Development of Sound Localization Mechanisms in the Mongolian Gerbil is shaped by Early Acoustic Experience

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Sound localization is one of the most important tasks performed by the auditory system. Differences in the arrival-time of sound at the two ears are the main cue to localize low-frequency sound in the azimuth. In the mammalian brain, such interaural time differences (ITDs) are encoded in the auditory brainstem; first by the medial superior olive (MSO) and then transferred to higher centers, such as the dorsal nucleus of the lateral lemniscus (DNLL), a brainstem nucleus that gets a direct input from the MSO. Here we demonstrate for the first time that ITD-sensitivity in gerbils undergoes a developmental maturation after hearing onset. We further show that this development can be disrupted by altering the animal's acoustic experience during a critical period. In animals that had been exposed to omnidirectional white noise during a restricted time period right after hearing onset, ITD-tuning did not develop normally. Instead, it was similar to that of juvenile animals three days after hearing onset, with the ITD-functions not adjusted to the physiological range. Animals that had been exposed to omnidirectional noise as adults did not show equivalent abnormal ITD-tuning. The development presented here is in contrast to that of the development of neuronal representation of ITDs in the midbrain of barn owls and interaural intensity differences in ferrets, where the representations are adjusted by an interaction of auditory and visual inputs. The development of ITD-tuning presented here most likely depends on normal acoustic experience and may be related to the maturation of inhibitory inputs to the ITD-detector itself.
INTRODUCTION

Differences in sound arrival times at the two ears (interaural time difference, ITD) are the dominant cue for localizing low-frequency sounds in the horizontal plane. In mammals, neurons in the medial superior olive (MSO; Fig. 1A) encode ITDs in the microsecond range (Goldberg and Brown 1969; Spitzer and Semple 1995; Yin and Chan 1990). Traditionally, it is believed that different ITDs are encoded by the maximal firing rate of different MSO neurons arranged to produce a topographic map of azimuthal space (Crow et al. 1978; Goldberg and Brown 1969; Spitzer and Semple 1995; Yin and Chan 1990). However, recent studies challenge this concept, indicating a population code rather than a topographic map of ITDs. In gerbils, guinea pigs, and cats, the maximal firing rates of ITD-sensitive low-frequency neurons are not distributed across the physiological range of ITDs (ITDs the animal experiences under natural conditions). Instead, many neurons respond maximally at ITDs far outside the physiological range of ITDs, with a distribution of “best ITDs” related to the neurons’ frequency tuning (best frequencies, BF, the frequency a neuron responds best to): Neurons in the MSO and the inferior colliculus (IC, part of the auditory midbrain) tuned to low frequencies tend to respond maximally at large ITDs, whereas high frequency neurons prefer shorter ITDs (Brand et al. 2002; Hancock and Delgutte 2004; McAlpine et al. 2001). For the gerbil, with its small inter ear distance, creating ITDs of maximally 120 µs (Maki et al. 2003), this relationship of BF and best ITD implies that almost all best ITDs occur outside the physiological range, thus at ITDs the animal never experiences. As a consequence, the maximal slope of almost all ITD functions lies within the physiological range, resulting in large spike rate changes across this range of ITDs. Therefore, different ITDs lead to
different spike rates of the MSO cell population, providing the basis for a population code (Hancock and Delgutte 2004; McAlpine et al. 2001).

Coincidence detection of binaural excitatory inputs is known to be of fundamental importance in producing the ITD sensitivity in MSO. Nevertheless, recent findings suggest that glycinergic inhibition is important for tuning single MSO cells to ITDs with the contralateral stimulus leading. Pharmacological blockade of glycinergic inhibition in vivo indicated that binaural excitation alone creates an ITD-sensitivity with maximal firing rates around zero ITD and maximal slopes mostly outside of the physiological range (Brand et al. 2002).

The glycinergic projections to MSO neurons undergo an experience-dependent refinement after hearing onset: Before hearing onset (in gerbils on postnatal day 12, P12), glycinergic synapses are dispersed equally on MSO cells, whereas after P25 inhibitory synapses are almost exclusively found on somata and proximal dendrites. This developmental refinement depends on normal early acoustic experience as it can be suppressed by rearing gerbils in the presence of omnidirectional noise as well as by removing one or both cochleae before hearing onset (Kapfer et al. 2002).

We therefore hypothesized that ITD-tuning in gerbils also develops only with normal acoustic experience. To this end, we compared ITD-sensitivity of single neurons in the dorsal nucleus of the lateral lemniscus (DNLL, Fig. 1A) in different experimental groups of gerbils. We recorded from the DNLL because MSO recordings are extremely difficult (compare: Yin and Chan 1990; Brand et al. 2002) and would not allow for a comparison of ITD sensitivity between different experimental groups of animals. A subpopulation of the DNLL is directly excited by MSO-neurons (Wu 1999) and therefore reflects its ITD-
properties (Fitzpatrick and Kuwada 2001). This study only provides data of DNLL cells that reflect MSO inputs. A careful description of low frequency DNLL cells in the gerbil including those receiving different inputs will be published independently. Focusing on cells showing “MSO-like” ITD functions we provide evidence for a developmental change of ITD-functions of single neurons shortly after hearing onset that depends on proper acoustic experience.

Material and Methods

All experiments were approved according to the German Tierschutzgesetz (Reg. Obb, AZ 2112531-40/01).

Animal treatment. We used Mongolian gerbils (*Meriones unguiculatus*), known for their good low-frequency hearing, for our experiments. All animals were raised in the animal facilities of the MPI of Neurobiology.

We examined ITD sensitivity of DNLL neurons in four different groups of animals:

1. Adult controls, which were at least 31 days old and never exposed to the noise as two of the other experimental groups of animals. 2. Juvenile animals, 14 or 15 days old. 3. Noise-reared animals that were exposed to omnidirectional white noise known to interfere with the normal development of glycinergic projections to MSO (Kapfer et al. 2002). Animals were exposed to noise from P10 (two days before hearing onset) to P25 and were allowed to recover for at least 7 days after noise-exposure before tested. 4. Animals that were exposed to omnidirectional noise as adults (>31 days of age). Duration and nature of noise-exposure was identical to animals under 3.
Noise-exposure. A cage with animals was placed in a 100 x 80 x 80 cm³ sound-attenuated box in a quiet room. White noise from two analogue generators (Rhode & Schwarz) was presented via 12 sets of speakers (2 high- and 2 low-frequency speakers at each of the six sides). Noise from two different sources thus was presented from each direction. The amplitude of the noise was about 80 dB SPL (rms-value, averaging time 30 sec) and never exceeded 85 dB SPL. During the first day of noise exposure the amplitude of the noise was increased stepwise to the desired level. During the entire period of noise exposure, the cage was time-lapse video recorded. The animals showed no signs of stress or abnormal behavior. After exposure, noise-reared animals were returned to their normal cages before recordings. This noise supposedly masked most spatial acoustic cues (Withington-Wray 1990a), but was set to a sound pressure level that does not cause any damage to the cochlea or the primary auditory centers (Withington-Wray 1990a).

Electrophysiology and stimulus presentation. Animals were anaesthetized with a mixture of Ketamine (10 mg per 100 g body weight) and Xylazine (2%) before recordings were carried out and throughout the experiment. Xylazine was reduced to 50% of the original amount for juvenile animals. Juvenile animals received an additional nutrient solution. Recordings were performed in a soundproof chamber and the animals were placed on a heating cushion maintaining body-temperature of 37-39°C (Field and Sibold 1999). The head was stereotaxically positioned and coated tungsten recording electrodes (1 or 5 MΩ resistance, WPI, Berlin, Germany, or Science Products, Hofheim, Germany, respectively) were inserted through a small hole drilled in the skull rostral and lateral of the transverse sinus (i.e. 500 - 2000 mm lateral and 200 - 1200 mm caudal of
the Lambda point). Current induced lesions (5mA for 5sec) were used to mark the recording sites.

Action potentials were recorded using conventional methods described elsewhere (Brand et al. 2000). In short, only action potentials from stable single neuron recordings with a signal-to-noise ratio > 5 were stored for offline analysis. Stimuli were delivered using TDT System II or III (Tucker Davis Technologies, Alachua, FL) via two Beyer dynamics speakers (model DT 990, Heilbronn, Germany) fitted to the ears via probe tubes. The stimulus delivery system and speakers were calibrated using a 1/4 inch microphone (Reinstorp VtS, Germany), a measuring amplifier (MV 302, Microtech, Gefell, Germany) and a waveform analyzer (Stanford Research Systems, model SR770 FFT network analyzer). Stimulus generation was controlled via custom made software (SPIKE, Brandon Warren) or Brainware (Jan Schnupp, TDT).

After electrophysiological isolation of a single neuron, the frequency response region and threshold were defined audiovisually or measured by a computer controlled generation of a frequency-tuning curve. Sensitivity to static ITDs was then tested for different stimulus frequencies within the tuning width at 20 dB above threshold. Stimuli were 100 or 200 ms long and were presented at an amplitude of 20 dB above threshold of the cell tested. The rise/fall-time was at least 5ms and stimuli were presented with a repetition rate of 4 Hz. The stimulus delay was constant at the contralateral ear and varied in 50 or 100 µs steps at the ipsilateral ear covering a range of at least one cycle of the stimulus frequency (20 - 80 repetitions per single ITD). All stimuli were presented randomly interleaved, thus there was no systematic delay in either one of the ears, ruling out asymmetrical ITD functions due to directional sensitivity. The relative highest
response of all measured ITD-functions determined a cell’s best frequency, hence, best frequency was determined by binaural stimulation.

**Data analysis and statistics.** Data were analyzed offline using the summed or normalized number of action potentials in response to the stimulus presentations for creating ITD or IPD functions. Individual spike times and a standard vector analysis were used for evaluating phase-locking (Goldberg and Brown 1969). Only statistically significant vector strength values that fulfilled the P < 0.05 criterion in the Rayleigh test (Batschelet, 1991) were used. Similarly, a neuron’s best ITD or IPD was calculated by defining the mean vector of the individual ITD or IPD functions, respectively. The maximal slope of ITD functions was obtained by calculating the inflection point (MATLAB, The MathWorks Inc., Natick, MA) of a fitted sigmoidal curve (STATISTICA, StatSoft Inc., Tulsa, OK).

Statistical differences between distributions of maximal slopes or best ITD or IPD between the different experimental groups of animals were calculated by the ANOVA single factor test on STATISTICA. All deviations and error bars are standard deviations (STDEV), except for the error bars of ITD functions in Fig. 2, displaying standard errors of the mean (SEM).

For defining a neuron as 'peak-type' we followed the definition by Yin and Kuwada (1983). In brief, the characteristic phase (CP), defined as the y-intercept in plots of the stimulus frequency (x-axis) versus the best interaural phase of the response (y-axis), was calculated from the individual IPD functions (at least three, mostly five or more). A linear regression line and a CP between -1.25 and + 1.25 indicated that the neuron was of 'peak-type'.
Results

We recorded from 152 low-frequency (BF< 2 kHz) DNLL neurons that could be driven by auditory stimulation. Of the 152 neurons, 83 showed a 'peak-type' ITD-sensitivity in their ongoing component of the response as well as in their onset component (see methods). A 'peak-type' ITD-sensitivity is almost certainly exclusively arising from the MSO and is typical for most MSO neurons (Spitzer and Semple 1995; Yin and Chan 1990; Yin and Kuwada 1983). The fact that the on-component and the ongoing response did not significantly differ in their ITD sensitivity (data not shown) excludes the possibility that the input from the contralateral DNLL had any significant influence. Hence, ITD-sensitive neurons in the DNLL showing such a feature are likely to receive their input from the MSO. Neurons showing any deviation from this type of ITD sensitivity either receive inputs from several MSO cells with different best ITDs, a mixture of inputs from different brainstem nuclei, or inputs from the lateral superior olive. Results from such neurons where omitted from any further analysis in this study. Therefore, in the following we will only consider neurons that had low BFs (< 2 kHz) and whose individual best ITDs were independent of stimulus frequency (‘peak-type’; Fig. 3A). Responses from these 83 neurons were used for further analysis: Adult controls: 39 neurons from 11 animals (39/11); juvenile animals: 9/8; noise-reared animals: 22/8 (three different litters); animals kept as adults in noise for 15 days: 13/4.

ITD sensitivity of adult DNLL neurons resembles that of MSO neurons.

To evaluate whether our sample of DNLL neurons reflect the physiological ITD-properties of MSO neurons we compared two key features of the ITD functions (Fig. 3A)
of 39 DNLL cells recorded from adult controls (Fig. 2A,B) with those of 20 MSO neurons from an earlier study (Brand et al. 2002): 1., mean best interaural phase differences (IPD) that were used to normalize for different stimulus frequencies indicating the relative position of the maximal response (best IPD [cycles] = best ITD [sec] x stimulus frequency [Hz]). 2., the spike rate’s modulation depth across the physiologically relevant range of ITDs, as a measure for the quality of a cell’s ITD-tuning. This physiological modulation depth is defined as the spike rate change between the two most extreme ITDs the gerbil can be expected to experience naturally (indicated by a gray bar in Fig. 2A,C,E,G) (Maki et al. 2003).

A representative example of an ITD sensitive DNLL neuron is shown in Fig. 2A. The neuron had a BF of 1000 Hz, its best ITD was at 118 μs (= 0.12 cycles), at the border of the physiological range, and the maximal slope was at -56 μs, well within the physiological range. For all 39 DNLL neurons the mean best IPD (at BF) was + 0.137 cycles (STDEV ± 0.067) (Fig. 2B). This corresponded well to the values shown for gerbil MSO neurons (+ 0.120 cycles, ± 0.047) (Brand et al. 2002) and also to the auditory midbrain in guinea pigs and cats (Hancock and Delgutte 2004; McAlpine et al. 2001). Thus, for most frequencies, best ITDs in DNLL, as in MSO (Brand et al. 2002), were outside the physiologically relevant range (Fig. 1B, dashed line). The maximal slopes, hence the range of ITDs at which neurons are most sensitive to changes, lay around a mean of -17 μs (± 87.60), well within the physiologically relevant range (3B and 5A). The average modulation depth across the physiologically relevant range of ITDs in DNLL was 68.4 % (± 22.27), somewhat larger than that found in MSO neurons (47.4 %, ± 19.55) (Brand et al. 2002). With lower BFs, best ITDs were larger (Fig. 1B), similar to
what is found in the gerbil MSO (Brand et al. 2002) and the IC of guinea pig and cat (Hancock and Delgutte 2004; McAlpine et al. 2001). Hence, for the reported cells, ITD-sensitivity in DNLL appears to reflect that of its MSO input.

Of the 39 neurons tested in the control group, we tested 12 for monaural activity: 5 could be driven by either ear, 4 by contralateral and 2 by ipsilateral stimulation alone. One neuron didn’t respond when stimulated monaurally. When monaurally stimulated at the same level with which we obtained the ITD-curves (i.e. 20 dB above threshold), 4 neurons showed no response at all. The sum of the monaural responses of the other 8 neurons tested was on average two thirds of the best binaural response at the same sound level (data not shown). These features also resemble those of MSO cells (Goldberg and Brown 1969; Yin and Chan 1990).

**ITD-tuning in juvenile gerbils.**

We recorded from P14 and P15 animals, 2 and 3 days after hearing onset, respectively, and about 6 to 7 days before the auditory brainstem is thought to be mature like (compare Sanes and Friauf 2000). DNLL recordings from juvenile animals turned out to be basically impossible at P14. The responses where too sloppy and only very few action potentials could be evoked via acoustic stimulation. We recorded 9 cells from eight 14 day-old animals with at least some responses, none of which turned out to be ITD-sensitive. Only one day later at P 15, some cells already showed ITD functions with a rather high modulation depth. Spike rates where still dramatically lower than in adults (Fig. 4B), which required many more stimulus repetitions and, therefore, made it very difficult to apply the full range of test stimuli. Nevertheless, at P 15 we tested ITD tuning
from 76 neurons, 19 of which were monaurally excitable from either ear (EE) and were ITD sensitive. Of those 19 neurons, 13 could be tested at several test frequencies and 9 of them where therefore categorized as ‘peak-type’. This is a low number of cells but sufficient to perform the necessary statistics. It should be stressed again, that we used very conservative standards, which is a prerequisite here.

An example of an ITD-sensitive cell of a juvenile animal is shown in Fig. 2C. The displayed cell had a BF of 1200 Hz, its best ITD was at $+ 51 \mu s$ (0.05 cycles) and the maximal slope at $- 104 \mu s$. In contrast to adult controls (Fig. 2A,B), the mean best IPD of the units recorded in the juvenile animals was much lower (Fig. 2C,D) and the maximal slopes were mostly outside the physiological range (Fig. 3B). The values significantly differed from those of adult animals. Although there were a few cells that were tuned as those in adult animals, the majority had steep slopes that only partially covered the physiological range like in adult animals (5 of 9). There was no significant correlation between animal body weight (indicative for the developmental stage) and best IPD ($R^2 = 0.0442$, data not shown). Interestingly the value for mean IPD in juvenile animals was not different from that found in MSO neurons during pharmacological blockade of inhibition (Brand et al. 2002) (DNLL juvenile animals: 0.082 cycles, ± 0.074; MSO: 0.021 cycles, ± 0.054; $P = 0.136$).

Development of ITD-tuning depends on early experience.

We next tested whether the development of the neural representation of ITDs is influenced by exposure to omnidirectional noise. Such noise is known to interfere with the maturation of inhibitory input patterns. The animals were exposed to the noise
between days P10 and P25, a critical period for the development of inhibitory inputs to MSO neurons (Kapfer et al. 2002). The ITD-sensitivity of DNLL neurons in gerbils reared in omnidirectional white noise indeed differed significantly from that found in adult controls, but shared important features with that in P15 gerbils. In our sample of 8 noise-reared animals, 22 out of 62 neurons showed 'peak-type' ITD-sensitivity. A typical example of an ITD-sensitive neuron of the noise-reared group is shown in Fig. 2E. A plot of all IPD functions of the 22 neurons at BF reveals that the neural representation of ITDs in the DNLL of noise-reared animals differs from that in adult controls (Fig. 2F). Across the population, the mean best IPD was significantly lower than in the control group ($P < 0.0005$; Fig. 3B) and the mean maximal slope was outside the physiologically relevant range. Moreover, 82% of ITD functions in adult controls but only 45% in the noise-reared animals had the maximal slope within the physiologically relevant range (in the control group always the “left” slope, in noise-reared animals the slope closest to 0 ITD, independent of whether it was the left or right slope).

In this study we assume that ITD-sensitive cells in the MSO are tuned to contralateral ITDs and we show that this property is transferred to DNLL. Yet we cannot a priori exclude the possibility that a subset of DNLL neurons receives inputs from the contralateral MSO and therefore is tuned to the other hemifield. Such neurons have been found in the IC of the guinea pig (McAlpine et al. 2001). In order to exclude the possibility that the different distribution of ITD functions in noise-reared animals was due to accidental over-sampling of reversed ITD functions, we additionally calculated the distributions after mirroring all ITD functions of noise-reared animals that had their peak at negative ITDs. Nevertheless, the mean best IPDs and maximal slopes significantly
differed between adult controls and noise-reared animals (best IPD: 0.080 cycles, ± 0.07, 
P \leq 0.005; maximal slope: -75.58 \mu s, ± 134.98, P < 0.05).

Interestingly, the mean IPD of noise-reared animals, like those of juvenile animals, was not statistically different from that measured in the MSO during antagonization of glycineric inhibition (0.021 cycles, ± 0.054; P = 0.912) (Brand et al. 2002). The modulation depth of the response rate across the physiologically relevant range of ITDs was significantly reduced compared to adult controls (P < 0.0005; Fig. 3b) but again comparable to MSO data during blockade of inhibition (20.5 %, ± 4.37 %; P = 0.984) (Brand et al. 2002) and to data found in DNLL of juvenile animals (P = 0.164). The total modulation depth in noise-reared animals was also different from control conditions (P < 0.0005) but close to the level measured in juvenile gerbils (Fig. 3B, P = 0.938) and in the MSO during blockade of inhibition (82.4 %, ± 12.90) (Brand et al. 2002). The 50 % widths of the ITD-tuning curves of noise-reared animals were increased compared to control conditions, and they were similar to those found in juvenile animals (Fig. 3B, P = 0.207).

**Noise exposure does not affect ITD-tuning in adults.**

To exclude the possibility that the effects resulting from noise-rearing were not related to the development of ITD-tuning but rather due to noise-exposure itself, we tested whether omnidirectional noise affects ITD-sensitivity in adult gerbils. We exposed adult gerbils to the same noise conditions as juveniles (see Methods). After recovery time we recorded from 26 neurons of which 13 showed a ’peak-type’ ITD-sensitivity. Values of modulation depth, 50 % bandwidth, and maximal slope of these 13 neurons were not
significantly different from those in control adult animals (Fig. 2G,H). The mean best
IPD, however, was significantly higher (Fig. 3B), but did not resemble the mean best ITD
in noise-reared animals. Preliminary experiments of noise-exposed adult animals with
longer recovery times (> 3 months) indicated that this effect might not be permanent, a
result that is confirmed by a larger sample from an unpublished study (Kollmar and
Grothe 2004).

**General characteristics are not different in juvenile animals or after noise exposure.**

In our sample of control animals the BFs ranged from 250 to 1250 Hz (mean 786 Hz,
Fig. 4A). 22 of the 39 neurons in this group showed phase-locked activity (56%). The
mean phase-locking strength (r) of the 22 cells was 0.53 (± 0.19).

The BFs of the 9 cells presented from juvenile animals lay between 400 and 1800
Hz, with a mean of 822 Hz, and were comparable to those of adult control animals (Fig.
4A). In general, discharge rates appeared to be slightly lower than those found in adult
animals, though not statistically significant (P = 0.12, Fig. 4B, compare also examples in
2A and 2C). Thresholds were significantly higher than in adult controls, though some
adult examples did reach comparable levels (Fig. 4C). Discharges of 5 out of the 9
neurons were phase-locked (r = 0.70, ± 0.20) and r was not statistically different from the
control group. The BF distribution of the cells showing phase-locked responses was not
different between the juvenile and the control group (data not shown).

Acoustic thresholds of neurons in the adult controls and noise-reared animals did not
differ (P = 0.62). The BFs of ITD-sensitive cells in noise-reared animals varied from 200
to 1300 around a mean of 911 Hz (Fig. 4A). The discharge rates were significantly lower
than in adult control animals ($P < 0.05$, Fig. 4B). 15 of the cells were phase-locked to auditory stimuli (68 %). The mean phase-locking strength $r$ was 0.58 ($\pm 0.14$) and was not different from the control group. Hence, no damage or overall persistent effect on the development of sound evoked responses were detectable, except that of ITD tuning. That our way of rearing gerbils in noise does not affect basic auditory functions in gerbils is confirmed by normal behavioral audiograms and other audiometric functions (Klump and Maier, pers. communication).

In noise-exposed adult animals BFs ranged between 500 and 1225 Hz (mean 825 Hz, Fig. 4A). The mean discharge rate was not different from adult control animals ($P = 0.65$). Responses of 5 out of the 13 neurons were phase-locked. The mean of $r$ and the distribution of BFs among the phase-locked neurons were not different from the control group.

**DISCUSSION**

In this study we assessed the development of ITD coding in the mammalian auditory system and the effect of early experience on ITD-tuning. Single neuron recordings from the DNLL showed that the neuronal representation of ITDs of neurons that show stable ITDs (‘peak-type’) closely matched that of ‘peak-type’ neurons in their potential origin, the MSO (Brand et al. 2002). From what is known about ITD processing circuits, the MSO is the only possible source of this type of ITD sensitivity in the DNLL (compare Yin 2002). The difference between monaural and binaural response rates, and the fact that some neurons didn’t respond in the absence of binaural stimulation supports the assumption that the recorded ‘peak-type’ cells were driven by the MSO. Moreover, since
correct MSO function has been shown to be essential for an animal's ability to perform azimuthal sound localization (Casseday and Neff 1975), it is unlikely that DNLL ITD functions are derived from sources other than MSO. The distribution of best ITDs we found in these cells is also consistent with results from the guinea pig and cat auditory midbrain (Hancock and Delgutte 2004; McAlpine et al. 2001). The fact that we found other types of ITD sensitivity and also cells that exhibited no ITD sensitivity at all reflects the fact that the MSO is not the only source of DNLL inputs. These cells will be described in detail in a different publication. Nevertheless, at least a subset of inputs seems to be segregated from other DNLL inputs and allow a comparison of ‘peak-type’ ITD sensitivity in the three different subgroups of animals.

We found that the majority of ITD functions in juvenile animals were not fully adjusted to the physiological range. Whereas in adult animals best ITDs are significantly shifted towards the contralateral hemisphere (Fig. 2B and Fig. 5A), this was only true for a subset of ITD-functions found shortly after hearing onset. Some ITD-functions resembled those found in adult control animals, whereas others had their maximal slopes not yet adjusted to the physiological range (Fig. 2D and Fig. 5B). Although there was no relationship between body weight and best IPD in P15 animals, it is conceivable that cells in animals of the same age were in different maturational stages (even within one animal) and, consequently, showed different ITD-tuning properties. The positioning of the best IPD, systematically realized in the adult animal, guarantees that the maximal slopes of the ITD functions of most neurons are close to the midline. This provides the basis for ITD-coding by population spike rate, rather than a map of peak-responses of a subset of MSO cells.
The second important finding of this study shows that the development of adult-like ITD-tuning can be suppressed during a critical period after hearing onset. DNLL neurons of animals reared in omnidirectional white noise showed ITD peaks scattered around zero ITD, similar to those of juvenile animals, indicating that masking of spatial acoustic cues prevents a crucial step in the development of ITD-tuning, without affecting most of the other cell characteristics (e.g. phase-locking and thresholds), hence, without damaging the auditory system as such. Indeed, the characteristics of these Neurons were otherwise comparable to those of adult control animals (Fig. 4). In contrast, noise exposure of mature animals does not persistently affect ITD-tuning.

Experience-dependent effects during ontogeny related to spatial processing have also been described in mammals and barn owls at the level of the superior colliculus (SC) (King et al. 1988) and external nucleus of the inferior colliculus (ICX) (review: Knudsen 2002). The observed experience-dependent effects on the ITD map in the barn owl midbrain involve bimodal sensory experience and occur in order to correct for mismatches of visual and auditory inputs. More specifically, in the barn owl a shift in ITD-tuning curves due to altered visual experience has been found: ITD-tuning in the ICX can be adjusted so as to align with prismatically displaced visual receptive fields (Gold and Knudsen 2000; Knudsen and Knudsen 1990). This plasticity of ITD-tuning in the barn owl has been shown to be instructed by visual inputs from the optic tectum (Hyde and Knudsen 2002). Experience-dependent plasticity shown for the representation of interaural level differences (ILD) in the guinea-pig SC seems also to depend on a bimodal interaction. There, exposure to omnidirectional white noise during a critical period disrupts the maturation of the ILD-map (Withington-Wray 1990a). However,
depriving guinea pigs of early visual experience alone also prevents the formation of the auditory map (Withington-Wray 1990b) proving again that an adjustment of auditory and visual inputs underlies the developmental effects in the SC.

In the present study, we show a development of ITD-tuning in the gerbil that most likely occurs due to plasticity at the level of the ITD detector itself, which therefore directly affects the mechanism underlying ITD processing. A visual contribution to the development of the MSO is unlikely as visual inputs to the MSO have not been found and the development of the visual system occurs too late to have an impact. Thus, it appears that ITD-development is self-organized. The omnidirectional noise apparently disturbs this self-organization, most likely by driving the MSO inputs at high rates in a binaurally uncorrelated manner. Learning rules for strengthening or eliminating “right” or “wrong” inputs would fail under such conditions.

ITD functions of noise-reared and juvenile animals strongly resembled those found in MSO neurons during blockade of glycinergic inhibition (Brand et al. 2002). Although we cannot exclude the contributions of other post hearing onset developmental changes, this further indicates that our finding of a developmental maturation may be related to our previous results of an experience-dependent refinement of glycinergic inhibitory inputs in the first days after hearing onset, leading to an almost complete removal of inhibitory synapses from MSO dendrites. It can be speculated that it is mainly the inhibition that tunes the slopes of adult ITD-functions of MSO cells to the physiologically relevant range and that this adjustment correlates with the structural rearrangement of inhibitory inputs (Brand et al. 2002; Kapfer et al. 2002) (Fig. 6). Hence, normal development of ITD-tuning seems to include a critical period in the first days after hearing onset at which
the inhibitory inputs are adjusted in order to tune the ITD functions to exhibit the maximal slopes within the physiologically relevant range. This allows high-acuity usage of the MSO ITD-sensitivity (Fig. 5) (Skottun et al. 2001). Compared to adult control animals with refined glycinergic synapses, unrefined inhibitory inputs, as found in juvenile and noise-reared animals, could cause an overall but unspecific impact of inhibition on single MSO cells inappropriate to fine tune ITD functions as in normal mature animals. This interpretation would be in agreement with the relative low response rates found in these two groups of animals (Fig. 4B).

The exact timing of inhibitory inputs may be crucial for the proper ITD tuning (Brand et al. 2002; Grothe and Sanes 1994). Therefore, it is possible that the post-hearing-onset refinement of glycinergic projections is based on temporal correlations of the naturally produced auditory activity, selectively eliminating inputs that are not contributing properly. In contrast to excitatory synapses, indications for long-term plasticity of inhibitory connections have been rarely reported (Stelzer et al. 1987). There is however evidence that GABAergic synapses in the cerebellum can undergo both potentiation and depression depending on the stimulation protocol (Aizenman et al. 1998), and GABAergic inputs on cortical pyramidal cells may switch between LTP (long-term potentiation) and LTD (long-term depression) depending on the temporal relation of pre- and postsynaptic activity (Holmgren and Zilberter 2001). Glycinergic synapses are also able to undergo LTP (Oda et al. 1998). It is coherent with our hypothesis that MNTB projections to another auditory nucleus adjacent to the MSO, the lateral superior olive (LSO), undergo LTD, which depends on pre- and postsynaptic activity and may account for developmental pruning of the glycinergic LSO inputs
This LTD is at least partly dependent on the release of neurotrophins (Kotak et al. 2001) and appears to be modulated by postsynaptic mechanisms (Chang et al. 2003). The selective potentiation or depotentiation could thus represent a means of discriminatory elimination of inhibitory inputs. This is contrary to a recent study according to which the developmental plasticity of inhibitory MNTB projections to the lateral superior olive is mainly due to early processes before hearing onset when the inhibition is still depolarizing (due to a different reversal potential for Cl⁻) (Kim and Kandler 2003) and is accompanied by a co-release of glutamate acting on NMDA receptors (Gillespie et al. 2005). Our previous results and the data presented here, however, support the idea that the structural and functional plasticity of MNTB projections to the MSO occur days after hearing onset, hence after they have become hyperpolarizing (Kapfer et al. 2002).

These results suggest that the maturation of sound localization encoding depends on patterned acoustic experience. This activity dependence occurs during a critical period when inhibitory inputs to MSO neurons are known to show a spatial refinement. Our data from the DNLL suggest that this plasticity might be necessary for proper ITD-tuning and represent a mechanism of direct adjustment of neuronal processing to behaviorally relevant cues. Future experiments will have to reveal whether noise rearing impairs the ability to localize low-frequency sounds or to separate low frequency sounds sources.
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REFERENCES


FIGURE LEGENDS

**Fig. 1.** (A) MSO neurons project directly to the ipsilateral dorsal nucleus of the lateral lemniscus (DNLL) and the auditory midbrain. Excitatory inputs solid, glycinergic inputs dashed. Note that the DNLL additionally receives inputs from other nuclei like both lateral superior olives and the contralateral DNLL. These projections are not shown here. (B) Relationship between BF and best ITD. Dotted lines indicates range of natural ITDs for the gerbil. Note that best ITDs are larger with lower BFs.

**Fig. 2.** ITD functions from DNLL neurons in the four experimental groups. (A) Example of a neuron from an adult control showing that the best ITD is independent of the stimulus frequency ('peak type'). The best ITD is at 118 µs, which corresponds to 0.12 cycles of its BF (1000 Hz). Its spike rate changes by about 82% across the physiological range of ITDs. (B) IPDs of all neurons recorded from adult controls (at each neuron's BF). The maximal slopes are close to zero ITD. (C) ITD function of a single cell (BF 900 Hz) in a 15 day old gerbil. Its best ITD is close to zero (+ 51 µs, corresponding to +0.046 cycles), the physiologically relevant modulation depth is only 39%. (D) IPD functions of all DNLL neurons recorded in juvenile animals (at each neuron's BF). (E) Example of ITD-tuning of a neuron in a noise-reared gerbil (BF 1200 Hz). The best ITD is close to zero (-53 µs, corresponding to -0.063 cycles). The ITD-sensitivity is independent of the test frequency. The change of the response rate across the physiological range at BF is only 23%. (F) IPD functions of all DNLL neurons recorded from noise-reared animals (at each neuron's BF). Only a minority of cells shows maximal slopes close to the midline.
Best ITDs are distributed around 0 ITD. (G) Recording from a DNLL neuron of an animal exposed to omnidirectional noise as an adult (BF 1050 Hz). ITD functions are of 'peak type' and the maximal slope is close to zero ITD, as in the adult controls. The best ITD, however, is larger than that of the control cell in (A), which had the same BF: 188 µs (corresponding to 0.197 cycles). (H) IPD functions of all DNLL neurons recorded from noise-adult controls do not resemble IPD functions of juvenile animals. The mean best IPD is larger than in control animals. Error bars: standard error of the mean.

**Fig. 3.** Quantification of ITD/IPD functions measured at each neuron's BF. (A) The parameters quantified were: best ITD in µs (see methods) and IPD (in cycles); width of the IPD functions at 50% discharge rate ('50% width') in cycles; total modulation depth (% decrease from maximal to minimal discharge); modulation depth within the physiologically relevant range of ITDs (gray area); x-value of the maximal slope closest to zero IPD. For details see methods. (B) Quantification of values in (A). Note that for almost all parameters quantified the ITD-sensitivity in adult controls significantly differs from that measured in juvenile and noise-reared animals. Asterisks indicate statistically significant differences (** *P < 0.0005, ** P < 0.005, * P < 0.05). Error bars: standard deviations.

**Fig. 4.** Distributions of best frequencies (BF) and average spike rates of single DNLL neurons in the different experimental groups. (A) BFs of all four groups cover similar frequency ranges. (B) Average discharge rates at best ITD in response to pure tones at BF and 20 dB above threshold. There was no difference between adult control
animals and noise exposed adults. However, spike rates of noise-reared and juvenile animals were smaller. (C) Mean thresholds of all four groups normalized to the adult control group. The square indicates the mean value, whereas the line stretches from the maximal to the minimal value. (** $P < 0.01$, * $P < 0.05$). Error bars in B: standard deviations.

**Fig. 5.** ITD-tuning within the physiologically relevant range. Spike rates are normalized and the x-axis shows the physiological range of the gerbil (ca. ±120µs). (A,D) In adult control animals, as in noise exposed adults, the maximal slopes are adjusted well to the physiological range, resulting in a constant spike rate change with increasing ITD. (B,C) In juvenile and noise-reared animals spike rates do not change with ITD in a systematically manner and the maximal slopes are not consistently adjusted to the range of ±120 µs.

**Fig. 6.** Correlation between development of inhibitory inputs and IPD-tuning. Only after the refinement of glycinergic synapses on MSO neurons during a experience-dependent development (Kapfer et al. 2002) are IPD response curves adjusted in a way that the maximal slope lies within the physiological range. Before (juvenile animals) or with reduced refinement (noise-reared animals), the mean best IPD is around zero and maximal slopes occur far from the midline. IPD-functions are taken from Fig. 2.
Figure 1

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Development of ITD-coding
Figure 3

A

B

Best IPD (cycles)

Total Modulation-depth (%)

50% width (cycles)

Physiol. Modulation-depth (%)

Adult controls
Juvenile animals
Noise-reared animals
Noise-exposed adults

ITD of maximal slope (µs)
Figure 4

(A) Best frequencies (Hz)
(B) Response rate (spikes per second)
(C) Threshold (dB relative control mean)

- Adult controls
- Juvenile animals
- Noise-reared animals
- Noise-exposed adults
Figure 5

A  Adult control

B  Juvenile

C  Noise-reared animals

D  Noise-exposed adults

ITD (µs)
Figure 6

Seidl and Grothe

Development of ITD-coding

Juvenile gerbil

White noise

Spike rate

0 IPD

Juvenile

Adult

Adult control

Noise-reared gerbil

Noise-exposed adult

White noise