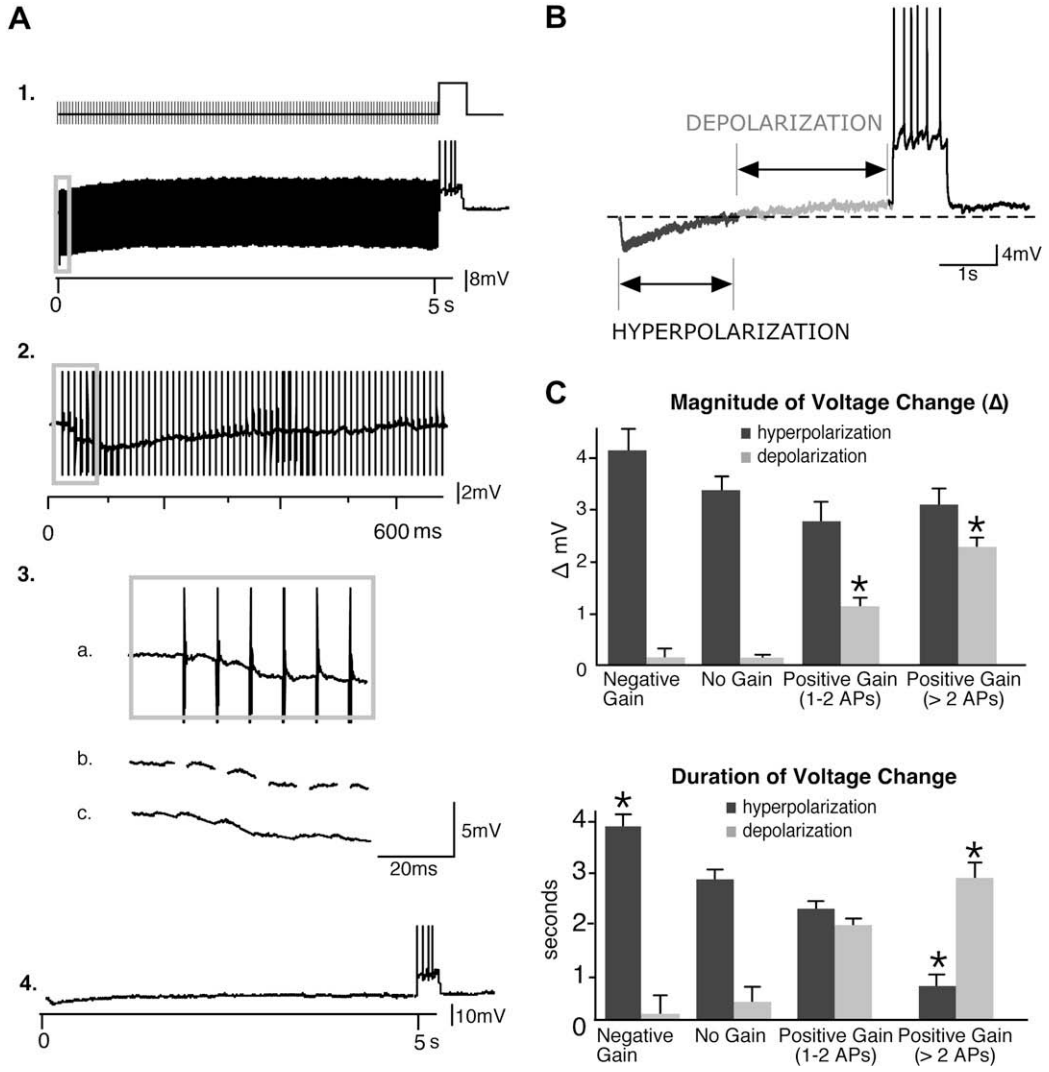

Fig. 3. (A) Demonstration of stimulus artifact removal. To facilitate measurement of membrane potential during the synaptic stimulation period, the stimulus artifact was removed. The duration of the stimulus artifact for each trace was measured by expanding the trace in the time domain, and then evaluating the difference between the large voltage artifact portion and the neuronal membrane response. The grey box in A1 corresponds to the same portion of the trace boxed in A2 and A3, where it is expanded further in the time domain. The stimulus artifact was removed with a chop command in Igor (A3b), then the trace was interpolated with a straight line between each segment (A3c). Panel A4 shows the same trace as in A1, after artifact removal. The creation of a continuous line in the trace was necessary for measurements of voltage amplitude and duration during the synaptic stimulus. (B) Example trace of deflections from V_{rest} during the synaptic stimulation train. For this cell, the 5 s, 100 Hz stimulation of the LL afferents in the presence of 4 mM Kynurenic acid (KYN, 4 mM) initiates a hyperpolarization from V_{rest} (hyperpolarization phase, black). As the synaptic train persists, a deflection back toward V_{rest} occurs. Gradually, the potential rises above V_{rest} (depolarization phase, grey), and stays there until the synaptic train ceases and the current pulse begins. The cell showed positive gain in action potentials following the synaptic train. (C) Measurements of magnitude and duration of voltage changes across gain subtypes. Deflections from V_{rest} were quantified for each cell and sorted by gain type. Gain types are defined as follows, for this and subsequent figures: No gain = no change in current-evoked action potential (AP) response after synaptic stimulation, Negative Gain = loss of AP in current-evoked response, Positive (1–2 AP) = increase of 1–2 action potentials, Positive (>2 AP) = positive gain of >2 action potentials. *Top graph*: the average magnitude of voltage change (ΔmV) during both the hyperpolarization (dark grey) and depolarization (light grey) phases of the synaptic stimulation period. *Bottom graph*: the duration of hyperpolarization and depolarization phases during the stimulation period. The trend in hyperpolarization and depolarization follows a similar pattern across gain type, for both magnitude and duration of voltage. Compared to the No Gain type, the Positive Gain type has larger measures of depolarization, and the Negative Gain type has larger measures of hyperpolarization. Neurons showing both types of Positive Gain (1–2 or >2 APs) had significantly larger amplitudes of depolarization, as compared to the No Gain type (ANOVA, $F = 13.16$, $p < 0.001$). The duration of depolarization was also significantly larger for the Positive Gain (>2 AP) type (ANOVA, $p < 0.001$). In addition, the duration of the hyperpolarization phase was significantly shorter for the Positive Gain (>2 AP) gain type (ANOVA, $F = 6.11$, $p < 0.0002$). Measures with significant difference from the No Gain type are indicated with an asterisk (*).

to recover for >20 min, and the depolarizing current injection alone was delivered. As illustrated in Fig. 4A, this stimulus did not change the number of action potentials evoked by the subsequent current pulse for any of the cells tested. As expected, when a tonic current injection persisted beyond the synaptic stimulation period, and throughout the current test pulse, there was an increase in the evoked response (Fig. 4B).

For both the Positive and Negative gain groups, the mean recovery time was 57 s. The recovery of Positive Gain varied widely (80 ± 57 s), and was significantly longer than that of the Negative Gain (40 ± 29 s) (ANOVA, $F = 21.4$, $r = 0.527$, $p < 0.001$). No significant correlations were found between age groups and recovery time (ANOVA, $p > 0.05$).

3.3. Lemniscus-evoked neurotransmission in the IC

We examined the role of several neurotransmitter systems with receptor-specific antagonists (Table 1). To determine which synaptic afferents were involved in the recruitment of the Positive Gain effect, we first focused on three major ionotropic systems known to

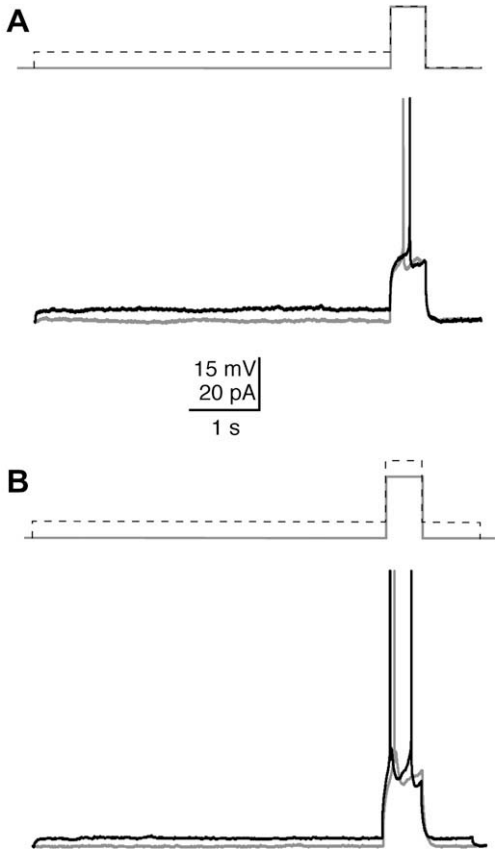

Fig. 4. Lack of positive gain effect with imposed depolarization. (A) Replacing the synaptic tetanus with a depolarizing current step does not cause a gain in APs. A current pulse (top, —) delivered at threshold elicited a single action potential (bottom, grey voltage trace). Delivery of a 5 pA tonic current (top, ————) did not change the evoked response (bottom, black voltage trace, superimposed). (B) Extending the tonic 5 pA current through the current pulse did increase the evoked response. A current pulse (top, —) delivered at threshold elicited a single action potential (bottom, grey voltage trace). Delivery of a 5 pA tonic current that persists throughout the test period and beyond the current pulse (top, ————) leads to an increase in the evoked response (bottom, black voltage trace, superimposed).

be present in the LL input pathway: GABA, glycine, and glutamate. Previous studies *in vivo* using focal application of GABA and glycine in the IC showed that single unit responses were briefly elevated following the cessation of a pulse of inhibitory neurotransmitter (Sanes et al., 1998). This post-inhibitory enhancement lasts up to 10 s, and implicates a GABAergic or glycinergic receptor-mediated mechanism. In the IC brain slice, blockade of inhibition with BIC and SN did not prevent the positive gain after synaptic stimulation ($n = 8$). In fact, this manipulation enhanced the positive gain effect observed prior to inhibitory antagonist treatment in five of the neurons tested (Fig. 5). Blockade of metabotropic $GABA_B$ receptors with the antagonist, SCH-50911 (20 μM), was also ineffective at reducing positive gain ($n = 4$).

Since ionotropic glutamate receptors were blocked in all experiments, they played no role in this effect. However, as an internal control, KYN was replaced by specific antagonists to AMPA and NMDA receptors (CNQX and APV). No change occurred in the gain effect under these receptor-specific manipulations ($n = 3$). Addition of MCPG (1 μM), a group I and II metabotropic glutamate receptor antagonist, also failed to block the positive gain ($n = 5$).

To test whether cholinergic pathways were involved, experiments were performed in the presence of either a nicotinic antagonist (5 μM mecamylamine) or a muscarinic antagonist (1 μM atropine) (Fig. 6). Neither of these agents was able to block the synaptically-induced increase in action potentials. In fact, each of these antagonists enhanced the gain effect in 5 of 7 neurons tested.

3.4. Synaptically-evoked serotonin mediates positive gain

To determine whether the LL-evoked synaptic response in IC neurons contained a serotonergic component, a 5-HT_3 receptor antagonist (LY-278, 584 at 1 μM) was added to the ACSF (KYN was not present). The LL-evoked EPSP amplitude decreased following application of LY-278,584, indicating the presence of ascending serotonergic afferents to the IC ($n = 3$) (Fig. 7A). Furthermore, in the absence of any synaptic stimulation, bath application of a 5-HT_3 agonist, 2-M-5HT (30 μM) enhanced the current-evoked discharge compared to control pulses (Fig. 7B).

To determine whether LL-evoked serotonergic transmission initiates the gain effect, slices were bathed in LY-278,584 prior to delivery of a 5 s afferent stimulation train. This 5-HT_3 antagonist led to a marked decrease or elimination of positive gain in 6 of 8 IC neurons tested (Fig. 8). Furthermore, the drug effect was reversible after a 30 min washout interval. Unlike all other pharmacological agents tested, the 5-HT_3 antagonist reduced the steady depolarization that occurred during the synaptic stimulation train (Fig. 8, bottom).

3.5. Role of calcium in positive gain

The 5-HT_3 receptor-coupled channel is permeable to Na^+ and Ca^{2+} ions (Barnes and Sharp, 1999), so other mechanisms that permit Ca^{2+} influx could also facilitate the positive gain effect. To

Table 1
Quantification of pharmacological antagonist effects on key gain parameters.

Drug	Site of block	Change in depolarization duration (ms)	Change in depolarization magnitude (mV)	Change in number of action potentials
LY-278,584	5-HT_3R	**−440 ± 150***	**−1.10 ±.17***	**−2.4 ± 1.4***
Verapamil	L-type Ca^{2+} channels	−250 ± 70	**−0.80 ±.001***	−1.0 ± .00
LY-278,584 + Verapamil	5-HT_3R & L-type Ca^{2+} channels	−367 ± 67	**−0.93 ±.07***	−1.3 ± .33
Bic, SN	GABAR, GlyR	+1.33 ± .213*	+0.96 ± .05*	+2.6 ± .86*
Atropine	mAChR	+1.06 ± .107*	+1.15 ± .01*	+2.0 ± 1.2
Mecamylamine	nAChR	+1.40 ± .058*	+1.00 ± .52*	+2.0 ± .58
MCPG	mGluR	+4.08 ± .116*	+1.98 ± .55*	+0.8 ± .37
DNQX, AP5	AMPAR, NMDAR	+0.30 ± .208*	0	0

For each parameter, the change from pre-drug value (in KYN) was calculated. For each value, "+" indicates an increase, and "−" indicates a decrease. All values are mean difference ± standard error of the mean. A significant change from the pre-drug value is indicated with an asterisk (*$p < 0.05$). LY-278,584 and verapamil were the only drugs that led to a *decrease* for all parameters, many of which were significant (upper section, bold). LY-278,584 and verapamil were also the only drugs that reduced the number of action potentials in the gain effect (last column). All parameters in each column heading have a significant association with positive gain. *Abbreviations:* 5-HT_3R, serotonin 3 receptor; GABAR, gamma-aminobutyric acid receptor; GlyR, glycine receptor, mAChR, muscarinic acetylcholine receptor; nAChR, nicotinic acetylcholine receptor; mGluR, metabotropic glutamate receptor; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; *N*-methyl-D-aspartic acid receptor; Bic, bicuculline; SN, strychnine; MCPG, a-methyl-4-carboxyphenylglycine; DNQX, 6,7-dinitroquinoxaline-2,3-dione; AP5, D(−)-2-amino-5-phosphonopentanoic acid.

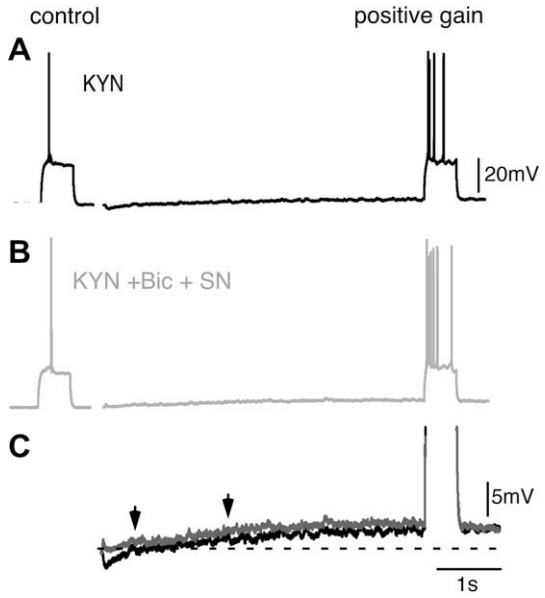

Fig. 5. Blocking GABA and glycine receptors does not eliminate the positive gain effect. (A) In this neuron, the control response to a current pulse at action potential threshold is shown before drug treatment (left, control trace, KYN). Immediately after the synaptic stimulation, the neuron exhibited positive gain, an increase in the number of current-evoked action potentials (right, positive gain). The neuron subsequently recovered to control level (not shown). (B) Addition of BIC and SN to block ionotropic GABA and glycine receptors enhances the gain response. (C) A closer look at the superimposed responses during synaptic stimulation in A and B shows a more depolarized membrane potential (arrows) in the presence of BIC + SN (grey).

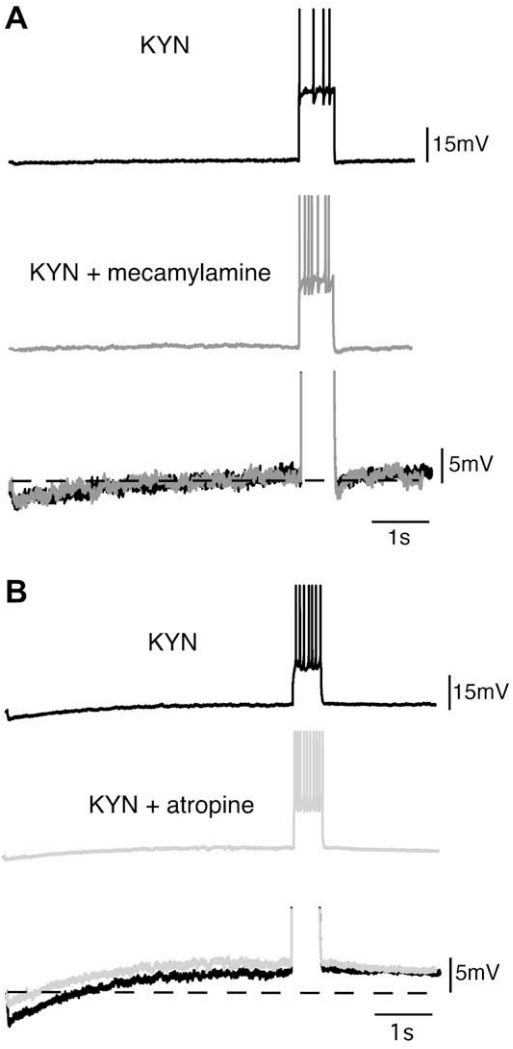

Fig. 6. Blocking Ach receptors does not eliminate the Positive Gain effect. (A) A neuron exhibits Positive Gain under conditions of blocked ionotropic glutamate (KYN, top, black trace). Application of mecamylamine, a nicotinic receptor antagonist, does not eliminate the Positive Gain. In fact, an increase in the gain is observed (middle, grey trace). Superimposition of both traces shows little difference, despite the larger number of action potentials elicited by the current pulse. (B) With application of atropine (grey trace), there is a similar increase in Positive Gain, and a V_m depolarization can be seen in the grey trace (bottom). For simplicity, the preceding control pulse (single action potential) is not shown.

examine the role of Ca^{2+}, experiments were performed in a low Ca^{2+} ACSF ($n = 3$). Though synaptic activation was present in 0.24 mM Ca^{2+} ACSF, there was an obvious reduction of positive gain in each neuron tested (Fig. 9A). Since this result cannot rule out a possible reduction in presynaptic release, the involvement of Ca^{2+} channels during synaptic stimulation was examined directly, with the L-channel blocker, verapamil (20 μM). In 3 of 5 neurons, verapamil reduced the duration of the positive gain effect; recovery time to pre-stimulation levels was shortened (Fig. 9B). In two neurons, the positive gain was completely eliminated. For the three cells in which LY-278,584 did not eliminate the positive gain, additional co-application of verapamil was able to eliminate the gain response. Thus, verapamil attenuated some aspect of the positive gain effect each time it was applied. The complementary effects of these two drugs are summarized in Fig. 9C.

The amplitude and duration of depolarization from V_{rest} that occurs during the synaptic train (Fig. 3), was examined for neurons treated with verapamil and LY. Direct measurements of gain in number of action potentials were also compared. Only verapamil and LY showed a consistent reduction in *all* of these parameters (Table 1).

4. Discussion

Previous studies demonstrated that the neural response to an acoustic cue depends on the recent history of stimulation (Malone and Semple, 2001; McAlpine et al., 2000; Spitzer and Semple, 1993, 1998). A period of stimulation with a specific sound level, frequency, or interaural phase can enhance the subsequent discharge rate of an IC neuron when any of these parameters are varied. More importantly, the enhanced discharge rate can persist for several seconds. Since persistent enhancement of discharge rate could influence the representation of all ongoing auditory stimuli, such as speech, the physiological basis for this short-term plasticity will be fundamental to our understanding of IC coding.

4.1. Characterization of the gain effect

Investigation of *in vitro* mechanisms that support acoustic conditioning led to the discovery of a novel form of short-term plasticity in IC neurons. After short periods of synaptic stimulation, there was an increase in the number of action potentials evoked by a current pulse, indicating a temporary change in postsynaptic excitability. This positive gain was observed in the majority of recorded neurons (66%), although negative gain did exist in a minority (12%) of neurons (Fig. 1E), and another minority displayed no change in gain (22%). Subsequent analysis showed that the positive gain effect is associated with a transient depolarization elicited by the synaptic train. These positive gain effects did not depend on the presence of either an outward cation current (I_h) that rectifies large negative changes from V_{rest}, or rebound action

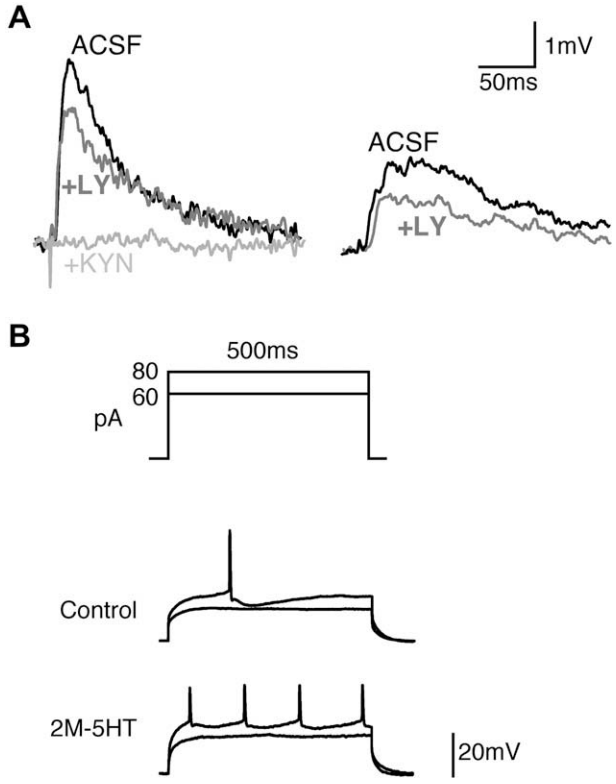

Fig. 7. (A) Effect of 5-HT_3 receptor antagonist LY-278,584 (LY) on evoked EPSPs. In both examples, a single stimulus pulse to the LL elicits an EPSP (black traces, ACSF). After delivery of LY, the maximum EPSP amplitude was reduced by 25% (dark grey traces, +LY), indicating that serotonergic afferents are activated by LL stimulation. For the neuron on the left, addition of KYN eliminated the remaining EPSP (left, light grey trace, +KYN). Each trace is the average of 3 evoked responses. (B) A 5-HT_3 agonist affects spike threshold. Top: Two current steps (60 and 80 pA) are delivered to an IC neuron, near AP threshold. Middle: An IC neuron responds to the 80 pA pulse with one AP. Bottom: After bath application of the 5-HT_3 agonist, 2 M-5HT, the 80 pA pulse yields more APs, showing an increased excitability in the neuron. The membrane potential was depolarized by 4.7 mV after application of 2 M-5HT.

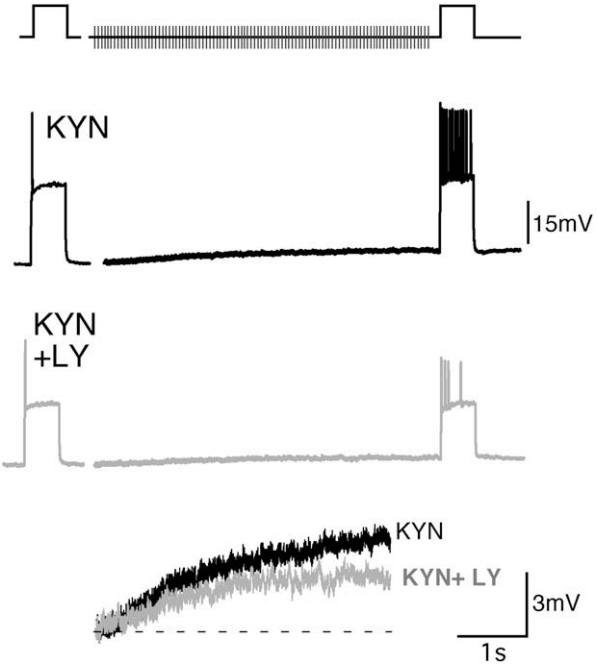

Fig. 8. The 5-HT_3 receptor antagonist LY-278,584 (LY) decreased the Positive Gain effect. In response to the stimulus paradigm (top row), the control response to a current pulse at action potential threshold (20 pA) is shown (middle row, left). A synaptic stimulus train to the LL pathway caused a large increase in the current-evoked response (middle, right), a Positive Gain in the presence of KYN. Following recovery, LY application did not alter the current-evoked response at threshold (grey trace, left). However, LY decreased the Positive Gain effect after synaptic stimulation (grey trace, right). A closer look at the membrane potential during the stimulus train (bottom row) shows that the gradual depolarization during synaptic stimulation is reduced in the presence of LY.

potentials (Fig. 2). Therefore, while certain intrinsic properties could add to the positive gain effect, they are not necessary for initiating it.

Periods of hyperpolarization and depolarization that occurred during the synaptic train suggested that a postsynaptic mechanism could underlie positive gain effects. In most cases, the magnitude of depolarization did predict a positive gain effect (Fig. 3C). While the hyperpolarizing forces are at least partly attributable to GABA and glycinergic transmission, the source of the depolarization is not ionotropic glutamatergic transmission, because KYN was present throughout the experiments. Furthermore, current injection controls (Fig. 4A) indicate that depolarization during the synaptic stimulation period is not sufficient to elicit a Positive Gain. Rather, this depolarization must be recruited by a seconds-long synaptic drive, and outlast the synaptic activation period. Thus, a persistent depolarization, when coupled with the depolarizing pulse, does lead to a greater output (Fig. 4B).

4.2. Pharmacological assessment of the positive gain effect

The lateral lemniscus is the primary ascending pathway to the IC and contains axons from many brainstem nuclei (Adams, 1979; Coleman and Clerici, 1987; Faingold, 1991; Oliver, 1991). Pharmacological manipulation of postsynaptic responses in the IC have also demonstrated the presence of a range of neurotransmitter systems, including serotonin (Faingold, 1991). Given this diversity, stimulation of the LL pathway at the ventral aspect of the IC could activate several neurotransmitter systems. Therefore, the chemical basis of positive gain effects were assessed with neurotransmitter antagonists.

All non-serotonin antagonists tested in this study were unable to block the Positive Gain effect. In contrast, incubation with BIC and SN led an *increase* in positive gain, indicating that GABA and glycinergic systems may serve to dampen the increase in gain observed following afferent stimulation (Fig. 5). Similarly, blockade of muscarinic and nicotinic acetylcholine receptors facilitated the positive gain effect. This result can be explained if the site of activation for acetylcholine in the IC is primarily on GABAergic synaptic terminals. Indeed, activation of muscarinic receptors upregulates the frequency of spontaneous GABAergic postsynaptic currents in the IC (Yigit et al., 2003), so it is likely that the increase in Positive Gain effects with atropine is due to down regulation of synaptic inhibition. Moreover, *in vivo* iontophoretic application of nicotinic antagonists increases discharge rates in IC neurons (Habbicht and Vater, 1996). As nicotinic receptors are known to gate calcium and facilitate postsynaptic depolarization, it is possible that their site of activation is also inhibitory synaptic terminals. Therefore, we cannot exclude disynaptic effects within the IC.

The IC contains a heterogeneous neuronal population, and the variability of pharmacological effects could reflect intrinsic differences among the recorded neurons, although each tested neuron displayed repetitive firing (Fig. 2). Since positive gain did occur in neurons that did not display rebound potentials, it is unlikely that our findings are due solely to Ca^{2+}-dependent rebound depolarization or anode-break excitation (Sivaramakrishnan and Oliver, 2001). The variability of the pharmacological effects may be partially explained by variability in membrane characteristics, such

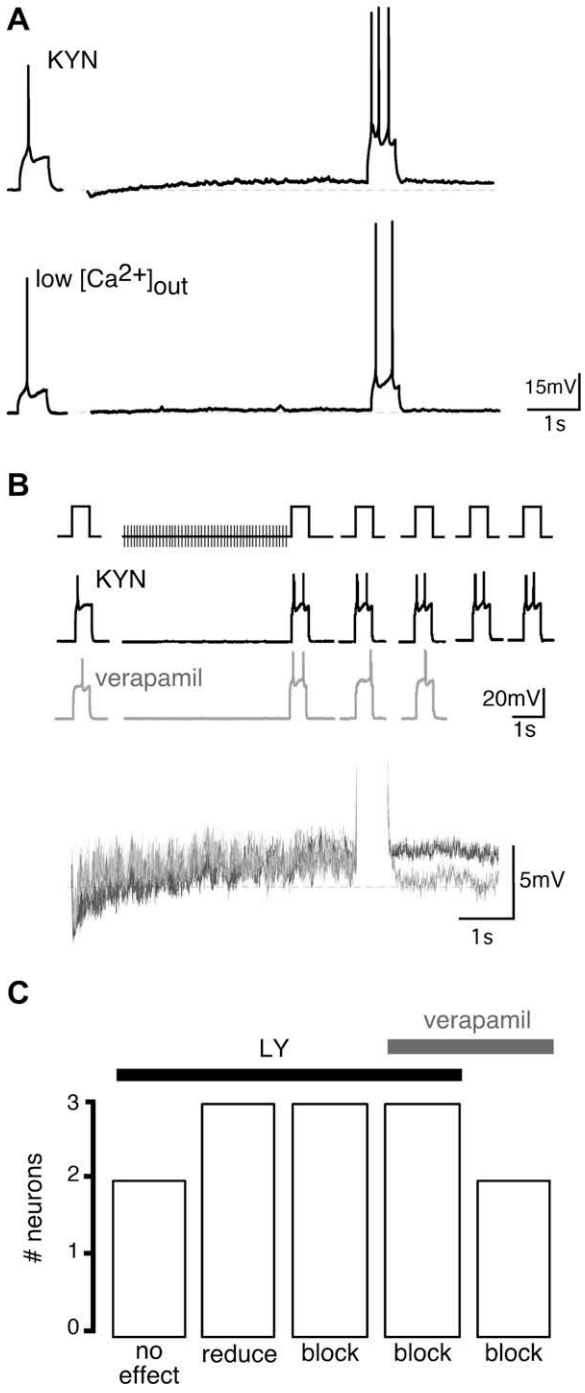

Fig. 9. (A) Lowering extracellular calcium to 240 μM reduces, but does not eliminate, Positive Gain. In KYN, the synaptic train increases the current-evoked response from 1 to 3 action potentials (top row). Following recovery, the gain effect in low Ca^{2+} is reduced to two action potentials. Note that the membrane potential change during the 5 s synaptic train is also reduced by the manipulation (flatter trace during synaptic stimulation, bottom row). (B) Verapamil reduces the duration of the gain effect. In KYN, the neuron displayed Positive Gain following the synaptic stimulation train (black). In the presence of verapamil (grey), the enhanced response remains, but recovery is shortened to <30 s. The persistent membrane potential depolarization observed in KYN (bottom row, black) was reduced by the addition of verapamil (bottom row, grey, superimposed). (C) LY and verapamil have additive effects on Positive Gain. In 6/8 cases, LY blocked or reduced the effect. For three cells in which LY did not block the effect, addition of verapamil (grey bar) led to complete blockade (overlap of black and grey lines). In some cases, verapamil alone eliminated the effect. These results indicate that both the 5-HT_3 receptor and voltage-gated calcium channels are involved in the Positive Gain effect.

as neurotransmitter receptor and Ca^{2+} channel density. Despite the similarity of results for animals of different ages, we must acknowledge that all recordings were obtained during a relatively early period of development, and many synaptic and biophysical properties do not yet display mature characteristics in the auditory central nervous system (Sanes, 1993; Balakrishnan et al., 2003: Scott et al., 2005; Vale and Sanes, 2000; Oswald and Reyes, 2008). Therefore, it is possible that the 5-HT_3R-dependent mechanism described above may display different kinetics in adults.

4.3. Serotonergic influence within the IC

Serotonergic fibers in the IC originate primarily from the dorsal raphe, and follow the LL afferent pathway to both the external cortex and central nucleus, where they display a wide distribution (Klepper and Herbert, 1991; Thompson et al., 1994). The existence of serotonin at synaptic sites is also supported by the morphological structure of serotonin-immunoreactive varicosities in the neuropil, some of which are apposed closely to IC cell bodies (Hurley and Thompson, 2001; Hurley et al., 2002). Indeed, serotonin fibers are found throughout the rodent SOC nuclei (Thompson and Hurley, 2004) and are known to modulate synaptic transmission in the LSO (Fitzgerald and Sanes, 1999).

A functional influence of serotonin on action potential generation in the brain slice preparation is consistent with previous observations *in vivo*. Local iontophoresis of serotonin into the IC can facilitate or depress discharge rates in both spontaneous firing *and* acoustically driven responses to modulated sounds (Hurley and Pollak, 1999). Even more interesting is the observation that local serotonin iontophoresis can increase the *duration* of responses to FM sweeps up to 3-fold, indicating a post-stimulus enhancement in excitability which outlasts acoustic stimulation (Hurley and Pollak, 1999). Serotonin iontophoresis is also more likely to enhance neural responses to FM sweeps than to pure tones, in a single neuron. This observation suggests serotonin preferentially affects acoustic signals that change over time.

Recent studies of pharmacologically-controlled serotonergic receptor activation and serotonin release in the IC have also demonstrated a role for serotonin in auditory coding (Hurley, 2006; Hurley, 2007; Hall and Hurley, 2007; Hurley et al., 2008). Thus, endogenous serotonin is present in the intact IC, and is likely to modulate acoustic processing. In step with these *in vivo* findings, our result that serotonin contributes to a portion of the evoked EPSP after LL stimulation is the first *in vitro* evidence of postsynaptic depolarization by serotonin in IC neurons. In this issue, a companion study of IC neurons *in vivo* reports enhancements in discharge rate following 5-HT_3R activation (Bohorquez and Hurley, 2009). Indeed, our report that 5-HT_3R activation exerts a depolarizing effect *in vitro* is consistent with this result. Our focus on gain changes near action potential threshold demonstrated that this depolarizing influence could change the input/output ratio of an IC neuron near rest. Interestingly, the *in vivo* study also reported a depressive effect for 5-HT_3R at high firing rates. While we did not observe any depressive effects of 5-HT_3R activation *in vitro*, this is likely because we examined postsynaptic gain at spike threshold, the lowest firing rate of a neuron. Furthermore, our pharmacological testing was restricted to neurons that could demonstrate the positive gain phenomenon, whereas the *in vivo* study, which demonstrated a variety of pharmacological effects, had no cell selection criteria. Therefore, it is not surprising that we observed less diverse effects than the *in vivo* study. An intriguing trend among these *in vivo* results was that, as the spike count gets higher, the effect of the 5-HT_3R agonist moves to less facilitation/more depression (Bohorquez and Hurley, 2009). Thus, our finding is consistent with pharmacological effects found for lower firing rates *in vivo*. Despite

the limitations in comparing *in vitro* with *in vivo* studies, our result of depolarizing 5-HT_3R activation leading to increased discharge rate supports the observation of enhancements in spike rate following pharmacological 5-HT_3R activation *in vivo*.

4.4. Consistency with serotonergic mechanisms elsewhere in the CNS

The gain effect in IC neurons is remarkably similar to findings obtained in turtle spinal motorneurons. In this system, a short synaptic train elicits a transient period of excitability, also measured as an increase in current-evoked action potentials (Hounsgaard and Kiehn, 1989). This temporary elevation in gain lasts for tens of seconds to minutes, and is attributed to an L-type Ca^{2+} plateau potential. Furthermore, this synaptically-evoked Ca^{2+} current is regulated by serotonin, and is independent of ionotropic glutamatergic and glycinergic transmission (Delgado-Lezama et al., 1999; Delgado-Lezama et al., 1997). The same transient states of excitability were also found in cat motorneurons, and were also regulated by serotonin (Hounsgaard et al., 1988).

The prominent reduction of excitability caused by the 5-HT_3 antagonist LY-278,584 strongly suggests a role for serotonin via its sole ionotropic receptor. This receptor is known to gate a fast-activating, nonselective cation channel, and cause rapid depolarization, making it a likely contributor to the net depolarization we observe in IC neurons (Barnes and Sharp, 1999; Davies et al., 1999; Dubin et al., 1999). Consistent with this observation, the 5-HT_3 agonist (2M5HT) increases membrane excitability enough to influence action potential generation (Fig. 7B). Indeed, the V_m depolarized 4.7 mV immediately after application of this 5-HT_3 agonist. Therefore, the contribution of serotonin via this ionotropic receptor may have a significant effect on discharge rate coding in the IC. Taken together, these results indicate that direct activation of 5-HT_3 receptors depolarizes the neuron enough to increase discharge rate.

5-HT_3 receptor-mediated excitability has been observed in other neural systems. Iontophoresis of a 5-HT_3 agonist increases mean discharge rate of amygdala neurons *in vivo*. In mouse neuroblastoma cells containing a native form of the 5-HT_3 receptor, local application of serotonin elicits depolarizing currents of monovalent and divalent cations, and increases $[Ca^{2+}]_{in}$ (Stewart et al., 2003). Also, contrary to the expectation that neuromodulator receptors are primarily extrasynaptic, electron micrograph studies of the hippocampus show postsynaptic 5-HT_3 receptor labeling preferentially localizes to the postsynaptic density (Miquel et al., 2002). Such a postsynaptic location suggests this receptor plays an active role in dendritic neurotransmission. Indeed, a survey of 5-HT_3 receptor antibody staining in the rat brain shows immunopositive cell bodies in the IC. (Morales et al., 1998). To date, there is no further *anatomical* evidence of 5-HT_3 receptor distribution in the IC. However, anatomical and physiological examination of 5-HT_1 and 5-HT_2 subtypes indicates a growing relevance to both auditory processing and the neural basis of anxiety (Castilho et al., 1999; Peruzzi and Dut, 2004).

4.5. The role of calcium

The duration of synaptically-elicited positive gain in IC neurons cannot be fully explained with a fast ionotropic receptor, such as 5-HT_3. The elevated response over tens of seconds is more likely due to a postsynaptic effect of Ca^{2+}. An intracellular rise in Ca^{2+} could be elicited by 5-HT_3 receptor activity or could independently result from other signals that depolarize the membrane. Our results with verapamil suggest that voltage-gated Ca^{2+} currents in IC neurons are involved in the enhanced excitability to postsynaptic current pulses. The membrane depolarization that is elicited by the tetanic synaptic stimulation is significantly reduced by verapamil (Fig. 9B), which shows a possible L-type channel contribution to this response. Sustained L-type voltage-activated Ca^{2+} currents in IC neurons have been described in approximately 63% of IC cells (N'Gouemo and Morad, 2003). Furthermore, L-type currents in these IC neurons displayed a slow voltage-dependent inactivation, causing them to last up to several minutes. Such a time course could account for the recovery periods observed in our experiments.

The role of Ca^{2+} in the long recovery of IC neurons in our stimulation paradigm could also reflect the presence of a plateau potential. An example of brief periods of synaptic drive causing elevated and sustained spiking activity in the entorhinal cortex shows that Ca^{2+} plateaus underlie enhanced responses to successive stimuli (Egorov et al., 2002). Similarly, serotonin application in ferret thalamus slice can cause minutes-long depolarizations, which were sufficient to elicit action potentials (Monckton and McCormick, 2002). Even more pertinent are results which demonstrate that serotonin application facilitates long, slow L-type Ca^{2+} plateau potentials, which increase the spike discharge rate to subsequent injections of current in spinal motorneurons (Perrier et al., 2002). While these plateaus are triggered by the activation of metabotropic serotonin receptors, it is possible that the positive gain effects we see involve serotonin and Ca^{2+} in a similar way. However, activation of a plateau potential would require depolarizations much greater than the typical 5 mV deflections seen in the current study. We have not ruled out the possibility of metabotropic serotonin involvement during our synaptic stimulation paradigm.

4.6. Limitations of brain slice studies

Meaningful *in vivo–in vitro* comparisons have been made in the visual system, wherein a slow potassium current observed in the slice is proposed as the basis for *in vivo* contrast adaptation, a temporary gain change in V1 neurons (Sanchez-Vives et al., 2000). In this case, a seconds-long subthreshold event directly impacts the discharge rate of a single neuron, and ultimately changes the neural coding of subsequent contrast stimuli. Similar to the positive gain phenomenon we see IC neurons, the mechanisms elucidated in the V1 slice experiments are also longer-lasting than those observed *in vivo*. This is an encouraging case of *in vivo* experiments motivating *in vitro* studies of cellular mechanisms underlying sensory function. Nonetheless, the IC slice model does fall short of direct comparison with the *in vivo* phenomenon of acoustic conditioning (Sanes et al., 1998) in several ways. Stimulation of the LL *in vitro* causes a broad activation of afferents, whereas acoustic stimulation presumably stimulates specific combinations of afferents. Also, the *in vitro* stimulation is always performed with ionotropic glutamate blocked (in KYN), making this broad afferent activation somewhat restricted. As ionotropic glutamate is known to influence the accumulation of postsynaptic calcium, the *in vitro* stimulation is likely to under-recruit postsynaptic calcium. Thus, the synaptic stimulation period *in vitro* does not have a specific acoustic equivalent. Furthermore, the *in vitro* model does not specifically address presynaptic mechanisms that may contribute to the *in vivo* phenomenon. Adaptation of afferents may influence postsynaptic gain effects during both acoustic conditioning and tetanic synaptic stimulation, however the contribution of afferents was not assessed with either stimulation paradigm.

4.7. Relevance to auditory processing

The prevalence of positive gain effects in IC neurons suggests a possible effect on many aspects of auditory processing. Although the kinetics of the slice are slow compared to *in vivo* conditions, our findings may point to the cellular bases of sensitivity to stim-

ulus order and context in IC neurons, particularly on the time scale of 100 s of ms. Stimulus context has proven important for neural coding in the IC, as shown with studies of increased sensitivity to gap detection in background noise, reflecting a form of IC gain control (Wilson and Walton, 2002), or the temporary enhancement of spike rate depending on the interval between two sequential tones (Finlayson, 1999). Indeed, temporal order of sound stimuli does affect our understanding of speech components (Holt and Lotto, 2002). Furthermore, speech recognition appears most dependent on low frequency components rather than complex spectral components (Shannon et al., 1995). Such low frequencies occupy temporal periods that would definitely be affected by neurophysiological changes on the order of 100 s of ms, such as those described herein.

Changes on an intermediate time scale (100 s of ms) are likely to have an important impact on auditory perception in the typical acoustic environment (e.g., reverberating). For example, human subjects report the apparent target location of a moving sound to be displaced in the direction of motion (Mateeff and Hohnsbein, 1988; Perrott and Musicant, 1981, 1977). Because the IC appears to integrate synaptic input over longer time intervals than lower brainstem auditory nuclei, it is a prime candidate for studies of short-term plasticity of coding properties.

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