Neural correlates of the precedence effect in the inferior colliculus of behaving cats

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Abstract
Several auditory spatial illusions, collectively called the precedence effect (PE), occur when transient sounds are presented from two different spatial locations, but separated in time by an inter-stimulus delay (ISD). For ISDs in the range of localization dominance (< 10 ms), a single fused sound is typically located near the leading source location only, as if the location of the lagging source were suppressed. For longer ISDs, both the leading and lagging sources can be heard and localized, and the shortest ISD where this occurs is called the echo threshold.
Previous physiological studies of the extracellular responses of single neurons in the inferior colliculus (IC) of anesthetized cats and unanesthetized rabbits with sounds known to elicit the PE have shown correlates of these phenomena though there were differences in the physiologically-measured echo thresholds. Here we recorded in the IC of awake, behaving cats using stimuli that we have shown to evoke behavioral responses that are consistent with the precedence effect (Tollin and Yin, 2003; J Neurophysiol 90: 2149-2162). For small ISDs responses to the lag were reduced or eliminated, consistent with psychophysical data showing that sound localization is based on the leading source, or localization dominance. At longer ISDs, the responses to the lagging source recovered at ISDs comparable to psychophysically-measured echo thresholds. Thus it appears that anesthesia, and not species differences, accounts for the discrepancies in the earlier studies.

Key words: sound localization, inferior colliculus, precedence effect, cat, psychophysics, anesthetics
Introduction

When transient sounds are presented from two locations and separated by an ISD, several spatial perceptual phenomena, collectively called the PE (Wallach et al., 1949), occur. We have shown that cats experience each of these phenomena (Populin and Yin, 1998; Tollin and Yin, 2003a,b), an example of which is shown in Figure 1. In cats, summing localization occurs for ISDs between ±400 µs, where a single fused ‘phantom’ sound is located between the sources, but biased towards the sound source that is leading in time, which, for brevity, we will refer to in this paper simply as the “lead.” Localization dominance occurs for ISDs of ~400 µs to 10 ms, where the paired sounds are localized near the lead with little effect of the sound source that is lagging in time, which we will call the “lag,” on localization. Finally, for ISDs > ~10 ms, the echo threshold is reached, the shortest ISD at which the two separate sound source locations are first perceived. The mechanisms that produce the PE illusion are thought to be responsible for the ability to localize sounds accurately in natural echoic environments.

The PE has been studied physiologically at virtually all levels of the auditory system, including the auditory nerve (Parham et al., 1996), the cochlear nucleus (Wickesberg, 1996; Fitzpatrick et al., 1995; Parham et al., 1998), superior olivary complex (Fitzpatrick et al., 1995), IC (Fitzpatrick et al., 1995; Yin, 1994; Litovsky and Yin, 1998a,b; Burger and Pollak, 2001), and auditory cortex (Fitzpatrick et al., 1999; Reale and Brugge, 2000; Mickey and Middlebrooks, 2001). At each stage, for small ISDs, neuronal responses to the lag are substantially reduced compared to responses to the same stimulus presented in isolation from the same location, yet the responses to the lead are generally unchanged. With increasing ISD, the lag responses recover to levels comparable to the response elicited when the lagging source is presented in isolation. However, the rate of recovery with increasing ISD is dependent on where in the auditory system the neurons are being recorded. At the auditory nerve and cochlear nucleus, neurons can respond to the lead and the lag for ISDs as low as 1-2 ms. Yet the behavioral responses of cats with such ISDs depend almost exclusively on the lead (Tollin and Yin, 2003b).

At the IC and the auditory cortex, neural correlates of the PE phenomena have been found: at short ISDs for which cats experience localization dominance, there is an accurate neural representation of the leading source, but the response to the lag is diminished or non-existent. We have focused our physiological studies on the IC because it is a site of major convergence of inputs from lower brainstem nuclei (Adams 1979), the neurons comprising many of these input nuclei are selectively sensitive to the acoustical cues to location (Yin 2002), and many IC neurons are sensitive to sound location (Irvine, 1986). Our previous studies (Yin, 1994; Litovsky and Yin, 1998a,b), performed in barbiturate-anesthetized cats with stimuli presented in the free-field, showed long-lasting (ISDs > ~ 30 ms) suppression of the response to the lag. However, lag responses in the IC of unanethetized rabbits recovered at substantially shorter ISDs (Fitzpatrick et al. 1995). Since it is not known over what ISD ranges (or even whether) rabbits experience the PE phenomena psychophysically, this difference in recovery times could be due to species and/or anesthetic-state differences. As one test of that hypothesis, we recorded from neurons in the IC of cats that were actively participating in a sound localization task using stimulus configurations we have shown to elicit the PE in cats (Tollin and Yin, 2003a,b). The IC responses to the lag recovered with ISDs virtually identical to that found in the unanesthetized rabbit, and much faster than our previous studies, demonstrating that anesthetics, and not species differences, were responsible for the prolonged recovery times found in our earlier studies.
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Methods
All procedures used were approved by the University of Wisconsin Animal Care and Use Committee and also complied with the National Institutes of Health guidelines for animal use. Five adult female cats were outfitted with a stainless-steel head-post, fine wire eye coils, and a recording cylinder to access the inferior colliculus with microelectrodes. Details of the surgical procedures can be found in Tollin and Yin (2003b) and Populin and Yin (2002).

Psychophysical tasks and physiological recordings
Detailed methods for the behavioral portion of these experiments can be found elsewhere (Tollin and Yin, 2003b). Briefly, the cats sat in a nylon bag in the center of a dimly illuminated (or dark) sound-attenuating chamber with their heads held fixed facing a bank of loudspeakers and light-emitting diodes (LEDs). Acoustic and visual stimuli were presented from one of 15 different locations situated within the oculomotor range of the cats (~±25°) via loudspeakers or LEDs, respectively. The speakers were located along an arc (62-cm radius measured from the center of the cats’ heads) in the horizontal and the median sagittal plane. Eye position was recorded using the scleral search coil technique. Acoustic stimuli consisted of five (sometimes 10) identical broadband (1.5-40 kHz) noisebursts of 10-ms in duration presented at a rate of 5 Hz. This particular stimulus was used in these experiments because it was identical to that used in our previous psychophysical studies in which we demonstrated the range of ISDs over which cats experience the various PE phenomena (Tollin and Yin, 2003a,b). Using this stimulus thus allows for a comparison between the psychophysical data from those studies and the physiological data from this study. On occasion, 100-µs clicks were used instead of the 10-ms noisebursts. In this paper, we refer to a “trial” as the presentation of one of these trains of 5 (sometimes 10) noisebursts or clicks. The stimulus was presented from single loudspeakers (single source) or with equal level from two different loudspeakers (paired source) connected in phase but with an ISD between the onsets. The paired sources were held constant at ±18° on the horizontal plane. During data collection, we always presented single-source trials at different speaker locations and paired-source trials with different ISDs randomly interleaved. In this way the cats could not anticipate the upcoming trial type and fluctuations in responses sensitivity and spontaneous rate were averaged over the course of the different stimulus conditions.

Standard extracellular recording techniques were used to record the discharges of well-isolated single neurons. We did not routinely assess the frequency selectivity of these neurons, aside from an estimate of best frequency, or attempt to determine the response type of the neuron (e.g., Ramachandran et al., 1999). Stimuli were 10-30 dB above each neuron’s threshold to the train of noiseburst or click stimuli, typically measured at 18° in the contralateral field, and held fixed for all trials. Recordings were taken from the sensory probe task to eliminate the possible confounding effects of eye position on the neural responses of the neurons; the responses of some IC neurons to sounds presented from identical spatial locations have been shown to be modulated with changes in eye position (Groh et al., 2001; Zwiers et al., 2004). Here, the initial fixation LED remained illuminated during stimulus presentation and the cats were required to maintain fixation on the LED throughout the stimulus duration to receive a reward. If the cat broke fixation of the LED at any time during stimulus presentation, the trial was discarded and was not analyzed further. Thus, there were often differing numbers of trials for each stimulus configuration which necessitated normalizing the responses by the number of stimulus presentations (see below). In the saccade task, the LED was extinguished simultaneously as the acoustic stimulus was presented and the cat was required to saccade to and maintain fixation at
the source’s apparent location. During training, both \textit{sensory probe} and \textit{saccade} tasks were presented randomly.

To summarize the responses of each neuron to the train of transients, the response rasters were "folded" on the 5-Hz period of the stimulus within trials (see definition of a trial above), and then summed across trials of the same type to produce a summary histogram (see Figure 2A). For example, in Figure 2A (top panels), the stimulus for each trial consisted of a train of 10 100-µs clicks presented at a rate of 5 Hz (i.e., 200-ms period). For this neuron there were 3 trials for the stimulus presented from speaker B and 4 trials presented from speaker A resulting across all trials in 30 and 40 presentations of the click from speakers B and A, respectively. For each neuron, responses were collected from 3-10 trials, and given that there could be 5 or 10 noisebursts or clicks comprising the train, this results in 15-100 total presentations of each individual noiseburst or click given the 5 Hz period. For the single-source condition (e.g., Figure 2A), this folding was done separately for each location and the number of spikes was counted in an \textit{analysis window} whose onset and duration was defined by the post-stimulus time at which the instantaneous discharge rate (computed in 1-ms bins) first exceeded 2 SD (upper arrows) of and then returned below, respectively, the mean spontaneous rate (lower arrows) computed 500 ms prior to each trial. The total number of spikes in the analysis window was divided by the number of individual stimulus presentations (i.e., each of the noisebursts or clicks comprising the train) yielding the mean number of spikes/stimulus. This allows comparisons between the responses of a neuron to different conditions where the numbers of trials might have differed across conditions. Response latency ('first-spike latency') was taken as the time of the onset of the analysis window (defined above) at the best azimuth (or at +18° in the contralateral field if that source was the most lateral tested). Computing the responses in this way was done because past physiological studies of the PE have used this method (Fitzpatrick et al., 1995; Litovsky and Yin, 1998a,b), thus allowing for a direct comparison of their data to the present data, and the method provides an objective measure of first-spike latency.

The sensitivity to sound source azimuth was quantified using the Modulation Index (MI), defined as \((R_{\text{contra}} - R_{\text{ipsi}}) / (R_{\text{contra}} + R_{\text{ipsi}})\) where \(R_{\text{contra}}\) and \(R_{\text{ipsi}}\) are the responses to the single-sources at +18° in the contralateral field and -18° in the ipsilateral field, respectively. We did not correct the responses for spontaneous activity. In effect, the MI indicates the size and direction of the response difference due to changes in source azimuth relative to the average discharge rate. The MI does not indicate whether the neuron being studied was monaural or binaural which is a difficult assessment to make when presenting stimuli from the free field (see Poirier et al., 2003).

For the paired-source condition, the dependent variable of interest was the response to the lag as a function of the ISD. To compute the lag response, we used the same analysis window defined for the single-source condition at the best azimuth (e.g., Figure 2A for single source), but shifted it in time by the size of the ISD (e.g., Figure 3A for paired source). The procedure was checked visually for all neurons to ensure that the window was placed over and captured all of the lagging response. Because the analysis windows for each neuron were computed separately for the different single-source locations tested, when the locations of the leading and lagging sources differed (as in Figure 3A), so too did the onset times and durations of the leading and lagging analysis windows. The lag responses at each ISD were computed and then normalized by the response to the "lag" presented by itself in isolation (e.g., single source condition) from the same location. A normalized response of 1.0 indicates the response to the lead had no effect on the response to the lag while values < 1.0 indicate a reduction in the lag
response. For large ISDs (e.g., 50- and 20-ms ISDs, Figure 3A), the leading and lagging analysis windows did not overlap and we were able to separately compute the response to the lag. For some smaller ISDs, the windows sometimes overlapped and the lag response was computed using the method outlined by Fitzpatrick et al. (1995) and Litovsky and Yin (1998a). Here, the number of spikes contained in a composite window, whose onset was determined by the onset of the window that was computed for the leading source and whose offset was determined by the offset of the window computed for the lagging source, was measured (see Figure 3A, 10-ms ISD). The response to the lag for these ISDs was estimated by subtracting the single-source response measured at the location of the lead from the paired-source response as computed through the composite analysis window. Note that this analysis technique assumes that the response to the lead is not affected by the presence of the lag, an assumption made by all previous studies of the PE (Yin, 1994; Fitzpatrick et al., 1995; Parham et al., 1996; 1998; Litovsky et al., 1997; Litovsky and Yin, 1998a, 1998b). When the ISDs are large enough that the leading and lagging responses can be discriminated, this assumption can be and usually is verified. However, at the shortest ISDs used here (1-2 ms), it is possible that in some neurons portions of the response due to the lead were affected by the presence of the lag. When the responses to the leading and lagging stimuli overlap at short ISDs, it is not possible to make this distinction. We adopted this analysis technique for overlapping ISDs primarily because it allows for a direct comparison to previous studies of the precedence effect.

Histology

After the completion of the experiments, the cats were euthanized and 2 metal pins were placed through the recording chamber near the ‘center’ of where most putative IC neurons were obtained. The pins were later used to block the midbrain for sectioning, after fixing the tissue in a solution of 10% formalin, and to confirm electrode penetrations through the central nucleus of the inferior colliculus (ICC). Although it was difficult to get precise localization of each electrode penetration due to the length of the experiments, each of the pins traversed the ICC, and assuming that electrode penetrations were parallel to the pins, all penetrations from which the data were taken were through the ICC.

Results

Results are based on 130 neurons from the ICC of 5 cats. Based on the orderly increase in best frequency (BF) with electrode depth, we believe that all neurons were from the ICC. All neurons had BFs > 1.5 kHz. For the 4 cats for which histology was available, we confirmed that the electrodes from which these data were taken traversed the ICC. One of the goals of this paper was to compare our results with similar studies in anesthetized or awake preparations. Data for comparison were from the IC of the barbiturate-anesthetized cat (Yin, 1994; Litovsky and Yin, 1998a) and from the IC of the unanesthetized, but non-behaving rabbit (Fitzpatrick et al., 1995). Another goal was to compare our previous psychophysical data on the PE in cats to the present physiological data. Four of the five cats used here participated in those psychophysical experiments with stimuli identical to those used here and all four exhibited the three phases of the PE (Tollin and Yin, 2003b); the average psychophysical data for the three cats tested most extensively in that study are shown in Figure 1.
Responses to single sources varying in azimuth

We examined the sensitivity to sound source azimuth in 85 neurons. Figure 2A-B shows an example of the responses of one neuron in the single-source condition for the two lead and lag positions used in this study, ±18°, on the horizontal plane. Due to the limited holding time for each neuron and the large number of stimulus conditions tested, responses to changes in source azimuth could not always be studied in detail. All neurons, however, were tested at ±18°, the two source locations for the paired-source conditions. The stimulus in Figure 2A-B was a train of 100-µs clicks. The sensitivity of these neurons to variations in azimuth was quantified using the Modulation Index (MI, see Methods). The MI for the neuron in Figure 2A-B was 0.12.

Figure 2C shows a histogram of the MIs for the neurons in this study; since there was no difference in MI for clicks (0.26 ±0.19, n = 18) and 10-ms noisebursts (0.23 ±0.23, n = 67) (t_{83} = 0.48, p = 0.63) we combined the results. Across the population, most neurons (76/85) had MIs > 0 preferring sources in the contralateral field and the mean MI (0.24 ±0.22) was significantly greater than 0.0 (t_{85} = 9.77, p < 0.00001). The MIs in the present study were not significantly different (t_{158} = -1.76, p < 0.08) from those computed for sources at ±15° from our previous study (Litovsky and Yin, 1998a) (Figure 2C) (mean MI = 0.33 ± 0.4, n = 75). Recall here that we studied sensitivity over a restricted range of azimuths, ±18°, because these sources were within the oculomotor range of the cats (±25°) and were also the locations (Figure 1) used for our psychophysical studies (Populin and Yin, 1998; Tollin and Yin, 2003a,b). In the few neurons that were studied over larger ranges, more complete modulations yielding MIs approaching 1.0 were typically observed. We did not determine whether each neuron was binaurally or monaurally responsive (see Methods).

Responses to paired-sources: the half-maximal ISD

Seventy of the 85 neurons were tested with paired-source stimuli as a function of ISD. We chose in these studies to concentrate on ISD ranges that evoked localization dominance and past the echo threshold, from 1-50 ms. Most (65/70) of these neurons had MI > 0. The responses of the neurons depended critically on the ISD. Figure 3 shows responses of the same neuron as in Figure 2A-B at three ISDs under two conditions, contralateral leading the ipsilateral source (left) and vice versa (right). As for most neurons, for large ISDs (20 and 50 ms), clear responses were seen to both the lead and the lag. But with decreasing ISD, lag responses were reduced as they overlapped with those of the lead. Figure 3B shows the normalized response to the lag as a function of ISD, which was approximately equally reduced whether the lead was ipsilateral or contralateral. Figure 4 shows the responses of a different neuron to the train of 10-ms noisebursts. Here, there was more reduction in the lag response when the lead was contralateral than when it was ipsilateral (Figure 4B). In a later section we investigate whether this asymmetry can be accounted for simply by the fact that contralateral sources produce greater responses than ipsilateral sources. Note that there were fewer trials for the 10-ms ISD conditions in Figure 4 than for the other ISDs, which was due to the fact that the cat broke fixation for most of the trials with a 10-ms ISD. [Because there were fewer trials, but plotted over the same distance on the ordinate, the responses (spikes/stimulus) in the 10-ms ISD condition in Figure 4 appear to be less robust than to the other conditions, when in fact the responses are quite similar.]

A common method to summarize how neurons responded to the lag as a function of ISD that we (Yin, 1994; Litovsky and Yin, 1998a) and others (Fitzpatrick et al., 1995) have used is the so-called half-maximal ISD. For each neuron, the half-maximal ISD represents the ISD at which the lag response reached 50% of the response to a single source at the lagging location.
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(e.g., Figure 3B). Larger half-max ISDs can be interpreted as indicating longer time periods of reduction in the response to the lag, or longer recovery times. Half-max ISDs were computed for the condition where the ipsilateral source was leading the contralateral source, as this allowed a direct comparison to the conditions tested in the previous experiments (Fitzpatrick et al., 1995; Litovsky and Yin, 1998a). Figure 5 shows a histogram of half-max ISDs for the present study for the 49 neurons tested over a sufficient range of ISDs (greater than four) and for Litovsky and Yin (1998a) and Fitzpatrick et al. (1995), studies that also used this metric. Data from our neurons studied with clicks and with noisebursts were pooled since we show in the next section that there was no significant effect of stimulus type on lag recovery with ISD for this particular condition. Within 5 ms, 35% of our neurons reached the half-maximal point, increasing to 63% by 10 ms, virtually identical to the proportion neurons found by Fitzpatrick et al. (1995). The close correspondence between the data in the current study and from Fitzpatrick et al. (1995) is confirmed by the similarities in the cumulative distributions of half-max ISD (Fig. 5, right ordinate). Only 12% of neurons in the Litovsky and Yin (1998a) study reached half-max by 5 ms, climbing to 32% by 10 ms. By 15-ms ISD, the approximate echo threshold for cats (e.g., Figure 1), 70-80% of the neurons in the two unanesthetized preparations had reached 50%, while only 34% had done so in the anesthetized preparation.

The half-max ISDs for this study and that of Fitzpatrick et al (1995) were similar in magnitude and both were clearly smaller than those observed by Litovsky and Yin (1998a). The ANOVA found a significant difference in the means of the half-max ISDs measured in the three studies \([F(1,2) = 26.9, p < 0.00001]\). The post-hoc analysis (Bonferroni) indicated that the half-max ISDs in this study (mean = 9.8 ±2.3 ms; median = 7.9 ms) were significantly lower than those obtained in the anesthetized cat (34.8 ±8.5 ms; 35.4 ms) \((p < 0.00001)\) but were not different from those obtained by Fitzpatrick et al. (1995) (11.9 ±3.3 ms; 6.6 ms).

Responses to paired-sources: population recovery functions

To show how our population of IC neurons responded to the lag as a function of ISD, we computed the mean (±1 SEM) normalized recovery curve, separately for a contralateral or ipsilateral leading source, for all neurons tested with 2 or more ISDs. Figure 6A shows the mean recovery curves for the 18 neurons tested with trains of 100-µs clicks (e.g., Figure 3), while Figure 6B shows recovery curves for the 52 neurons tested with 10-ms noisebursts (e.g., Figure 4). The hatched region indicates the range of ISDs over which cats experienced behaviorally the localization dominance aspect of the PE. For these ISDs, the cats always localized the paired sources to the leading source location only (Figure 1). The end of the hatched region in Figure 1 indicates the echo threshold (~10-15 ms), where the cats first began to localize the lagging source on some trials. For the data in Figure 6A, the two-factor ANOVA showed a significant main effect of ISD on the mean normalized response to the lag \([F(1,5) = 7.05, p < 0.00001]\), but no significant main effect of whether the lead was contralateral or ipsilateral to the lag \([F(1,1) = 0.39, p = 0.53]\); the interaction of ISD and lead/lag side also did not reach significance \([F(1,5) = 0.094, p = 0.99]\). In other words, when clicks were used, the population response in the IC to the lag recovers with ISD independent of the side containing the lead. Lag responses were ~50% of normal by 5 ms and nearly 85% recovered by 20 ms ISD. When the stimulus was the train of 10-ms noisebursts (Figure 6B), there was a significant main effect of ISD \([F(1,5) = 41.37, p < 0.00001]\) as well as a significant effect of the side of the lead \([F(1,1) = 38.29, p < 0.00001]\); the interaction did not reach significance \([F(1,5) = 1.07, p = 0.38]\). With these stimuli, while the response to the contralateral stimulus recovered quickly when the ipsilateral source was leading,
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like that found with clicks, the response to the ipsilateral stimulus remained reduced longer when
the contralateral source was leading. The test of whether there was an effect of stimulus type
(100-µs clicks vs 10-ms noisebursts) revealed that when the ipsilateral source was leading, there
was no significant effect of stimulus type \([F(1,1) = 0.79, p = 0.38]\), but when the contralateral
source was leading, there was an effect \([F(1,1) = 23.57, p < 0.00001]\). In other words, the
reduction of the response to the lag lasted longer with 10-ms noisebursts than with clicks, but
only when the contralateral source led the ipsilateral source.

Responses to paired-sources: effect of leading source location

In general, there were at least two factors contributing to the reduction of the lagging response in
these experiments. First, the lag response was reduced for smaller ISDs and recovered at larger
ISDs. Second, with the 10-ms stimuli and for a given ISD, there was a further reduction of the
lagging response when the lead source was contralateral and the lag ipsilateral relative to the
lagging response when the lead source was ipsilateral and the lag contralateral (Figure 6B),
which could imply a directional-dependence of the leading source on lag-source reduction
(Litovsky and Yin, 1998b). Alternatively, the latter findings might simply be due to extended
refractory or adaptation-like effects in that contralaterally-placed sources result in increased
responses relative to ipsilateral sources (e.g., \(MI > 0\), Figure 2C) and the neuron is less likely to
respond after this. To address this, in 16 neurons from two cats we varied the location of the
lead source, either ±18° on the horizontal plane, relative to a fixed lag location, also either ±18°,
resulting in four stimulus configurations: contra leading-contra lagging, contra leading-ipsi
lagging, ipsi leading-contra lagging, and ipsi leading-ipsi lagging. The stimuli were 10-ms
noisebursts and the ISD was fixed at 20 ms because the responses to the lead and the lag in all 16
neurons were separable at this ISD. These 16 neurons were representative of the larger
population in two ways. First, varying the spatial location of the lead from ipsilateral to
contralateral locations significantly increased the response to the leading source \([paired t(15) =
2.5, p < 0.02]\). Moreover, the mean normalized response to the lag at 20 ms ISD for these
neurons were characteristic of the larger population shown in Figure 6B with means as follows:
contra-lead, ipsi-lag 0.58; ipsi-lead, contra-lag 0.81.

As an example of these manipulations, Figures 7A and 7B show the folded rasters and
histograms for one neuron to single-sources and for the four possible lead-lag combinations in
the paired-source configurations, respectively. Does the response to the lag at a fixed position
depend on the changes in the lead response brought about by changing its location? To test this
hypothesis, we adapted the technique of Litovsky and Delgutte (2002) and computed the change
in lag response for the two conditions, contra-lag (Figure 7C, filled circles) or ipsi-lag (Figure
7C, open circles), as a function of the change in lead response when it was changed from ipsi to
contra, which yields an increased response. The computations for the neuron in Figure 7B are
shown as the two large symbols in Figure 7C and demonstrate that even though the leading
response varied substantially and by approximately the same magnitude in the two conditions
(~5 spikes/stim), the effect on the lag was not the same but rather seemed to have depended upon
which side the lag was on. This finding held across the populations of neurons tested: although
the response to the lead varied, there was no systematic effect on the response to the lag and
none of the correlations (lines in Figure 7C) reached significance. If increases in lead discharge
rates always led to proportional decreases in lag discharge rates, then the data in Figure 7C
should lie in the lower right-hand quadrant. As a further test, paired t-tests were performed on
the leading and the lagging responses for the contra-lead, contra-lag condition and the ipsi-lead,
contra-lag condition. As expected, there was a significant difference in response to the leading source brought about by changing source azimuth [paired t(15) = 3.29, p < 0.005], but there was no difference in the response to the lag [paired t(15) = -0.59, p = 0.56]. At this ISD under the limited conditions studied in this paper, the reduction of the response to the lag was not solely dependent on the excitation, as measured by the discharge of the IC neurons, produced by the lead.

**Barbiturate anesthetic, not species difference, prolongs recovery to the lag**
As shown in Figure 5, we found similarities in the recovery of the responses of the lag in different species (cat vs rabbit), but differences within the same species (cat). We suggested that the barbiturate anesthetic was the likely cause for this and not species difference. Figure 8 shows the mean normalized recovery functions, computed across the population of neurons, to the lag ± 1 SEM for the condition where the ipsilateral source was leading and contra lagging for this study and two others, Fitzpatrick et al. (1995) and Yin (1994). The population responses to the lag were similar for all studies for small ISDs (< 5 ms), but the responses from the Yin (1994) experiments began to differ by 10 ms. From 10-40 ms, the population response was substantially less than that seen in the two unanesthetized experiments, which exhibited nearly identical recovery rates. These results are likely not due to sampling bias in the IC for the following reasons. First, there is consistency in the results of our previous studies (Yin, 1994; Litovsky and Yin, 1998a,b) in that recovery times were similar and long. Second, there is consistency in the present results and those of Fitzpatrick et al. (1995) in that the experiments were conducted in unanesthetized preparations and recovery times were short. Finally, in the present study we used analysis techniques, stimuli, and apparatuses similar to that we used in our previous studies (Yin, 1994; Litovsky and Yin, 1998a,b). These data, then, support the hypothesis that barbiturate anesthetic can prolong the recovery times of IC neurons to paired-source stimuli that produce the PE illusions.

Anesthetic may not affect other response characteristics, as we showed above that spatial sensitivity (over a limited range) did not differ at the population level. However, there were significant differences (Figure 9A; t_{236} = 11.15, p < 0.00001) in the distributions of spontaneous responses (SRs) from this study (mean = 14.6 ± 11.7 sp/sec) and from Litovsky and Yin (1998a) (1.6 ± 3.3 sp/sec). In contrast, the SRs from four studies in unanesthetized preparations were similar to ours (rabbit, Fitzpatrick et al., 1995; monkey, Ryan and Miller, 1978; cat, Bock et al., 1972; Ramachandran et al., 1999). The species and experimental techniques used by Bock et al. (1972) were most similar to that here (e.g., cats presented with sounds from loudspeakers) and their SR distribution (14 ± 11 sp/sec) was virtually identical to ours. While we cannot discount entirely the possibility that some responses might be due to sounds related to the psychophysical task (e.g. chewing), the role of such sources was small: in addition to the similarities in SRs in other preparations, our cats rarely vocalized and, for a few seconds prior to each trial, they adopted a ‘ready’ position in which they remained virtually motionless in anticipation of the impending trial (Populin and Yin, 1998b). Figure 9B shows the distribution of first spike latencies to transient stimuli at the best azimuth for this (mean = 8.9 ± 4.5 ms) and Litovsky and Yin (1998a) (9.2 ± 5.2 ms): differences in first spike latency were not significant (t_{179} = 1.21, p = 0.23). In the present study, there was no relationship between the SR of a neuron and its first spike latency ($r^2 = 0.006$).
Discussion

We studied the responses of single neurons in the ICC of cats, simultaneously engaged in a psychophysical sound localization task, to acoustic stimuli that we have shown to produce the perceptual illusions associated with the PE (Populin and Yin, 1998a; Tollin and Yin, 2003a,b). With paired sources, neural responses to the lag as determined by our analysis technique were substantially reduced in all neurons for small ISDs (< 10 ms) corresponding to localization dominance. Since the responses of the ICC neurons for these ISDs were dominated by the leading source, to the extent to which these neurons are involved in localization, the localization ability of the cats would be expected to be determined mainly by the spatial characteristics of the leading sound source. Indeed, this is what we and others (Cranford, 1982; Kalmykova, 1993) have found psychophysically in cats. Thus, at the level of the ICC, localization dominance is correlated with the reduction in the response to the lagging source and preservation of the response to the leading source.

We showed previously that the characteristics of the saccadic eye movements of the cats (i.e., latency and final eye position) to paired sources during localization dominance were virtually the same as those to single sources at the leading source location (Tollin and Yin, 2003b), suggesting that the neural representation of sound location may also be similar in both stimulus conditions at some level of the auditory system (see Mickey and Middlebrooks, 2001). The results here suggest that the absolute discharge rates of single ICC neurons, however, are insufficient to explain the apparent location of both the single and the paired source stimuli during the illusions of the PE. For ISDs corresponding to localization dominance (~1–10 ms), while the cats’ responses were consistently towards the position where they localized the leading source when presented in isolation (Figure 1), the discharge rates of ICC neurons to these paired stimuli changed considerably due to the graded recovery of the response to the lagging source (Figure 6). In other words, during localization dominance, the discharge rates of the neurons to paired sources could be very different than that to single sources at the leading location even though the behavioral responses to both were consistent. A thorough investigation and discussion of other potential neural ‘codes’ for sound location is beyond the scope of this paper.

With increasing ISD, the lag responses recovered towards normal, consistent with the echo threshold. At the psychophysically-defined echo threshold (10-15 ms, Figure 1), the population response to the lag was 60-75% of normal for the condition where the ipsilateral led the contralateral source and 35-50 % of normal for the contra-ipsi condition. Clearly echo threshold occurs prior to the ISD at which total recovery was obtained across the population, although many individual neurons recovered completely within 10-20 ms (e.g., Figures 3 and 4). In total, our data from behaving cats are in support of previous studies demonstrating that correlates of the PE exist in the neural responses of IC neurons (Yin, 1994; Fitzpatrick et al., 1995; Litovsky and Yin, 1998a,b; Burger and Pollak, 2001; Litovsky and Delgutte, 2002). Similar results have been reported for neurons in the auditory cortex (Fitzpatrick et al., 1999; Reale and Brugge, 2000; Mickey and Middlebrooks, 2001). It has not escaped our notice that the psychophysical echo thresholds measured in cats for these stimuli were comparable to the duration of the 10-ms noisebursts comprising the stimuli used in the psychophysical and the present experiments. Since we did not systematically vary the duration of the noisebursts in both our previous psychophysical studies and the current physiological studies, we cannot discount the possibility that a correlate of echo threshold in the responses of IC neurons is simply the ISD.
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at which there are two distinct neural responses, one due to the lead and one due to the lag (e.g., as in Figure 4a, top row, 20-ms ISD).

The reduction in the response to the lag was more prolonged with the 10-ms noisebursts than with the clicks consistent with previous studies (Litovsky et al., 1998a), which correlate with psychophysical studies in humans in which clicks yield shorter echo thresholds than longer-duration stimuli (Litovsky et al., 1999). While there was no effect of stimulus type on recovery in the ipsi-leading, contra-lagging condition, there was a difference in the contra-leading, ipsi-lagging condition (Figure 6). Based on this and the psychophysical data just cited, we propose that the IC ipsilateral to the lead (contralateral to the lag) governs echo threshold. Our hypothesis is supported by a patient with a lesion of the dorsal midbrain, which included the IC, whose echo thresholds were similar to normals when the lead was contralateral and the lag was ipsilateral to the lesion, but substantially elevated when stimuli were reversed (Litovsky et al., 2002). Based on animal lesion studies (Jenkins and Masterton, 1982; Kelly and Kavanagh, 1994), we propose that single-source localization and localization dominance is governed primarily, but not exclusively, by the IC contralateral to the single source or the leading source. Although there have been no behavioral studies of the PE after ablation of the IC in animals, behavioral deficits, such as reduced echo thresholds, do occur when the auditory cortex is lesioned unilaterally (Cranford et al., 1971; Whitfield et al., 1978; Kalmykova, 1995).

A delayed, inhibitory input to the ICC from the dorsal nucleus of the lateral lemniscus (DNLL) may contribute to the neural correlates of PE observed in ICC (Carney and Yin, 1989; Yin, 1994; Fitzpatrick et al., 1995; Kidd and Kelly, 1996; Litovsky and Yin, 1998b; Burger and Pollak, 2001; Litovsky and Delgutte, 2002). The neurons of the DNLL are sensitive to the binaural cues to sound location (Brugge et al., 1970; Markovitz and Pollak, 1994; Yang and Pollak, 1994; Kelly et al., 1998) and are GABAergic (Adams and Mugnaini, 1984; Gonzalas-Hernandez et al., 1996). Although the DNLL projects to both ICCs (Shneiderman et al., 1988; Hutson et al., 1991; Shneiderman et al., 1999), the input from the contralateral DNLL seems particularly relevant. For example, the time course of the reduction of the response to a lagging stimulus in the ICC can be reduced through pharmacological manipulations at the contra-DNLL (Kidd and Kelly, 1996; Burger and Pollak, 2001), or sectioning the afferent inputs (van Adel et al., 1999), but these same manipulations have little effect on the responses in the ipsilateral ICC. This mechanism is consistent with the findings that the magnitude of the reduction of the response to the lag is dependent on the spatial or binaural properties of the lead (Yin, 1994; Fitzpatrick et al., 1995; Litovsky and Yin, 1998b; Burger and Pollak, 2001; Litovsky and Delgutte, 2002).

Our results concur with the above hypothesis. Changing lead source azimuth by 36° resulted in insignificant changes in the lag responses even though the lead responses changed significantly (Figure 7). This finding argues against the hypothesis that adaptation or refractory-like effects caused the reduction in the response to the lag since a more reduced response might have been expected when the response to the lead was greater. We did not manipulate the lead response magnitude independently of source azimuth (by changing the overall sound level, for example), so we cannot say whether spatial location per se has an effect on lag response. However, in anesthetized cat, for the vast majority of neurons where lag suppression was dependent on the binaural cues to location, interaural time (ITDs) or level differences, or azimuth of the lead, the reduction in lag response was almost always greatest when lead cues or azimuths produced the greatest responses (Yin, 1994; Litovsky and Yin, 1998b; Litovsky and Delgutte, 2002). On the other hand, only about half of the neurons in the unanesthetized rabbit exhibited
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that trend, while the others showed the opposite trend where lag responses were reduced the greatest when the lead yielded the lowest response (Fitzpatrick et al., 1995). In fact, all these studies have found neurons where the response to the lag was reduced even though the neuron did not respond at all to the lead. These data support the hypothesis that the putative inhibitory inputs to ICC themselves are sensitive to the cues to location, consistent with the DNLL studies cited above. However, similar responses have also been predicted by a recent model (Hartung and Trahiotis, 2001; Trahiotis and Hartung, 2002). In that model, an apparent reduction in the response to the lagging source in low-frequency IC neurons could theoretically result not from explicit inhibition of the lagging response by the leading response but rather from the way that the leading and lagging stimuli are processed by and represented at the auditory periphery. On the other hand, the model pertains only to low-frequency IC neurons that are sensitive to the ongoing interaural time delays in the fine structure of the acoustic stimulus. All of the neurons in this paper where sensitive to only high-frequencies (BF > 1.5 kHz) and would likely not be sensitive to such interaural delays. Hence, it is difficult to extrapolate the model to these data. While our data here are limited, there does seem to be a difference between the present results, along with those of Fitzpatrick et al. (1995), and the previous studies, which might be due to anesthesia. All of these results suggest that localization dominance should persist psychophysically irrespective of the relative locations of the lead and lag sources. We have recently shown psychophysically that this indeed the case in cats (ML Dent, DJ Tollin, and TCT Yin, Abstr. 33rd Meeting Soc. Neurosci., 2003).

Finally, anesthetic state, and not species differences, was the reason for the large differences in the responses of IC neurons to stimuli that evoke the PE since our results here are virtually identical to those reported in the unanesthetized rabbit (Fitzpatrick et al., 1995) in all respects but significantly different than our previous studies (Yin, 1994; Litovsky and Yin, 1998a,b). It is important to note that this result was not a forgone conclusion; from a neuroethological perspective, a nocturnal predator such as the cat may have more need to suppress acoustical reflections for a longer time period (i.e. greater ISDs) in order to accurately localize its prey whereas an herbivore like the rabbit may have less stringent sound localization requirements. Unfortunately, little is known about the sound localization abilities of rabbits or whether they experience the various PE phenomena. Given the striking similarities between our physiological results here and those obtained in the rabbit, to the extent to which the responses of the IC neurons we recorded from contribute to sound localization, we hypothesize that with similar stimuli, rabbits would indeed experience localization dominance for ISDs up to ~10 ms. Barbiturate anesthetics have been known for some time to affect the responses of IC neurons, including suppressing or abolishing spontaneous activity (Bock and Webster, 1974; Kuwada et al., 1989), reducing the level of response and response pattern during stimulus presentation (Kuwada et al., 1989; Walker and Teas, 1974), and altering the sensitivity to interaural cues to location, like ITDs (Kuwada et al., 1989). These results are consistent with known pharmacological action of barbiturates which potentiate inhibition produced in GABA-ergic pathways (Barker and Ransom, 1978). A major source of GABAergic inputs to the ICC arise from the DNLL, and it is hypothesized that this input provides a mechanism for the PE. Barbiturate anesthetic would be expected to have an effect on this pathway, and may potentially have prolonged the suppression. However, at the population level, the spatial sensitivity and first-spike latency of ICC neurons in the behaving cat was comparable to the spatial sensitivity and first-spike latency observed in other free-field studies of the ICC in anesthetized cats using transients (Litovsky et al., 1998a) suggesting that anesthesia may not affect these response
characteristics over the restricted range of azimuth tested here. Data collected in anesthetized preparations should be interpreted in regard to their behavioral correlates with caution.
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Figure legends

**Figure 1.** Cats experience the three phenomena of the precedence effect: summing localization, localization dominance, and the echo threshold. Mean response azimuth ±1 SD for three cats for paired sources (filled circles and error bars) delivered from loudspeakers A and B as a function of ISD and for the two single source locations, A and B (solid and dashed horizontal lines). Positive ISDs indicate that source A was leading B, negative ISDs indicate source B was leading A. The hatched region (± 400 µs) corresponds to summing localization and localization dominance extends from 400 µs to ~10 ms. At echo threshold (ISDs > 10 ms) the cats sometimes localized the lagging source which shifts the mean response azimuth towards 0° and increases the variance of the response. Data from Tollin and Yin (2003b).

**Figure 2.** Responses of single IC neurons are modulated with sound source azimuth. **a.** Responses of one neuron at two single-source azimuths to a train of 10 transients shown as “folded” dot rasters (top panels) and summary histograms (bottom panels). An analysis window was defined, separately for each location, by those times at which the number of spikes in each bin significantly exceeded the mean spontaneous discharge rate. The upper horizontal line (shown in right panel only) shows 2 SD of the mean spontaneous rate (lower arrows). **b.** Mean number of spikes/stimulus ± 1 SEM for the neuron in a for the two azimuths used most extensively in this report, ±18°. **c.** IC neurons respond more for contralateral than ipsilateral sources. Distribution of the Modulation Index for this study (grey bars) and our previous study in the anesthetized IC (Litovsky and Yin, 1998a; black bars). MIs > 0 indicate that contralateral sources elicited greater responses than ipsilateral sources.

**Figure 3.** Responses to lagging sources are reduced in the paired-source condition. **a.** Responses of the same neuron as in Figure 2a-b at three different ISDs to the two paired-source conditions, contra leading ipsi (positive ISDs, left column) and ipsi leading contra (negative ISDs, right column). Rasters and histograms as in Figure 2. Analysis windows, appropriately shifted for latency and ISD, are shown at two ISDs. Responses to the lag were computed at each ISD as indicated in the Methods. **b.** Normalized response to the lag as a function of ISD for the two paired-source conditions. Half-maximal ISD indicates the ISD at which the lagging response recovers to 50% of the response to a single source at the lagging location. The stimulus was a train of 100-µs clicks.

**Figure 4.** Responses to paired sources from another neuron. The axes and symbols are the same as in Figure 3, but the stimulus was a train of 10-ms noisebursts.

**Figure 5.** Histogram of half-maximal ISDs for our sample of IC neurons from behaving cats (grey bars) and the sample of IC neurons from the anesthetized cat (Litovsky and Yin, 1998a; black bars) and the unanesthetized non-behaving rabbit (Fitzpatrick et al., 1995; open bars). The symbols and error bars show the mean half-maximal ISD ± 95% confidence intervals for the three studies. Cumulative half-max ISDs are also shown with the corresponding filled symbols (top abscissa, right ordinate). Barbiturate anesthetic leads to an increase in half-maximal ISD.

**Figure 6.** Population recovery functions. Population recovery functions were constructed by averaging the individual neuron recovery functions (as in Figures 3b and 4b) for the two paired-
source configurations, ipsi leads contra (filled circles) and contra leads ipsi (open squares). Error bars indicate $\pm 1$ SEM. Hatched region indicates the time course of localization dominance as measured psychophysically in cats with these stimuli; the offset between 10-15 ms ISD indicates the psychophysical echo threshold (Figure 1).  

**Figure 7.** The effect of the magnitude of the response to the lead on the suppression of the lag.  

**a.** Responses of one IC neuron to single sources at two azimuths. **b.** Responses of the same neuron to four different paired-source configurations: ipsi leads ipsi, contra leads contra, ipsi leads contra, contra leads ipsi. The ISD was 20 ms. **c.** Change in response to the lag (sp/stim) at a fixed location (contra, filled circles; ipsi, open circles) as a function of the change in response to the lead brought about by changing the lead source from ipsi to contra (from bottom to top in **b**). Large circles show the data points for the neuron in **a** and **b**. If increases in lead response systematically reduced the response to the lag, the data points should lie in the lower right-hand quadrant. Lines show regression fits for the two conditions.

**Figure 8.** Barbiturate anesthetic and not species difference prolongs lag recovery times. Mean normalized population responses to the lag $\pm 1$ SEM as a function of ISD for this study (filled circles), our previous study in the IC of the barbiturate anesthetized cat (Yin, 1994; filled triangles), and from the IC of the unanesthetized, non-behaving rabbit (Fitzpatrick et al., 1995; open squares). Data from all three studies are from the ipsi leads contra paired source configuration. Population recovery functions in the two unanesthetized conditions are short and virtually identical whereas that in the anesthetized IC is prolonged, particularly for ISDs from 5-40 ms. Hatched region same as in Figure 6.

**Figure 9.** Spontaneous rates (SRs) and first spike latencies. **a.** Distribution of SRs for this study (grey bars) and for our previous study in the IC of the barbiturate anesthetized cat (Litovsky and Yin, 1998a; black bars). The symbols and error bars show the mean SRs $\pm 95\%$ confidence intervals when available for our two studies and four previous studies in unanesthetized preparations (Listed to the right of each symbol). **b.** Distribution of first spike latencies for the same two studies as in **a**. Symbols and bars show mean $\pm 1$ SD. Latencies for each study were corrected for the acoustic delay due to the distance from the loudspeaker to the ear.
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