Synaptic Physiology in the Cochlear Nucleus Angularis of the Chick

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MacLeod, Katrina M. and Catherine E. Carr. Synaptic physiology in the cochlear nucleus angularis of the chick. J Neurophysiol 93: 2520–2529, 2005. First published December 22, 2004; doi:10.1152/jn.00898.2004. Nucleus angularis (NA), one of the two cochlear nuclei in birds, is important for processing sound intensity for localization and most likely has role in sound recognition and other auditory tasks. Because the synaptic properties of auditory nerve inputs to the cochlear nuclei are fundamental to the transformation of auditory information, we studied the properties of these synapses onto NA neurons using whole cell patch-clamp recordings from auditory brain stem slices from embryonic chickens (E16–E20). We measured spontaneous excitatory postsynaptic currents (EPSCs), and evoked EPSCs and excitatory postsynaptic potentials (EPSPs) by using extracellular stimulation of the auditory nerve. These excitatory EPSCs were mediated by AMPA and N-methyl-D-aspartate (NMDA) receptors. The spontaneous EPSCs mediated by AMPA receptors had submillisecond decay kinetics (556 μs at E19), comparable with those of other auditory brain stem areas. The spontaneous EPSCs increased in amplitude and became faster with developmental age. Evoked EPSC and EPSP amplitudes were graded with stimulus intensity. The average amplitude of the EPSC evoked by minimal stimulation was twice as large as the average spontaneous EPSC amplitude (~110 vs. ~55 pA), suggesting that single fibers make multiple contacts onto each postsynaptic NA neuron. Because of their small size, minimal EPSPs were subthreshold, and we estimate at least three to five inputs were required to reach threshold. In contrast to the fast EPSCs, EPSPs in NA had a decay time constant of ~12.5 ms, which was heavily influenced by the membrane time constant. Thus NA neurons spatially and temporally integrate auditory information arriving from multiple auditory nerve afferents.

INTRODUCTION

The synaptic properties of auditory nerve inputs onto their postsynaptic targets in the cochlear nuclei are fundamental to the transformation of auditory information. In birds, acoustic cues for sound localization are segregated into parallel streams: in vivo recordings showed that cochlear nucleus magnocellularis (NM) encodes timing cues, whereas cochlear nucleus angularis (NA) encodes intensity cues (Konishi et al. 1985; Sullivan and Konishi 1984; Takahashi et al. 1984). Recent work studying the morphology, physiology, and auditory responses in NA suggest that this nucleus is also important for encoding sound for nonlocalization tasks, such as sound recognition and discrimination (Köppl and Carr 2003; Soares et al. 2002).

At the brain stem level, the cellular and synaptic specializations that allow temporal coding of sound phase for the computation of interaural time differences are well understood (Carr et al. 2001; Trussell 1999). Studies of nucleus magnocellularis neurons have revealed a suite of anatomical and physiological specializations that enable the precise encoding of the temporal properties of auditory nerve inputs, including large, calyceal synapses, very fast AMPA receptor (AMPAR)-mediated synaptic currents, short membrane time constants, and fast synaptic potentials (Carr et al. 2001; Jhaveri and Moster 1982; Parks 2000; Trussell 1999; Zhang and Trussell 1994a,b). The time course of these AMPAR excitatory postsynaptic currents (EPSCs) is due to the presence of “flop” splice variants of glutamate receptor subunits GluR3 and GluR4 and to the absence of subunit GluR2, which together confer faster desensitization kinetics, as well as permeability to calcium ions (Dingledine et al. 1999; Geiger et al. 1995; Parks 2000; Sugden et al. 2002).

In contrast, much less is known about the synaptic properties in NA. Auditory nerve terminals in NA make anatomically smaller, bouton-like synapses (Carr and Boudreau 1991). Immunohistochemical evidence from the barn owl suggests GluR3 and GluR4 are the most prominent AMPAR subunits in NA (Kubke and Carr 1998; Levin et al. 1997). AMPA receptors expressed by chick NA neurons have fast desensitization kinetics (Raman et al. 1994) and are also calcium permeable (Zhou et al. 1995). These data suggest that the synaptically evoked responses should also display fast kinetics, such as those in NM and other auditory brain stem areas. However, such fast kinetics would seem excessive and unnecessary in a nucleus whose neurons have comparatively slow membrane time constants (Fukui and Ohmori 2003; Soares et al. 2002) and is not thought to be involved in encoding timing cues.

We studied the synaptic properties in NA using whole cell intracellular recordings from auditory brain stem slices of chicken embryos during auditory nerve (AN) electrical stimulation. Both spontaneous and evoked AMPAR-mediated EPSCs in NA were very fast, with submillisecond decay time constants, similar to most other auditory brain stem nuclei. Excitatory EPSCs and excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation of the AN were small and graded with stimulus intensity. Evoked EPSPs in NA were much slower than the underlying synaptic currents due to filtering by the membrane time constant. We further provide physiological evidence that NA neurons receive inputs from at least several AN fibers and that each fiber probably makes several synapses onto its postsynaptic target in NA. These data together show that NA neurons spatially and temporally integrate auditory information arriving from multiple AN afferents to a much greater degree than NM neurons.

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METHODS

Brain slice preparation

Chicken embryos of ages E16–E20 were rapidly decapitated, and an ~4-mm segment of the caudal skull containing the brain stem was removed with a razor blade and quickly submerged in artificial cerebrospinal fluid (ACSF) containing (in mM) 130 NaCl, 26 NaHCO₃, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, and 10 dextrose. The brain stem segment was dissected out and transferred to a vibrating tissue slicer (Campden Instruments, Leicester, UK) where it was mounted with cyanoacrylate glue, supported by a gel solution (4% agarose in H₂O) and cut in ACSF. Transverse slices (250–300 μm) containing both NA and afferent fibers of the eighth nerve were collected and maintained in a holding chamber at room temperature (24–25°C) in oxygenated (95% O₂-5% CO₂) ACSF. For recordings, slices were placed in a submersion-type recording chamber continuously perfused with oxygenated ACSF (1–2 ml/min) heated to 28–31°C with a Warner TC 324B (Warner Instruments, Hamden, CT), except for current-voltage relation experiments, which were conducted at room temperature. All experiments were performed in accordance with the guidelines approved by the University of Maryland Institutional Animal Care and Use Committee.

Whole cell recordings and physiological analyses

Whole cell patch-clamp recordings were made from visually identified NA cells using infrared/differential interference contrast (IR/DIC) video microscopy (Stuart et al. 1993). Our standard potassium gluconate intracellular solution was (in mM): 120 potassium gluconate, 20 KCl, 0.1 EGTA, 2 MgCl₂, 10 HEPES, and 0.1% bovine serum albumin. We also used a cesium intracellular solution for voltage-clamp experiments to determine the I-V curve of the synaptic currents (in mM): 70 cesium sulfate, 5 BAPTA, 10 HEPES, 1 MgCl₂, 1 Na₂ATP, and 4 NaCl. All reagents were from Sigma unless otherwise indicated.

Electrophysiological recordings were made using an Axoclamp 2B or AxoPatch 200B (Axon Instruments, Foster City, CA) in voltage-clamp or fast current-clamp mode. Extracellular stimulation of the eighth nerve was produced with a tungsten metal monopolar or bipolar electrode. Stimulation and recordings were controlled by a computer running custom software written with IGOR Pro (WaveMetrics, Lake Oswego, OR) and delivered via a National Instruments A/D board (National Instruments, Austin, TX). Stimuli were precisely controlled biphasic waveforms run through an analog stimulus isolation unit (World Precision Instruments, Sarasota, FL).

We report data from a total of 105 individual NA neurons. Neurons were judged healthy if they had a resting potential below ~50 mV and rejected if the initial series resistance was >25 MΩ. For recordings with potassium gluconate electrodes, the resting potential determined immediately after establishing whole cell mode was ~60.3 ± 6.5 mV, and whole cell capacitance was 48.5 ± 15.0 pF. NA neurons were classified by their action potential responses to step current injections in current clamp. We limit our analysis to three broad groups: 1) single-spiking, which always fired one action potential at the step onset; 2) damped, which fired multiple action potentials that attenuated in amplitude with stronger stimulation; and 3) tonic, which fired multiple, full amplitude action potentials. Although tonically firing neurons may be further distinguished into three subgroups (Soares et al. 2002), we lump them here. A junction potential of ~10 mV was subtracted posthoc.

Current-voltage experiments were carried out in 20 μM bicuculline and 3 μM strychnine; some were also done in 50–100 μM 2-amino-5-phosphovalerate (APV). The synaptic reversal potentials were calculated from linear regression of fast AMPA receptor (AMPAR) component of the EPSC across all voltages and the x-intercept of the linear fit of slow N-methyl-d-aspartate (NMDA) receptor component across voltages from 0 to +40 mV. The ratio of slow to fast EPSC components was measured from average EPSCs during a +40-mV step; in cases in which a clear secondary current peak could not be distinguished, we measured the amplitude at 10 ms. All other experiments were carried out on pharmacologically isolated AMPAR-mediated events (in the presence of APV, bicuculline, and strychnine) unless otherwise noted. Voltage-clamp investigation of evoked and spontaneous EPSCs was performed with series resistance correction set to 70–95%. Any recordings that could not be corrected to a residual series resistance of <4 MΩ were rejected. We used 6,7-dinitroquinoxaline-2,3-dione (DNQX) and GYKI-52466 (Tocris Cookson, Ellisville, MO) for pharmacological identification of AMPAR-mediated currents.

Spontaneous EPSCs were recorded under voltage-clamp at ~80 mV without TTX; miniature EPSCs (mEPSCs) were recorded with the addition of 0.1–1 μM TTX. Spontaneous and miniature EPSC analysis was carried out with the program MiniAnalysis (Synaptosoft, Decatur, GA) using an amplitude threshold of 8–20 pA. All events were scanned by eye to remove errors and closely spaced EPSCs for kinetic analysis. A minimum of 30 events per cell were required to be included in the analysis, but typically 100–1,000 events per cell were analyzed. Analysis of dendritic filtering was restricted to neurons with ≥80 events.

We used a minimal stimulation protocol similar to that used in hippocampal and other slice preparations (Allen and Stevens 1994). Briefly, the extracellular stimulus level was increased until a threshold level could be determined and a stable, small evoked EPSC or EPSP could be elicited (e.g., see Fig. 8A). The response was probed with stimulus level steps of 5–15% of threshold level with 10–30 trials for each level, and an average failure rate and “potency” (amplitude not including failures) were calculated. The minimal response was considered the lowest stable potency plateau with a range >10% of threshold level. In most cells plateau failure rates were nonzero (n = 9 of 11). To find an accurate threshold for a single fiber, we used ACSF containing 3 mM Ca²⁺ and 1 mM Mg²⁺ to maximize probability of release. However, this technique does not eliminate the possibility that the minimal response actually represents two or more fibers with similar thresholds.

All statistical analyses were performed in Microsoft Excel 2001 for Mac (Student’s t-test) or with Statview (ANOVAAs). Values reported are mean ± SD, unless otherwise specified.

Anatomy

Slices containing biocytin fills were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight and processed with Vectastain ABC (avidin-biotinylated HRP complex) Elite Kit (Vector Labs, Burlingame, CA). The boundary of NA, the location of filled neurons, and their morphological reconstructions were drawn using the computer-assisted tracing program Neurolucida (Microbrightfield, Williston, VT) or photographed. Photographs were composited and adjusted for brightness and contrast in Adobe Photoshop.

RESULTS

Evoked EPSCs: voltage dependence and pharmacology

NA was easily recognizable as a bean-shaped area of neuropil at the dorsolateral margin of the transverse slice through chick auditory brain stem (Fig. 1A). Individual neurons in NA were clearly identified with video microscopy using IR-DIC. Whole cell recordings were established as previously described (Soares et al. 2002). A metal electrode was placed in the auditory nerve tract, which was clearly visible under light or DIC microscopy, at the dorsomedial or medial margin of NA where AN fibers enter the nucleus (Fig. 1B) (Boord and Rasmussen 1963; Fukui and Ohmori 2003; Köppl 2001).
Because NA is known to receive GABAergic input locally and from the superior olive (Carr et al. 1989; Monsivais et al. 2000; Takahashi and Konishi 1988; Yang et al. 1999) and also stains from the superior olive (Carr et al. 1989; Monsivais et al. 2000; Soares et al. 2002).

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The voltage dependence of the excitatory synaptic current in NA was examined in slices from an E18 chick. Evoked EPSCs were recorded during a series of voltage steps in the presence of GABA receptor and glycine receptor blockers (Fig. 2A).

In the absence of APV, two components could be observed. First, there was a fast component, which peaked and decayed in <2 ms at negative voltages (Fig. 2A, ■). This component had a linear I-V relationship, which reversed at +5.3 mV (n = 6; Fig. 2B, ■). Second, there was a slowly decaying, voltage-dependent component, sometimes observed as a secondary peak in the EPSCs during depolarizing voltage steps (Fig. 2A, □). This slowly decaying component had a nonlinear I-V relationship with a negative peak at ~30 mV, which reversed at +2.3 mV (n = 6; Fig. 2B, □), as would be expected for an NMDA receptor-mediated current. The amplitude of the slowly decaying current was large: the ratio of the fast EPSC component to slow EPSC component, measured during the voltage step to +40 mV, was 0.94 ± 0.34 (range, 0.44–1.42; n = 6; Fig. 2B).

To determine the identity of these components, we applied pharmacological agents. When APV (75–100 μM) was bath applied, the slow component apparent at +40 mV was blocked (n = 4; Fig. 2C, top traces), leaving a fast outward EPSC; APV had no effect on the inward EPSC at ~80 mV (Fig. 2C, bottom traces). The APV-resistant EPSC was reversibly blocked by the AMPA/kainate antagonist DNQX (5–20 μM, n = 8; Fig. 2D) and had an I-V relationship similar to the fast component (n = 4). The fast APV-resistant EPSC was also blocked by GYKI-52466, an AMPAR-specific antagonist (data not shown; n = 4). When DNQX was bath applied, the fast component of the control currents was blocked, but not the slow, voltage-dependent component (n = 4; Fig. 2E). Thus the slow component is a direct NMDA receptor-mediated effect and not due to polysynaptic activity. These results show that the excitatory AN synapses onto NA are glutamatergic, with a fast AMPA-mediated component and a large, slow NMDA receptor-mediated component.

**sEPSCs**

Previous work showed that the desensitization kinetics of AMPA receptors expressed by NA neurons are fast in response to glutamate exposure (Raman et al. 1994). To determine whether the kinetics of the synaptically evoked responses are also fast, we measured sEPSCs in 40 NA neurons from animals aged E16–E20. These recordings were done in the absence of TTX to determine the physiological firing properties: 22 tonically firing, 10 single-spike firing, 4 damped, and 4 undetermined. Neurons were voltage clamped at −80 mV, and the series resistance was corrected by 65–95%. Frequent spontaneous events could be observed at 29–31°C and in the presence of GABA receptor, glycine receptor, and NMDA receptor antagonists, which were completely blocked with DNQX, and thus were AMPA receptor-mediated (Fig. 3A). Event frequency ranged from 0.08 to 6.1 Hz among NA neurons (1.5 ± 1.5 Hz; see Fig. 3D for an interval histogram for 1 NA neuron).

**TABLE 1. Spontaneous EPSC properties by NA cell type**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>n</th>
<th>Amplitude, pA</th>
<th>Rise Time, μs</th>
<th>Decay τ, μs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic</td>
<td>6</td>
<td>46.3 ± 10.3</td>
<td>400 ± 68</td>
<td>933 ± 206</td>
</tr>
<tr>
<td>Damped</td>
<td>3</td>
<td>53.6 ± 17.9</td>
<td>489 ± 67</td>
<td>1281 ± 258</td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td>46.9 ± 16.6</td>
<td>371 ± 95</td>
<td>941 ± 260</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data are from 13 E17 NA neurons. Rise time, 10–90% rise time; Decay τ, exponential fit.
The results reported below are based on ~30–2,000 individual events per cell (average: 490 events per cell).

A broad range of sEPSC amplitudes was evident across individual traces (Fig. 3B) and in the amplitude histogram (Fig. 3C) for single NA neurons. In Fig. 3, for example, the sEPSC amplitudes for this neuron ranged from 8.2 to 101.9 pA, with a mean of 43.8 ± 18.9 pA (n = 545 events). The amplitude histogram was highly skewed (skew, 0.84; Fig. 3C), such that the median amplitude was less than the mean (38.9 pA). To determine the kinetics of synaptic transmission in NA we measured the 10–90% rise time and the exponential decay time constant of the sEPSCs. The decay of both individual and average sEPSCs in NA could be fit well by a single exponential function.

Developmental effects on sEPSCs

Developmental age contributed to the variability in the amplitude and kinetic measurements of sEPSCs from NA neurons. Average EPSC traces from E19 recordings were larger and faster than those from E17 (Fig. 5A). ANOVA of sEPSC amplitude showed that this effect was significant, with an age-dependent effect (P < 0.05, single-factor ANOVA). Posthoc comparisons showed significant differences between E16 and E17 versus E18 and E16 versus E19 (P < 0.05, Fisher’s PLSD test, Fig. 5B). Across NA recordings, the amplitude of the average sEPSC increased with age, from 40.0 ± 9.3 pA at E16 to 57.6 ± 9.5 pA at E19 (P < 0.01; r = 0.43, simple regression; Fig. 5C). ANOVA also showed significant changes in 10–90% rise times and decay time constants (P < 0.05, single-factor ANOVA). Posthoc comparisons for rise times and decay time constants showed significant differences between E16 and E17 versus E19 (P < 0.05, Fisher’s PLSD test, Fig. 5B). Rise times decreased from 320 ±
sEPSCs by nucleus and cell type

Previous work has shown that mEPSCs in chick nucleus magnocellularis are among the fastest recorded synaptic currents (Raman et al. 1994; Trussell 1999). To make direct comparisons with the literature and our NA sEPSC data, we acquired sEPSCs from NM at E17 and E19. NM sEPSC amplitudes were significantly larger than those recorded from NA neurons at E19 (110.9 ± 23.3 pA for NM, n = 3; P < 0.001, Student’s t-test; Fig. 6, A and B), although there was no significant difference at E17 (49.1 ± 13.2 pA for NA, n = 15; 57.9 ± 31.9 pA for NM, n = 5; P > 0.05, Student’s t-test). The kinetics of the sEPSCs were significantly faster in NM than in NA at both E17 (data not shown) and E19 (Fig. 6, A and B). At E17, the average decay time constant was 391 ± 92 μs for NM neurons compared with 875 ± 279 μs for NA neurons (P < 0.01). At E19, the average decay time constant was 178 ± 19 μs for NM neurons compared with 556 ± 201 μs for NA neurons (P < 0.01). These data suggest that while both sEPSCs become larger and faster with development in both nuclei, the size and speed of NA sEPSCs may lag behind those in NM.

We examined sEPSCs from NA neurons for evidence of dendritic filtering by plotting the rise time for each event.
against its decay time constant and amplitude (Fig. 7, A and B). While in a few cells a correlation between rise and decay tau was observed (e.g., in Fig. 7A), it was relatively weak overall ($r$ values ranged from $-0.22$ to $0.58$; average, $0.22 \pm 0.18; n = 33$). Data from most NA neurons showed no correlation between rise time and amplitude ($r$ values ranged from $-0.19$ to $0.51$; average, $0.15 \pm 0.14$). Thus only weak dendritic filtering, at best, was observed in our recordings of spontaneously occurring synaptic events. However, substantial variance in the kinetics and amplitude of quantal EPSCs at individual sites may obscure filtering effects observable with this method (Bekkers and Stevens 1996).

**Evoked synaptic currents by minimal stimulation of auditory nerve**

In NM neurons, stimulation of single auditory nerve fibers evokes large, all-or-none synaptic currents, due to their giant calyceal synaptic terminals (Hackett et al. 1982; Trussell et al. 1993). In NA, however, contacts are made by small, bouton-like synapses (Carr and Boudreau 1991). To determine the impact of a typical auditory nerve input to NA, we recorded EPSCs from 11 NA neurons while extracellularly stimulating the nerve tract at the dorsolateral or lateral margin of NA. These neurons were composed of the following cell types: one single-spiking, three damped, five tonic, and two undetermined.

Minimal stimulation protocols were used to measure responses from single, or a small number, of input fibers (Fig. 8A; see METHODS). Evoked EPSCs from putative single fibers were recorded at a stimulation level 10–20% above threshold stimulation ($-75–200$ trials; average, $123 \pm 52$ trials). Minimal evoked EPSCs from one NA neuron are shown in Fig. 8, B and C. In this neuron, the minimal stimulation strength always evoked an EPSC with no failures. Minimal stimulation more typically evoked an EPSC with a probability of $<1$ (range, $0.15–0.71$) despite the relatively high calcium levels (see METHODS; $n = 9$ of 11).

For all NA neurons, the peak amplitude of the evoked AMPAR-mediated synaptic current, including failures, was $109.3 \pm 48.6$ pA at a voltage of $-80$ mV, which corresponds to a peak conductance of $1.36 \pm 0.6$ nS ($n = 11$; Fig. 8D). The average amplitude, including failures, was $87.3 \pm 50.0$ pA. The average CV of the amplitude, excluding failures, was $0.35 \pm 0.07$ (range, $0.25–0.50$). The decay of the average evoked EPSC was fit well with a single exponential (Fig. 8C). The evoked synaptic responses had a 10–90% rise time of $0.3 \pm 0.07$ ms, while the peak latency was $2.2 \pm 0.4$ ms.

To compare the minimal evoked EPSC with the quantal EPSC, we also recorded sEPSCs from the same neurons in between evoked responses (Fig. 8, E and F). Evoked EPSCs were larger than these spontaneous EPSCs ($54.2 \pm 11.5$ pA; $P < 0.01; n = 11$). We calculated that the average ratio of evoked EPSC amplitude to spontaneous EPSC amplitudes was

![Diagram of EPSC amplitudes and kinetics](image-url)
2.3 (range, 0.9–5.5), suggesting that each auditory nerve fiber makes at least two synaptic contacts, on average, onto its postsynaptic target.

The kinetics of evoked EPSCs and spontaneous EPSCs recorded in the same neurons were identical (sEPSCs rise times: 454/81 ms; P < 0.05; sEPSCs decay time constants: 1179/338 ms, P < 0.05; n = 11). Both rise times and decay time constants were well correlated between evoked EPSCs and spontaneous EPSCs within the same neurons (rise time, r = 0.65; decay time constant, r = 0.88; n = 11).

Excitatory postsynaptic potentials in NA are graded and subthreshold

The large amplitude of auditory nerve inputs onto NM neurons results in reliable, superthreshold postsynaptic responses (Hackett et al. 1982; Zhang and Trussell 1994a). We now show that NA neurons receive graded subthreshold excitatory inputs, consistent with the observation of smaller terminals from the auditory nerve onto NA neurons (Carr and Boudreau 1991).

In current-clamp recordings from NA neurons, subthreshold EPSPs were always evident with low to moderate levels of stimulation before reaching action potential threshold at higher stimulation levels (n = 19). In 11 NA neurons, we analyzed EPSPs from a large number of trials (100–200) across the full range of postsynaptic responses, from failure to spiking. Figure 9 shows that, for one cell, at least two EPSP amplitude levels can be observed before reaching action potential threshold (Fig. 9, A and B), with a large variation in amplitude within each level. In 8 of 11 NA neurons, EPSPs responses could be grouped into two to four discrete, but broad, clusters according to peak amplitude (with high variance in amplitude within a cluster). These results suggest that NA neurons receive and must sum at least three to five active auditory nerve fiber inputs to reach spiking threshold. In the remaining NA neurons, the range of subthreshold amplitudes did not show discrete clusters, and instead blended into a continuous range. Multiple EPSP amplitude levels were also routinely observed in voltage clamp recordings of evoked EPSCs with increasing stimulus intensity (Fig. 9, C and D).

The short time course of the EPSPs in NM, due to the fast underlying kinetics coupled with the short membrane time constant, is thought to contribute to efficient phase-locking of NM neurons to the auditory nerve input by maintaining a narrow window for temporal integration (Zhang and Trussell 1994a). We have investigated the time course of the evoked EPSPs in NA with minimal stimulation. A comparison of the kinetics of the EPSPs with those of the EPSCs reported above suggests a strong filtering effect due to the membrane time constant of NA neurons. We made recordings from 16 NA neurons in current clamp, which had the following cell types: three single-spiking, three damped, nine tonically firing, and one undetermined. We measured the kinetics of small EPSPs likely to result from a single or small number of AN fiber inputs, using the same minimal stimulation methods described above, and in the presence of GABA-, NMDA- and glycine-receptor blockers (Fig. 10). These EPSPs had an onset (10%
these. Discrete EPSP amplitude clusters are apparent, with large variability within passant synapses in NA, unlike the large, calyceal synapses in NM with the anatomical observation of small, bouton-like, and en passant. EPSCs also had a substantial NMDA receptor–mediated component due to the membrane time constant of the NA neurons. Evoked glutamatergic EPSPs that were small, graded, and sub-threshold for cell in A. Threshold stimulation occurred at ~330 µA; at least 2 discrete EPSP amplitude clusters are apparent, with large variability within these. C: overlay of individual EPSCs evoked with increasing stimulation strength. Different neuron than in A and B. D: EPSC amplitudes vs. stimulation strength for cell in C.

**FIG. 9.** NA neurons received excitatory postsynaptic potentials (EPSPs) and EPSCs of graded amplitude with extracellular stimulation strength. A: overlay of individual subthreshold EPSPs evoked with increasing extracellular stimulation strength. Note clustering of traces in distinct groups. *Action potential, clipped for clarity. B: subthreshold EPSP amplitudes vs. stimulation strength for cell in A. Threshold stimulation occurred at ~330 µA; at least 2 discrete EPSP amplitude clusters are apparent, with large variability within these. C: overlay of individual EPSCs evoked with increasing stimulation strength. Different neuron than in A and B. D: EPSC amplitudes vs. stimulation strength for cell in C.

**DISCUSSION**

To better understand how sound information is encoded in the avian cochlear nuclei, we have identified and characterized the excitatory synaptic inputs from the auditory nerve onto chick nucleus angularis neurons. Stimulation of the auditory nerve evoked glutamatergic EPSPs that were small, graded, and sub-threshold. Spontaneous and evoked AMPAR-mediated EPSCs had fast kinetics, similar to those that characterize other auditory brain stem regions in both avian and mammalian systems. Each NA neuron appeared to receive multiple small EPSPs from a number of AN fibers, which were filtered by the time constant of the NA membrane and summed to reach firing threshold. Evoked EPSCs also had a substantial NMDA receptor–mediated component.

The small size of the evoked EPSCs and EPSPs is consistent with the anatomical observation of small, bouton-like, and en passant synapses in NA, unlike the large, calyceal synapses in NM (Carr and Boudreau 1991). We observed individual evoked currents as small as 14 pA and evoked EPSPs as small as 0.2 mV. We used minimal stimulation procedures to estimate the evoked EPSC and EPSP amplitudes from the input of a single AN fiber. Since the minimal evoked EPSP amplitude in NA neurons was larger than that of the spontaneous EPSC amplitude, we conclude that the each auditory nerve fiber may make multiple synaptic contacts onto its postsynaptic target. While the data show the number of contacts to be at least two, this is a lower bounds estimate; the actual number should be higher because the probability of release is most likely less than one under normal physiological conditions. Because this procedure cannot guarantee stimulation of single fibers, however, we cannot exclude the possibility that the larger evoked EPSCs are composed of inputs from multiple fibers with similar thresholds, even though we took care to reject clearly compound EPSCs. The graded nature of the EPSPs and EPSCs with increasing stimulus intensity shows that each NA neuron also likely receives input from multiple auditory nerve fibers. Our results showing the small, graded excitatory inputs are similar to those found for T-stellate and octopus neurons in the mammalian ventral cochlear nucleus (VCN) (Ferragamo et al. 1998; Golding et al. 1995).

NA neurons express fast AMPA receptor kinetics, both in spontaneous and evoked EPSCs. In the older, E19 embryos, spontaneous EPSCs had a mean 10–90% rise time of 213 ± 28 µs, with some cells on average as fast as 170 µs. The EPSC decays could be well fit by a single exponential with a mean time constant of 556 ± 201 µs, with some cells as fast as 300 µs. These results place NA AMPAR currents well within the category of fast excitatory currents that characterize the auditory brainstems of birds and mammals, which, for comparison, have decay time constants of ~200 µs in NM (Raman et al. 1994; Zhang and Trussell 1994b); ~600 µs in chick nucleus laminaris (Kuba et al. 2002); ~340–400 µs in the VCN (Bellingham et al. 1998; Gardner et al. 1999, 2001); and ~700 µs in the mammalian brain stem regions (Bollmann et al. 2005).

**FIG. 10.** Kinetics of evoked EPSPs in NA. A: average minimal EPSP from 1 NA neuron and the single exponential fit to its decay. B: summary data of average amplitude, peak latency, 10–90% rise time, and exponential decay time constant for NA neurons (n = 16). C: scatter plot shows the correlation between minimal EPSC decay time constant and neuronal membrane time constant. Open symbols, minimally stimulated EPSPs; line, linear fit of minimal EPSPs (y = 1.48x –2.50, r = 0.82; n = 16); closed symbols, supraminimal EPSPs shown for comparison (n = 7).
medial nucleus of the trapezoid body (Barnes-Davies and Forsythe 1995). It should be noted that some variation among these results is due to temperature and age differences. Our data are consistent with previous studies on GluR subunit distributions (Levin et al. 1997) and receptor desensitization kinetics (Raman and Trussell 1992) in NA. These “auditory” currents (Parks 2000) are faster than most AMPAR-mediated currents reported from many other areas in the brain, which have decay time constants of several milliseconds or more. These include synapses in hippocampus (Hestrin et al. 1990; Jonas et al. 1993; Walker et al. 2002), cerebellum (Llano et al. 1991; Silver et al. 1992), neocortex (Hestrin 1992, 1993; Stern et al. 1992), and nonauditory brain stem (Raman et al. 1994; Titz and Keller 1997), with few exceptions (but see Silver et al. 1996). Even using techniques designed to circumvent voltage-clamp error ( Háusser and Roth 1997; Walker et al. 2002), most decay time constants of EPSCs recorded outside the auditory brain stem are not in the submillisecond range.

When we compared spontaneous EPSC kinetics in NA with those we recorded in NM under similar conditions, reproducing the results of Zhang and Trussell (1994b), we found that the kinetics in NA were significantly slower than in NM. There could be several reasons for this difference. Receptors in the two nuclei may be composed of a different complement of GluR subunits, such that NM receptors are simply faster. This may also be a developmental difference (Lawrence and Trussell 2000; Sugden et al. 2002), where the NA AMPAR currents simply lag NM AMPAR current development. Another possible explanation for slower EPSC kinetics in NA is that the dendrites of NA neurons may lead to voltage-clamp error or dendritic filtering. However, we found only weak evidence for dendritic filtering and no correlation between decay time constant measurements and any voltage clamp settings (e.g., series resistance, whole cell capacitance, or speed of voltage clamp). Further experiments with older animals and improved techniques will be needed to resolve this discrepancy.

We observed no differences in spontaneous EPSC receptor kinetics or amplitude by NA neuronal cell type. Similarly, bushy, T-stellate, tuberculoventral, and octopus cells in the mammalian VCN were reported to have nearly identical kinetics to one another (Gardner et al. 1999). Neither did we observe any bimodal kinetic distributions, as have been found in some fusiform cells of the dorsal cochlear nucleus (DCN), where the population of slower EPSCs are thought to have a nonauditory origin (Gardner et al. 1999).

We also used minimal stimulation to estimate the time course of the AMPA receptor–mediated EPSP. The 10–90% rise times and exponential decay time constants of the EPSP were much slower than those of the underlying synaptic currents, and we showed that the decay time constant was directly correlated to the membrane time constants in NA neurons (Fukui and Ohmori 2003; Soares et al. 2002). Due to their shorter membrane time constants, the EPSP decay times reported for NM neuron (with a half-decay of <1 ms) (Zhang and Trussell 1994a) are faster than even the fastest we report here for NA (~4 ms). Thus while all these neurons all receive fast underlying synaptic currents, the membrane time constant is the governing factor for how these EPSPs decay. The broad range of EPSP decay time constants we show across different NA neurons combined with differential expression of voltage-dependent currents (Fukui and Ohmori 2003; Soares et al. 2002) could furthermore lead to differential integration of auditory inputs within NA. NA neurons with the slower EPSPs are precluded from encoding the precise temporal information present in the auditory nerve inputs, thus contributing to the segregation of acoustic information in the two nuclei.

It is unclear why a nucleus that appears to be better suited for integration would be equipped with receptors that display such fast kinetics. We propose that fast kinetics may be required to manage the high rates of input without saturation: in hatching chick, the driven rates for AN fibers exceed 100–300 spikes/s (Saunders et al. 2002). EPSP kinetics at synaptic sites distant from the soma may be considerably faster than measured and contribute to local dendritic computation. Faster EPSCs in NA might contribute to the in vivo firing responses of some NA neurons having onset and primary-like properties, which are characterized by temporal precision (Köppl and Carr 2003; Sachs and Sinnott 1978; Sullivan 1985; Warchol and Dallos 1990). Alternatively, with fast currents, the degree of temporal integration is largely determined by the time constant of the membrane, which is known to decrease over development (Fukui and Ohmori 2003), or could be easily modulated or voltage-dependent, providing a greater degree of computational flexibility.

In mammalian cochlear nucleus, T-stellate cells are thought have recurrent excitatory connections (Ferragamo et al. 1998; Oertel et al. 1990) and provide feed-forward excitation to neurons in the DCN (Zhang and Oertel 1993a,b). In contrast, we did not observe any evidence of intrinsic circuitry, such as large numbers of delayed or polysynaptic EPSPs, as seen in T-stellate recordings (Ferragamo et al. 1998). In many NA neurons for which the morphology was recovered, axons were observed to leave the nucleus, but no axon collaterals within or near the NA neuropil were found (personal observation). Furthermore, there is no avian cytoarchitectural division like the mammalian dorsal cochlear nucleus (Carr and Soares 2002). However, in vivo recordings from NA show a similar range of response types to sound stimuli as in mammalian cochlear nucleus (Köppl and Carr 2003). These data suggest that NA may contain a simplified circuit that still retains essential coding features to sound.

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