NMDA and AMPA Receptors in the Dorsal Nucleus of the Lateral Lemniscus Shape Binaural Responses in Rat Inferior Colliculus

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Kelly, Jack B. and Sean A. Kidd. NMDA and AMPA receptors in the dorsal nucleus of the lateral lemniscus shape binaural responses in the rat inferior collicus. J. Neurophysiol. 83: 1403–1414, 2000. Binaural responses of single neurons in the rat’s central nucleus of the inferior colliculus (ICC) were recorded before and after local injection of excitatory amino acid receptor antagonists (either 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[1]quinoxaline-7-sulfonamide disodium [NBQX], (±)-3-(2-carboxypropiazin-4-yl)-propyl-1-phosphonic acid [CPP], 6-cyano-3-nitroquinoxaline-2,3-dione [CNQX], or (±)-2-amino-5-phosphonovaleric acid [APV]) into the dorsal nucleus of the lateral lemniscus (DNLL). Responses were evoked by clicks delivered separately to the two ears at interaural time delays between −1.0 and +30 ms (positive values referring to ipsilateral leading contralateral click pairs). The neurons in our sample were excited by contralateral stimulation and inhibited by ipsilateral stimulation, and the probability of action potentials was reduced as the ipsilateral stimulus was advanced. Binaural inhibition resulted in response suppression that lasted up to 30 ms. Injection of excitatory amino acid antagonists into the DNLL contralateral to the recording site reduced the strength of binaural inhibition in the ICC. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist NBQX preferentially affected responses at small interaural time intervals (0–1.0 ms), whereas the N-methyl-D-aspartate (NMDA) antagonist CPP preferentially affected responses at longer intervals (1–30 ms). Both CNQX and APV produced a release from binaural inhibition, but neither drug was selective for specific intervals. The data support the idea that binaural inhibition in the rat ICC is influenced by both AMPA and NMDA receptor–mediated excitatory events in the contralateral DNLL. The results suggest that the AMPA receptors contribute selectively to the initial component of binaural inhibition and the NMDA receptors to a longer lasting component.

INTRODUCTION

Previous studies have shown that the dorsal nucleus of the lateral lemniscus (DNLL) exerts an inhibitory influence on the contralateral central nucleus of the inferior colliculus (ICC) and that blockade of synaptic activity in the DNLL by local injection of the excitatory amino acid antagonist kynurenic acid or other pharmacological agents reduces the strength of the binaural inhibition imposed on ICC neurons (Faingold et al. 1993; Kelly and Li 1997; Kidd and Kelly 1996; Li and Kelly 1992). Injection of kynurenic acid affects responses to dichotically presented sounds with either binaural time or intensity differences and reduces the strength of inhibition in the contralateral ICC of rats at either short (0–1 ms) or long (1–30 ms) interaural time delays (Kidd and Kelly 1996; Li and Kelly 1992). No changes have been found in binaural responses in the ICC ipsilateral to the injection site. These results indicate that the excitatory responses in the DNLL make an important contribution to binaural responses in the contralateral ICC.

Brain slice studies have shown that both N-methyl-D-aspartate (NMDA) and non-NMDA receptors are involved in the synaptic excitation evoked in the DNLL by electrical stimulation of the lateral lemniscus (Fu et al. 1997; Wu and Kelly 1996). A single-current pulse delivered to the lateral lemniscus elicits both early and late excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs), which can then be blocked respectively by non-NMDA and NMDA receptor antagonists. The early component of the synaptic response is thought to be mediated primarily by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and the longer lasting component by NMDA receptors in the DNLL.

Given that most neurons in the DNLL are GABAergic (Adams and Mugnaini 1984; Glendenning and Baker 1988; González-Hernández et al. 1996; Moore and Moore 1987; Roberts and Ribak 1987; Shneiderman et al. 1988; Thompson et al. 1985; Vater et al. 1992; Winer et al. 1995; Zhang et al. 1998) and that most of these neurons (70% in the rat) project directly to the contralateral ICC and DNLL (Adams 1979; Bajo et al. 1993; Beyerl 1978; Brunso-Bechtold et al. 1981; Coleman and Clerici 1987; Covey and Casseday 1995; Hutson et al. 1991; Ito et al. 1996; Kudo 1981; Merchan et al. 1994; Oliver and Shneiderman 1989; Ross et al. 1988; Shneiderman and Oliver 1989; Shneiderman et al. 1988; Tanaka et al. 1985; van Adel et al. 1999; Zook and Casseday 1982), it seems likely that both NMDA and AMPA receptors contribute to the release of GABA in the contralateral auditory midbrain. The time course and duration of the resulting inhibition would be shaped by the pattern of activation of the NMDA and non-NMDA receptors in the DNLL.

The purpose of the present study was to examine the relative influence of NMDA and non-NMDA receptors in the DNLL on binaural inhibitory responses in the rat ICC. To achieve this objective, NMDA and AMPA receptor antagonists were pressure injected locally into the DNLL, and binaural responses to paired clicks at various interaural time delays were recorded from neurons in the contralateral ICC before and after blocking the receptors.

METHODS

Physiological procedures

The methods of recording responses and injecting drugs were similar to those reported previously by Li and Kelly (1992) and Kidd...
and Kelly (1996). Briefly, binaural responses to paired clicks were recorded from single neurons in the ICC before and after pressure injection of AMPA antagonists (either 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[1]quinazoline-7-sulfonamide disodium [NBQX] or 6-cyano-7-nitroquinoxaline-2,3-dione [CNQX]) or NMDA antagonists (either [(±)-3-[2-carboxyamidotetrazol-4-yl]-propyl]-1-phosphonic acid [CPP] or [(±)-2-amino-5-phosphonovaleric acid [APV]]) into the contralateral DNLL (Fig. 1). The response probability of ICC neurons was determined by manipulating the interaural time difference (ITD) between the clicks over the range from −1.0 ms to +30 ms, where positive values refer to ipsilateral leading contralateral ITD intervals. The laterality of the stimulus (contralateral and ipsilateral) refers to the position of the ear relative to the recording site in ICC.

Successful experiments were performed on 51 male Wistar albino rats (250−450 g) from Charles River, St. Constant, Quebec, Canada. The animals were initially anesthetized with pentobarbital sodium (60 mg/kg, ip) and subsequently maintained in an areflexive state by injections of Equithesin [0.5 ml/kg, ip; see Sally and Kelly (1992) for preparation]. The animals were placed in a head holder that left the external ear canals free for insertion of earphone drivers. A midline incision was made in the scalp, and the tissue was retracted to expose the skull. Two craniotomies were made to allow penetration of recording and injection pipettes into the inferior colliculus and the DNLL, respectively.

The stereotaxic coordinates for positioning the recording and injection pipettes were referenced from lambda with the skull flat (Paxinos and Watson 1997). For placements in the DNLL, the injection pipette was tilted 30° in the sagittal plane and lowered into the brain to a depth of 7.8 mm from a point 6.7 mm lateral and 0.3–0.4 mm rostral to lambda. The injection pipette doubled as a recording electrode to monitor neural activity in the DNLL as described previously by Li and Kelly (1992), and the position of the pipette was fine-tuned to give the best acoustically driven response.

Recording pipettes were pulled from single-barrel glass tubing (Sutter, 1.0 mm OD, 0.5 mm ID) to a tip diameter of approximately 2 μm. They were back-filled with 2 M saline and had impedances between 1.5 and 2.5 MΩ.

The recording pipettes were inserted into the inferior colliculus with the use of a Kopf Model 650 micropositioner. The final position was adjusted to obtain short-latency, acoustically driven responses that exhibited narrow-frequency tuning and a clearly defined characteristic frequency (CF, the frequency to which a neuron responded at the lowest sound pressure level). As previously reported (Kelly et al. 1991; Syka et al. 1981), the CFs of neurons in the inferior colliculus showed a reliable progression from low to high frequencies as the recording pipette was lowered through the central nucleus. Histological reconstructions confirmed the location of all electrode placements in the ICC.

Injection pipettes were pulled from single-barrel glass tubing (Sutter, 1.0 mm OD, 0.5 mm ID) to a tip diameter of 20–40 μm. The pipettes were back-filled with excitatory amino acid receptor antagonists in normal saline. They were connected by a short length of flexible tubing to a small chamber constructed from two disposable hypodermic needles positioned back to back. A thin wire was fitted and glued in place between the two needles with one end extending within and along the shaft of the needle and into the tubing so that it could make contact with the solution in the pipette. The other end of the wire emerged from the junction between the two needles and was connected to a preamplifier for electrical recordings. The end of the second needle was connected by a longer length of flexible tubing to a 5-ml syringe for pressure injections. The volume of the injection (1.5−2.0 μl) was controlled by monitoring the progression of the solution along the length of the pipette, and the flow was stopped by releasing pressure through a three-way stopcock.

The drugs used were NBQX, CNQX, CPP, and APV. All were obtained from Research Biochemicals International (catalog numbers N-183, C-127, C-104, and A-110, respectively). The drugs were applied at various concentrations to determine the limits of their effectiveness under the conditions of our experiment. The vehicle for delivery of the drug was physiological saline. To avoid possible accumulated effects associated with multiple injections, only one drug at one concentration was tested per animal. The single neurons tested with different pharmacological agents were recorded from separate rats.

Physiological potentials were amplified by a Dagan EX4-400 amplifier, displayed on oscilloscopes, and monitored acoustically over a loudspeaker. Neural responses were digitized and processed by MALab 881, a data acquisition system designed and produced by Steve Kaiser (Department of Neurobiology, University of California, Irvine; Kaiser Instruments) for use with Macintosh computers (in our case, a Quadra 700). The program provided a digital window discriminator for selection of action potentials and displayed poststimulus time histograms on-line. Physiological responses were stored on optical disk and processed later with standard database and graphics software.

All procedures were approved by the Carleton University Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

**Stimulus parameters**

Sounds were presented separately to the two ears through sealed headphones (Pioneer SE-50D) coupled to hollow specula that were inserted into the rat’s external ears. Sounds were generated digitally by a Kaiser Instruments DA interface controlled by MALab 881 to produce either tone pulses (100 ms with 10-ms rise and fall times) or clicks (50 μs square waves). The clicks had a broad spectrum from 0.1 to 25 kHz, essentially flat up to 4.0 kHz and rolling off at higher frequencies. The sound pressure of tone pulses was referenced to a cell’s threshold at CF, and the sound pressure of clicks was calibrated in dB sound pressure level (SPL; re 0.0002 dynes/cm²) using a 0.5-in. B&K microphone with the headphone speculum inserted into a Tygon enclosure that served as an artificial ear. For most experiments the sound pressure level of clicks delivered to either ear was fixed at 20 dB above the threshold for eliciting a contralateral excitatory response.

All recordings were obtained from well-isolated single units defined by action potentials of constant amplitude and waveform. Before investigating responses to binaural time differences, we examined the neural response to tone pulses. First, the CF was determined with monaural stimulation of the contralateral ear. Then the binaural response pattern to either tone or click stimulation was determined by setting the contralateral stimulus level at 20 dB above threshold and presenting ipsilateral sounds simultaneously in steps of increasing intensity. In some cases, both ipsilateral and contralateral stimulation...
produced excitation, and combined stimulation resulted in facilitation. The majority of cells, however, showed binaural suppression—i.e., the contralateral response was strongly inhibited by simultaneous ipsilateral stimulation. Among the neurons that exhibited binaural suppression, some showed a slight response facilitation at low levels of ipsilateral stimulation, but strong suppression as the ipsilateral level was increased. All the recordings in the present study were made from neurons showing strong binaural suppression.

The rat’s range of hearing is restricted primarily to high frequencies, and most of the neurons in its central auditory pathway have CFs above 1 kHz (Kelly and Masterton 1977; Kelly and Phillips 1991; Kelly et al. 1991; Sally and Kelly 1988). Because phase-locking is not secure at these frequencies, most of the neurons in the rat ICC are insensitive to the ongoing time (i.e., phase) differences between tones presented to the two ears. Therefore, after the responses to tone bursts had been recorded, ICC neurons were tested with transients (clicks) to determine their response to binaural time differences. The clicks were delivered to the left and right ears in pairs at various ITD intervals, and each click pair was repeated 30 times at a rate of one per second.

The sound pressure level of the clicks was the same in both ears. The probability of a spike was determined for a wide range of ITDs from –1.0 to +30 ms (positive values representing ipsilateral leading contralateral time differences). The data were plotted separately for small ITDs (+1.0 to –1.0 ms) and large ITDs (1.0–30 ms). The smaller intervals were chosen to span the range of ITDs produced by a single sound source located at various free-field positions in the azimuthal plane and to bracket the dynamic range of responses of neurons to small ITD intervals. The larger intervals were explored to determine the duration of the inhibition produced by stimulation of the ipsilateral ear. For each neuron, responses to both short and long binaural time intervals were examined before and after injection of pharmacological agents into the DNLL.

Quantitative and statistical analysis

The effects of drug injection on each neuron were expressed as indices of response change for short and long binaural time intervals separately. For the short intervals the index was calculated by measuring the difference in spike counts before and after drug injection for ITDs of 0.0, 0.25, 0.50, 0.75, and 1.0 ms and averaging over the five ITD periods to give a single measure of response change, with positive values indicating an increase in the number of spikes. For the long time intervals the index was calculated by measuring the differences in spike counts for 2.0, 4.0, 6.0, 8.0, 10, and 12 ms and averaging over the intervals to obtain a single mean value. A positive index reflects an increase in spikes. The effects of antagonists were evaluated by plotting the indices of response change as a function of drug concentration. Statistical comparisons were made using the Kruskal-Wallis nonparametric ANOVA to determine the effect of drug concentration. Further comparisons between short and long binaural time intervals were made using the Mann-Whitney U test.

Histology

The positions of recording and injection pipettes were marked by passing positive current through the electrode to produce a small lesion. In preparation for histology the animals were given an injection of pentobarbitol (120 mg/kg ip) and perfused through the heart with normal saline followed by 10% formalin. The brains were removed, stored in 20% sucrose-10% formalin and cut serially at 40 μm in the frontal plane on a freezing microscope. The location of pipette tracks and lesions at the pipette tips were determined microscopically from cresyl violet–stained sections. All data presented here were obtained from cases in which the injection and recording pipettes were clearly in the DNLL and ICC, respectively.

RESULTS

NBQX

Injection of the AMPA antagonist NBQX produced a consistent release from binaural inhibition as shown in Fig. 2. Responses to click pairs are plotted separately for short (–1.0–1.0 ms) and long (1–30 ms) ITD intervals. The CFs as determined by the response to contralateral tone bursts are shown at the top of each pair of graphs. The binaural response pattern for each of these cells was contralateral excitatory and ipsilateral inhibitory (EI). Before drug injection, the cells were strongly inhibited by acoustic stimulation of the ipsilateral ear. Click pairs with contralateral lead times usually evoked action potentials after each stimulus presentation, but as the binaural time difference was shifted in favor of the ipsilateral ear, the probability of an action potential progressively decreased. The duration of the inhibitory effect varied from cell to cell but in most cases lasted for 15–20 ms.

At concentrations of 5.0 and 2.5 mM NBQX reduced the strength of the binaural inhibition imposed on cells in the contralateral ICC. At both concentrations the effect of NBQX was most evident at short time delays with relatively little effect at longer ITD intervals. Release from inhibition was seen for cells with widely varying CFs ranging from 3.5 to 22.6 kHz. At a concentration of 1.25 mM NBQX had an inconsistent effect on binaural responses. In two cells (1.2 mM, Fig. 2, A and C) there was a release from inhibition that was most pronounced at short interaural time delays, but in two other cells (B and D) there was no effect.

As shown in Fig. 3, A and B, a 0.75-mM concentration of NBQX had no systematic effect on binaural responses of neurons in the contralateral ICC. The results indicate that the injection procedure itself was without effect and that the action of NBQX was concentration dependent.

CPP

Injection of a 10 mM concentration of the NMDA antagonist, CPP, produced a consistent release from binaural inhibition in each of the five cells tested (Fig. 4). The effect was most apparent at long time intervals. The strength of inhibition produced by stimulation of the ipsilateral ear was reduced at intervals between 1 and 30 ms in all five cases (Fig. 4, A–E). In three of these cases (A, B, and E) there was no corresponding release at shorter time intervals, although some effect was apparent in two other cases (C and D). Injections of CPP at lower concentrations (5.0, 2.5, and 1.25 mM) had no consistent effect on binaural responses (see Table 1 for summary).

CNQX and APV

Injection of 10 mM CNQX into the DNLL resulted in a release from binaural inhibition at both long and short ITD intervals (Fig. 5). Binaural responses recorded from the contralateral ICC were less strongly suppressed after the drug injection, but there was no indication of a selective effect on short versus long ITD intervals. There was a less pronounced release from inhibition after injection of 5.0 mM CNQX. Little or no effect was recorded with concentrations of either 2.5 or 1.25 mM (see Table 1 for summary).

Injection of 30 mM APV resulted in a release from binaural
inhibition that was apparent at both short and long ITD intervals (Fig. 6). A less pronounced release from inhibition was seen after injection of 15 mM APV. At this concentration the effect was most evident at long ITD intervals, but there was also some release at short ITD intervals. Lower concentrations (7.5 or 3.7 mM) were without effect on binaural responses of neurons in ICC (see Table 1).

The mean response change produced by each of the four excitatory amino acid antagonists is shown in Fig. 7. Response change is plotted as a function of drug concentration for both long and short binaural time delays. For each of the drugs the magnitude of the release from binaural inhibition increased with concentration. The AMPA receptor antagonist NBQX had a greater effect at short time intervals, whereas the NMDA antagonist CPP had a greater effect at long time intervals. CNQX and APV had effects at both long and short ITD intervals, although there was some tendency for selectivity at moderate concentrations.

Statistical results

ANOVA (Kruskal-Wallis) showed a significant effect of drug concentration for each of the four excitatory amino acid antagonists used in this study (CNQX, NBQX, APV, and CPP). For CNQX there was an increased release from inhibition at both short and long interaural time intervals ($H = 8.36, P < 0.01$ and $7.72, P < 0.01$ respectively). For NBQX the increase was significant for the short intervals only ($H = 6.24, P < 0.05$); no differences were found for the longer time intervals ($H = 0.76$). For both APV and CPP significant differences were found for long intervals ($H = 6.72, P < 0.05$ and $H = 6.76, P < 0.05$, respectively), but the differences for short intervals were not statistically significant ($H = 3.95$ and $H = 1.45$ respectively).

The magnitude of response change produced at the highest drug concentrations was compared for long and short ITD intervals using the Mann-Whitney $U$ test. Injection of NBQX at 5.0 and 2.5 mM concentrations was found to have a significantly greater effect on responses to short time intervals ($U = 6, P < 0.002$). A significant difference was also found for 5.0 mM CPP ($U = 1, P < 0.008$) with the greater change occurring at long time intervals. There were no statistically significant differences between long and short intervals for either APV or CNQX at the concentrations used in this study.

DISCUSSION

The DNLL-ICC circuit

The results of our study show that the DNLL plays an important role in the regulation of binaural responses in the contralateral ICC. Pharmacological block of excitatory activity in the DNLL by local injection of either AMPA or NMDA receptor antagonists results in a release from binaural inhibition in the contralateral ICC. In our sample of EI neurons, binaural responses were altered in every case provided that a sufficient concentration of drug was injected into the DNLL. After pharmacological blockage, the ICC neurons were less strongly inhibited by stimulation of the ipsilateral ear, and their binaural response curves were shifted relative to normal.

These data confirm the results of earlier studies in which kynurenic acid was injected into the DNLL (Kelly 1997; Kelly and Kidd 1997; Kidd and Kelly 1996; Li and Kelly 1992). Local injection of kynurenic acid, a nonspecific excitatory amino acid antagonist, resulted in a consistent release from binaural inhibition and a consequent shift in binaural response curves of ICC neurons located contralateral to the injection site. The reduction in inhibition was apparent over a wide range of interaural time delays and affected the sensitivity of cells to either binaural time or intensity differences (Kidd and Kelly 1996; Li and Kelly 1992). Evidence of disinhibition was found in both the contralateral ICC and DNLL, but no effect was seen when the recordings were made in the ICC ipsilateral to the injection site (Kelly and Kidd 1997; Li and Kelly 1992).

Our interpretation of the contribution made by the DNLL to binaural responses in the ICC is presented in Fig. 8. First, we recognize that binaural responses in the mammalian auditory system are established in the superior olivary complex through neural circuits in the lateral and medial superior olivary nuclei.
(LSO and MSO, respectively) (Irvine 1992). Neurons within the LSO are typically excited by stimulation of the ipsilateral ear and inhibited by stimulation of the contralateral ear (Bou-dreau and Tsuchitani 1968; Caird and Klinke 1983; Finlayson and Caspary 1989; Tsuchitani 1988). The contralateral inhibition of LSO neurons is imposed through a glycinergic projection from the medial nucleus of the trapezoid body (MNTB), which itself receives an excitatory projection from the cochlear nucleus on the opposite side of the brain (Sanes 1990; Wu and Kelly 1991). The neurons in LSO then project either ipsilaterally or contralaterally to the auditory midbrain through the lateral lemniscus. The contralaterally projecting LSO neurons are considered to be excitatory, and the ipsilaterally projecting neurons are predominantly glycinergic and presumably inhibitory (Glendenning et al. 1992; St. Marie et al. 1989). Therefore, preferential stimulation of one ear over the other, due to either more intense or earlier acoustic input, would cause a net excitation of midbrain structures contralateral to the preferred ear. A net inhibition of structures ipsilateral to the preferred ear would also be expected, because of the glycinergic projection from LSO (see Fig. 8A). In addition, the MSO contributes to early binaural processing through converging projections from the left and right cochlear nuclei (Kuwada et al. 1997a; Yin and Chan 1990). The neurons in MSO are sensitive to binaural phase differences and are maximally excited by sounds that favor the contralateral ear. Because of the circuitry within the superior olivary complex (SOC), a free field sound that results

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**TABLE 1. Index of response change after injection of excitatory amino acid antagonists**

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<tr>
<th></th>
<th>n</th>
<th>Short</th>
<th>Long</th>
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<tr>
<td><strong>NBQX</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5.0 mM</td>
<td>4</td>
<td>9.50 ± 2.78</td>
<td>2.38 ± 1.61</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>4</td>
<td>7.70 ± 2.29</td>
<td>0.83 ± 1.35</td>
</tr>
<tr>
<td>1.25 mM</td>
<td>4</td>
<td>2.90 ± 2.18</td>
<td>1.04 ± 1.54</td>
</tr>
<tr>
<td>0.75 mM</td>
<td>2</td>
<td>0.90 ± 0.50</td>
<td>0.33 ± 1.15</td>
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<tr>
<td><strong>CPP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>5</td>
<td>1.36 ± 0.61</td>
<td>7.13 ± 1.10</td>
</tr>
<tr>
<td>5.0 mM</td>
<td>3</td>
<td>0.60 ± 0.70</td>
<td>2.30 ± 1.43</td>
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<tr>
<td>2.5 mM</td>
<td>2</td>
<td>2.60 ± 0.40</td>
<td>1.42 ± 2.75</td>
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<tr>
<td>1.25 mM</td>
<td>3</td>
<td>−0.86 ± 0.18</td>
<td>1.17 ± 0.66</td>
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<tr>
<td><strong>CNQX</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10 mM</td>
<td>4</td>
<td>15.15 ± 2.45</td>
<td>16.25 ± 3.67</td>
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<tr>
<td>5.0 mM</td>
<td>4</td>
<td>6.65 ± 2.12</td>
<td>1.04 ± 1.53</td>
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<tr>
<td>2.5 mM</td>
<td>2</td>
<td>−0.30 ± 0.50</td>
<td>1.17 ± 1.65</td>
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<tr>
<td>1.25 mM</td>
<td>2</td>
<td>−1.40 ± 0.60</td>
<td>3.25 ± 0.05</td>
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<tr>
<td><strong>APV</strong></td>
<td></td>
<td></td>
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<tr>
<td>30 mM</td>
<td>4</td>
<td>6.60 ± 3.10</td>
<td>7.46 ± 2.50</td>
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<tr>
<td>15 mM</td>
<td>4</td>
<td>3.40 ± 2.75</td>
<td>6.04 ± 1.76</td>
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<tr>
<td>7.5 mM</td>
<td>2</td>
<td>1.40 ± 0.60</td>
<td>0.83 ± 0.35</td>
</tr>
<tr>
<td>3.75 mM</td>
<td>2</td>
<td>0.10 ± 1.10</td>
<td>−0.92 ± 1.25</td>
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</tbody>
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Data are means ± SE. See METHODS for description of excitatory amino acid antagonists.
in earlier and/or more intense stimulation of the ear closer to the sound source would be expected to excite neurons primarily in the contralateral auditory midbrain (DNLL and ICC).

The DNLL provides a second level of binaural processing that reinforces the contralateral bias already established by the SOC (Fig. 8B). The neurons in the DNLL are GABAergic and project heavily to the DNLL and ICC on the opposite side of the brain through the commissure of Probst (CP). A separate population of DNLL neurons projects to the ipsilateral ICC (Adams and Mugnaini 1984; Glendenning and Baker 1988; González-Hernández et al. 1996; Moore and Moore 1987; Roberts and Ribak 1987; Shneiderman et al. 1988; Thompson et al. 1985; Vater et al. 1992; Winer et al. 1995; Zhang et al. 1998). In the rat retrograde tract tracing shows that 70% of the neurons in the DNLL that project to the ICC have crossed connections, and the remaining 30% have uncrossed connec-

**Fig. 5.** Effects of injecting 10 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into the DNLL. Separate panels are shown for short and long ITDs, and the CFs for each neuron (A–D) are shown above the left panels. Conventions and symbols are as indicated in Fig. 2.

**Fig. 6.** Effects of injecting 30 mM APV into the DNLL. Separate panels are shown for short and long ITDs and the CFs for each neuron (A–D) are shown above the left panels. Conventions and symbols are as indicated in Fig. 2.
tions (Ito et al. 1996; Merchán et al. 1994). Also, nearly 70% of the neurons in the DNLL degenerate after cutting the decussating fibers in the rat CP (van Adel et al. 1999). Many of the DNLL neurons with crossed projections to ICC have collateral connections with neurons in the contralateral DNLL (van Adel and Kelly 1998). Thus, the DNLL is in an excellent

![Graph of mean index of response change for short and long ITD intervals with various concentrations of four excitatory amino acid antagonists (NBQX, CNQX, CPP, and APV). A positive change reflects an increase in spike count and a release from inhibition relative to the preinjection control.](http://jn.physiology.org/)

![Diagram of basic circuits that influence neural activity in the DNLL and ICC. A: the main structures that project from the lower brain stem to the DNLL and ICC, i.e., the medial superior olive, the lateral superior olive, and the anteroventral cochlear nucleus (MSO, LSO, and AVCN, respectively). Binaural interactions and crossed projections within the auditory lower brain stem result in a net excitatory activity in the lateral lemniscus (LL) contralateral to the source of acoustic stimulation. There is also a source of inhibition in the LL ipsilateral to the sound source arising from a population of glycinegic neurons in the LSO. B: pathways projecting to and from the DNLL. Excitatory activity within the LL results in AMPA and NMDA receptor–mediated excitation of neurons in the DNLL. The DNLL neurons in turn send an inhibitory (GABAergic) projection to the contralateral DNLL and ICC via the commissure of Probst. A separate population of DNLL neurons (~30% in the rat) sends projections to the ipsilateral ICC. MNTB, medial nucleus of the trapezoid body.](http://jn.physiology.org/)
position to impart a GABAergic inhibitory influence on the DNLL and ICC on the opposite side of the brain. This contralateral inhibition would have the effect of magnifying any difference in neural activity that might arise between auditory structures on the left and right sides of the brain. The laterality of responses in the central auditory system first established by circuits within the superior olive would be enhanced by the contralateral inhibitory projections of the CP. This role of the CP has been demonstrated recently by recording binaural evoked responses in the rat ICC before and after surgical transection of the decussating fibers as they pass the midline just below the medial longitudinal fasciculus (van Adel et al. 1999). The result is a reduction in the strength of the inhibition produced by stimulation of the ipsilateral ear as reflected in the amplitude of binaural evoked responses. Pharmacological blockade of excitatory activity in the DNLL by local injection of the excitatory amino acid antagonist, kynurenic acid, produces a similar effect, presumably through disruption of the crossed projections in the CP. Thus, the DNLL and its efferent fibers in the CP play a role in maintaining the laterality of responses in the central auditory system and can be considered a part of the “acoustic chiasm” described previously by Glen-dennning and Masterton (1983) (see also Glen-dennning et al. 1985, 1992).

The concept of a progressive refinement of binaural responses in the ascending auditory pathway is consistent with the recent results of Spitzer and Semple (1998) showing emergent properties in the ICC of the gerbil. The response of ICC neurons to time-varying phase disparities between the two ears is strongly influenced by dynamic aspects of the stimulus, whereas responses of binaural neurons in the SOC are largely predictable on the basis of static stimulus properties. One possible source of this transformation in binaural response characteristics may be the GABAergic projection from the DNLL. Furthermore, the neurons in the gerbil ICC show lasting changes in their response to interaural level differences (ILDs) depending on prior exposure to binaural stimulation. Dynamic changes in the balance of binaural stimulation can produce a “conditioned enhancement” or a “conditioned suppression” of subsequent binaural responses. Comparable conditioning is not found in the gerbil superior olive (Spitzer and Semple 1993, 1995). The dynamic conditioning of responses in the ICC can also be induced pharmacologically by the release of GABA-glycine from the recording pipette, which shows the importance of inhibition in shaping the emerging properties of binaural responses in ICC (Sanes et al. 1998). The crossed GABAergic projection from DNLL is a possible source of inhibition for generating these dynamic binaural response properties.

The effects of blocking activity in the DNLL are also consistent with the finding that binaural responses in ICC can be altered by release of the GABA_{A} antagonist, bicuculline, at the recording site (Klug et al. 1995; Park 1998; Park and Pollak 1993, 1994). Bicuculline results in a release from inhibition that is probably due in part to a reduction in the influence of crossed GABAergic projections from the DNLL. Park (1998) has recently suggested on the basis of his comparison of responses in the SOC and ICC that binaural inhibitory responses can be formed locally in the auditory midbrain, a conclusion that is supported by intracellular recordings from the ICC (Covey et al. 1996; Kuwada et al. 1997a,b; Nelson and Erulkar 1963; Pedemonte et al. 1997) and extracellular recordings of binaural responses following SOC lesions (Kelly and Sally 1993; Li and Kelly 1992b; Sally and Kelly 1992). One possible source of local binaural inhibition in the ICC is the crossed GABAergic projection from the DNLL through the CP.

**NMDA and AMPA receptors**

The present results show that injection of the receptor specific excitatory amino acid antagonists, NBQX and CPP, into the DNLL alters the response of ICC neurons to sounds with short and long ITDs selectively. The AMPA receptor antagonist NBQX results in a preferential release from binaural inhibition at short ITD intervals (0–1 ms) and has little effect at longer intervals (1–30 ms). In contrast, injection of the NMDA antagonist CPP produces a release from inhibition at the long intervals and has little or no effect at the shorter intervals. Under the same experimental conditions, neurons tested with kynurenic acid show a generalized release from inhibition at both short and long ITD intervals with no indication of selectivity for specific time delays (Kidd and Kelly 1996).

We attribute the effects of NBQX and CPP to their selective action on AMPA and NMDA receptors in the DNLL. Both receptor types are present, and their role in generating postsynaptic responses has been shown by intracellular recordings in brain slice preparations (Fu et al. 1997; Wu and Kelly 1996). Excitatory potentials evoked in the DNLL by electrical stimulation of the lateral lemniscus have two distinct components: an early response that can be blocked by AMPA antagonists and a later response that can be blocked by NMDA antagonists (Fu et al. 1997; Wu and Kelly 1996). The AMPA antagonists selectively eliminate short latency action potentials, whereas NMDA antagonists selectively block longer latency action potentials. Because most of the neurons in the DNLL are GABAergic, their excitation would be expected to result in the inhibition of structures to which they project. The early and late components of excitatory responses in the DNLL would be translated into an early and late inhibitory action on target neurons in the ICC and contralateral DNLL. Thus, blockade of AMPA and NMDA receptors by injection of receptor specific antagonists into the DNLL would be expected to have selective effects on binaural responses of ICC neurons to short and long ITD intervals. The results of the present study confirm this expectation.

Unexpectedly, CNQX was not highly selective in its effect on binaural responses at specific ITD intervals. At concentrations of 5.0 and 10 mM, it caused a release from binaural inhibition at both short and long ITDs. One explanation for this apparent nonselectivity may be that CNQX exerted an indirect effect on NMDA as well as AMPA receptors at the concentrations used in this study. It is well known that activation of the NMDA receptors in the hippocampus and other CNS structures is dependent on a concomitant depolarization mediated by AMPA receptors. In DNLL, as in these other structures, the NMDA component of excitatory responses is voltage dependent and subject to a magnesium block that can be overcome by membrane depolarization (Fu et al. 1997). Thus, block of AMPA receptors may have resulted in the elimination of both NMDA and non-NMDA receptor–mediated excitation in the DNLL. On the other hand, two observations
mitigate against this possibility. First, our brain slice studies of the DNLL show that AMPA antagonists can block the early phase of excitatory responses without eliminating the longer lasting, NMDA receptor–mediated responses. These data indicate that the NMDA receptors in the DNLL are normally available at or near resting potential and do not require depolarization through AMPA receptors for their activation (Fu et al. 1997; Wu and Kelly 1996). Second, the injection of the AMPA antagonist NBQX into the DNLL in fact produced a selective release from binaural inhibition at short ITD intervals. Thus, even though some interaction between NMDA and non-NMDA receptor–mediated events almost certainly occurs, this mechanism alone cannot explain why the effects of CNQX are not selective, whereas those of NBQX under similar experimental conditions are.

A more likely explanation of the nonselective effect of CNQX is its relative nonspecificity as a receptor channel antagonist compared with NBQX. At high concentrations CNQX exerts an antagonistic action at glycine receptors as well as AMPA receptors (Lester et al. 1989). Because the activation of the NMDA receptor requires the presence of both glycine and glutamate (Asher and Johnson 1989), an inadvertent block of the glycine binding site by CNQX would eliminate both NMDA and non-NMDA receptor–mediated responses. In contrast, NBQX does not block the glycine receptor at high concentrations. Thus, for our studies, which require a relatively high concentration of drug to produce an effect, NBQX is a superior antagonist and a better choice than CNQX for selectively blocking AMPA receptors.

Injection of the NMDA antagonist APV into the DNLL was also relatively nonselective in its effect on binaural responses in the contralateral ICC. At the concentrations used in this study the drug resulted in a release from binaural inhibition at both long and short ITD intervals. Although there was some indication of a larger release at long ITDs, the effect was not statistically significant. It is not known why APV was less selective than CPP in producing a shift in binaural responses. However, another investigator has reported greater selectivity for CPP than APV in studies of other CNS structures (Kita 1996).

**Functional implications**

The ability to localize sounds in space is dependent on central processing of small binaural time and intensity cues (Heffner and Masterton 1990). For mammals with small heads, the interaural time delays produced by sound sources at various positions in the horizontal plane are considerably <1 ms. For the rat, with an interaural distance of 3.5 cm, the maximum ITD associated with a sound positioned on the left or right is at most ±130 μs (Kelly and Phillips 1991). In the present study, injection of the AMPA antagonist NBQX into the DNLL primarily affected binaural responses to ITDs within the range that is useful for localization of a single sound source (±1.0 ms). The release from inhibition produced by blocking the AMPA receptors in the DNLL resulted in a shift in the binaural response of neurons in ICC that would likely degrade the ability to localize a single sound source. Indeed, our behavioral studies have shown that the crossed projection from the DNLL is important for maintaining accurate sound localization. Either surgical transection of the crossed fibers in the CP or kainic acid lesions of the DNLL result in elevated minimum audible angles for localization of a brief noise burst (Ito et al. 1996; Kelly et al. 1996). The acuity for sound localization in the horizontal plane is significantly reduced by either lesion.

Binaural time differences >1.0 ms are well beyond the range that is useful for localization of single sound sources. However, several investigators have suggested that binaural interactions at ITDs >1 ms may play an important role in localization of multiple sound sources or the suppression of reflected sounds as demonstrated by the “precedence” effect (Carney and Yin 1989; Fitzpatrick et al. 1995; Kelly and Kidd 1997; Kidd and Kelly 1996; Yang and Pollak 1994a,b; Yin 1994). The results of the present study indicate that responses in ICC to binaural stimuli with ITDs in the range of 1–20 ms are selectively influenced by activation of NMDA receptors in the DNLL. Pharmacological blockage of the NMDA receptor in the DNLL reduces the strength of long-lasting binaural inhibition in the contralateral ICC, but has relatively little effect on responses to binaural stimuli with ITDs in the range of 0–1 ms. These observations suggest that the NMDA receptor in the DNLL plays a role in sensory processing and serves to regulate the period of GABAergic inhibition imposed on neurons in the ICC. We suggest that one functional contribution of the NMDA receptor in the auditory midbrain is to extend the period of excitation in the DNLL and thus prolong the period of inhibition in the contralateral ICC, providing a neural mechanism for echo suppression.

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