GABAergic Inhibition Influences Auditory Motion-Direction Sensitivity in Barn Owls

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Kautz, Dirk and Hermann Wagner. GABAergic inhibition influences auditory motion-direction sensitivity in barn owls. J. Neurophysiol. 80: 172–185, 1998. Many neurons in the barn owl’s inferior colliculus (IC) exhibit auditory motion-direction sensitivity (MDS), i.e., they respond more to motion of a sound source in one direction than to motion in the opposite direction. We investigated the cellular mechanisms underlying the phenomenon of auditory MDS by microiontophoretically applying γ-aminobutyric acid (GABA) or the GABA-antagonist bicuculline methiodide (BMI) while recording from neurons in the owl’s midbrain. In most cases GABA reduced the overall firing rate, whereas BMI increased it. In addition, 29% of the motion-direction–sensitive cells completely lost their selectivity for the direction of auditory movement during administration of BMI. It had been proposed that auditory MDS in the owl is due to inhibition. The present results show that GABAergic inhibition plays a role in the strengthening of MDS. We discuss the data within the framework of the acoustic motion detector and with respect to microiontophoretic studies on visual motion detection and on inhibitory mechanisms in the inferior colliculus.

INTRODUCTION

Sound localization is of crucial importance for the survival of barn owls that hunt small rodents in dawn and dusk. To effectively catch a moving prey in the dark, owls often need to make midflight course corrections, and they do so with impressive accuracy (Konishi 1973). In addition, it is important for them to get a firm grip on their prey, best achieved when the rectangle formed by their talons aligns with the body axis of the prey. Payne (1971) showed that in total darkness the owl aligns its talons according to the prey’s axis of motion that it had to infer from auditory cues alone. Both of these achievements suggest that the neural circuitry underlying sound localization is influenced by auditory motion.

The barn owl is a uniquely well-suited animal to study the neural representation of auditory motion signals since the neuroanatomy and neurophysiology of stationary sound localization are well understood (Konishi 1993). Barn owls possess a two-dimensional neural map of auditory space in the external nucleus of the inferior colliculus (ICx) (Knudsen and Konishi 1978b). This map is formed by so-called space-specific neurons that have spatially restricted receptive fields similar to neurons in the visual system.

A subpopulation of the midbrain neurons is also sensitive to the motion direction of an apparent sound source (Wagner and Takahashi 1990) as well as to continuously varying interaural phase differences (Wang and Moiseff 1992). Those neurons respond differently depending on whether motion occurs from left to right or vice versa.

Wagner and Takahashi (1992) proposed a motion-detecting model for the auditory system of the barn owl (Fig. 1), which is similar to a model for visual motion-direction sensitivity (MDS) in the rabbit’s retina first suggested by Barlow and Levick (1965). The acoustic motion detector has two stages where inhibition plays a role: in a first stage (“Nonlinearity”), a direct excitatory input from receptor 1 (see Fig. 1) and a delayed inhibitory input from receptor 2 together create MDS. The conclusion that inhibition plays a role at this stage was based on the finding that the response of a cell to motion in the preferred direction was about the same as the response to stimulation with stationary sounds, whereas the response to motion in the null direction was diminished (Wagner and Takahashi 1992). This would not be expected in an alternative model where excitatory inputs from the two receptors interacted in a facilitatory fashion. At the final level of the model (“Further processing”), a direction-insensitive inhibition lowers the overall response of the cell and thereby increases MDS.

To better understand the cellular mechanism of MDS, standard extracellular recording techniques were combined with microiontophoresis. Because the existence and functional importance of GABAergic inhibition in the barn owl’s midbrain is well established both anatomically (Carr et al. 1989) and physiologically (Adolphs 1993; Fujita and Konishi 1991; Mori 1997), the γ-aminobutyric acid-A (GABA_A) antagonist bicuculline methiodide (BMI) was used to block GABAergic inhibition. In addition, application of GABA itself was used to investigate the effects of stimulus-independent inhibition.

In the following, we present evidence for the contribution of GABA-mediated inhibition to the enhancement of MDS. The results suggest similar mechanisms for auditory and visual motion sensitivity.

METHODS

Twelve adult barn owls (Tyto alba) were used in this study. The owls were reared in the colony at the Max-Planck-Institute for Biological Cybernetics in Tübingen; one owl came from the university research animal facility of the TU Munich. We recorded from each owl more than once. Except for the use of microiontophoresis, which is described in detail here, procedures were equivalent to those described earlier (Wagner and Takahashi 1992;
FIG. 1. Hypothetical auditory motion-detector proposed by Wagner and Takahashi (1992). The numbers 1 and 2 denote receptors, e.g., neurons with auditory spatially restricted receptive fields. The detector creates motion-direction sensitivity (MDS) in the following way: when stimulated by motion in the preferred direction (indicated by the arrow on top), receptor 1 is activated first. The input from receptor 2 arrives at the nonlinear integration stage much later. For motion within a certain range of speeds in the null direction, however, the input from receptor 2 can suppress input from receptor 1. inh., inhibitory; exc., excitatory connection.

Wagner et al. 1994) and are, therefore, only briefly summarized in the following.

Preparation for electrophysiological recording

All recordings in this study were performed under anesthesia. Anesthesia was started by an initial dose (im) of 40 mg/kg body wt of ketamine and maintained by doses of 20 mg/kg body wt. Diazepam was initially given as a muscle relaxant (1 mg/kg im). During the experiment, Diazepam was applied only when needed, because Diazepam reduces brain excitability by increasing inhibition mediated by GABA receptors (Brainard and Knudsen 1993). Atropine sulfate (0.05 mg/kg ip) was also given initially to avoid bronchial secretions.

Auditory stimuli

Experiments were carried out in a sound-attenuating chamber. As in earlier studies (Wagner and Takahashi 1992; Wagner et al. 1994), dichotic stimuli (broadband noise with a duration of 100 ms and rise-fall times of 5 ms) presented via stereo earphones (Sony MDR-E 272) were used to search for auditory evoked responses. These stimuli were also used to obtain response functions for (stationary) interaural time difference (ITD) and interaural level difference (ILD).

Auditory-MDS in the horizontal plane was investigated with free-field stimuli. Seven loudspeakers were placed in a semicircular array around the owl at a distance of 100 cm and a separation of 30° (Fig. 2A, inset). Apparent auditory motion in the horizontal plane was generated by sequential activation of the speakers in either a counterclockwise (CCW) or a clockwise (CW) direction.

FIG. 2. Example of a motion-direction-sensitive response. A, inset: top view of the stimulus configuration with free-field speakers numbered 1–7 and the owl in the center of the semicircle (radius 100 cm). A: a cell was stimulated with apparent motion by activating the speakers 1st in the counterclockwise (CCW) direction (1–7) and then in clockwise (CW) direction (7–1). B: to check for onset or adaptation effects, the stimulus sequence was reversed (1st 7–1, then 1–7). C: when only speaker 4 was connected (this corresponds to a repeated, stationary stimulus), the cell showed a symmetrical response. Response refers to number of spikes summed over all repetitions. Individual peristimulus time histograms (PSTHs) are not normalized to make changes due to the drugs directly visible. The ticks on the Time-Axis give the onset times of the respective speakers (on-on time: 95 ms) whose numbers are given in the top row of each figure. Vertical dashed line represents the middle portion of the stimulus cycle where the motion direction is reversed. Binwidth is 19 ms, which is 1/5 of the interstimulus interval. Data from 10 stimulus repetitions.
We used two stimulus paradigms to avoid possible onset asymmetries: in paradigm 1, the sound first moved in the CCW direction and then in the CW direction; in paradigm 2, the acoustic motion started in the CW direction and ended in the CCW direction. Stimuli from the individual speakers consisted of noise bursts (0–25 kHz) having a rise-fall time of 5 ms and a duration of 100 ms. Because of overlap in speaker activation, the interstimulus interval of 95 ms provided a constant overall sound pressure level during the stimulation. This stimulus configuration resulted in an apparent speed of 310°/s, which was used throughout this study. Note that most motion-direction sensitive neurons are broadly tuned to speed calculated (Wagner and Takahashi 1992). To prohibit the influence of click artifacts produced by the relays used for switching from one speaker to the next, we continuously presented a background noise of constant level (25.4 dB SPL) via all seven speakers (see Wagner et al. 1994).

Recording and iontophoresis

Three- or five-barreled glass micropipettes (World Precision Instruments) were used for simultaneous microiontophoresis and extracellular recordings of action potentials of single cells and multiunits in the midbrain. The microelectrodes were made using methods described by Armstrong-James and colleagues (Armstrong-James et al. 1981; Armstrong-James and Millar 1979; Fox et al. 1980; Wallis 1993). One of the barrels contained a single carbon fiber (7-μm diam, SGL Carbon, Meitingen, Germany, fiber C-40) for recording. Electrodes were pulled on a custom-made vertical microelectrode puller from the blanks with the carbon fiber in place. After pulling, the tip of the electrode assemblies was usually bumped back under microscopic control to obtain an outer diameter of 10–15 μm, and excess carbon fiber was cut away flush or slightly protruding with the barrels. The end of a silver wire was coated with conductive paint to the carbon fiber and fixed in place inside the recording barrel by a drop of acrylamide glue. The resistance of the carbon fiber electrode was ~1 MΩ. The time of action potentials, relative to the onset of the stimulus was recorded with a 1-ms resolution.

The iontophoresis barrels were filled with GABA (0.5 M; pH 3.0; Sigma), BMI (5 mM; pH 3.0; Sigma), or saline (pH 3.0) for controls. The formation of salt bridges between the barrels was prevented by coating the end of the barrels with paraffin (Shi and Bunny 1990). All solutions were passed through Millipore filters. Iontophoretic ejection and retaining currents were generated and monitored by a Neurophore BH-2 System (Medical Systems). Retaining currents of −10 to −20 nA were employed to minimize spontaneous drug diffusion from the tip. Ejection currents ranged from 5 to 100 nA. Drug barrel resistance (8–30 MΩ) could be tested during the experiment to identify blocked barrels. Current balancing to decrease possible current effects was not found to be necessary as others have noted (Fujita and Konishi 1991). Several experiments with saline in one of the barrels also confirmed that with the ejection currents used in this study, current artifacts do not occur.

In a few cases, GABA and BMI were applied simultaneously as a control. Usually, it was possible to silence the cell completely with GABA; it was then possible to counteract the effect of GABA with BMI.

In the majority of cells, different intensities of ejecting current were applied to compare effects of different levels of disinhibition (Fig. 5).

Data analysis

The responses to apparent movement were examined before, during, and after drug application. Data were taken about every 3 min so that the time course of the changes in the response of the cell could be quantified (see Fig. 3).

It was not always possible to hold the cell long enough to restore the predrug control levels. In these cases, cells were included in the analysis if they showed a decrease in response magnitude of >30% from maximum drug levels. Whenever control values are reported, they mean that the cell was back to control levels as judged by a t-test. A Wilcoxon signed-rank test was used for comparisons on pairs of measurements from one neuron. To test for correlations, the Spearman rank-order correlation coefficient r, was calculated. The significance level used throughout was P < 0.05, and the tests are two-tailed unless noted otherwise.

MDS was defined by comparing the temporally integrated number of spikes elicited by motion in the preferred direction with the number of spikes elicited by motion in the null direction. Averaging over 5–25 stimulus repetitions was done. To exclude onset responses (Wagner 1990; Wagner and Takahashi 1992), MDS was computed for responses to speakers 2–6 and quantified by a directionality index (DI)

\[
DI = 1 - \frac{R_{\text{Null}}}{R_{\text{PD}}} \tag{1}
\]

where \(R_{\text{PD}}\) and \(R_{\text{Null}}\) denote the relative response strengths (R) elicited by motion in the preferred direction (PD) and the null direction (Null), respectively. We defined the signed directionality index (SDI) to be positive (SDI = DI) when CCW was the preferred direction and to be negative (SDI = –DI) when CW was the preferred direction. A χ² test was used to test for statistical significance of the MDS (Wagner and Takahashi 1992).

We tried to separate effects of the drugs on the generation of MDS of a cell and on the enhancement of MDS. To do so, we first normalized all responses to the number of spikes per 20 repetitions. To reveal the response due to the drug, we subtracted the spikes obtained in the normal condition from the spikes in the drug condition for each direction. From the remaining responses we obtained again an index that we term specificity index, SI

\[
SI = \frac{(R_{\text{Null, Drug}} - R_{\text{Null, normal}}) - (R_{\text{PD, Drug}} - R_{\text{PD, normal}})}{(R_{\text{Null, Drug}} - R_{\text{Null, normal}}) + (R_{\text{PD, Drug}} - R_{\text{PD, normal}})} \tag{2}
\]

To avoid possible adaptational artifacts, we averaged over the responses obtained with paradigms 1 and 2.

As noted in earlier studies (Wagner and Takahashi 1992; Wagner et al. 1994), MDS was not different in multiunit and single-cell recordings. Iontophoresis results obtained from recordings on small cell groups also did not differ from those on single cells (see Fig. 7) and for summary statistics, the data from multiunit and single-cell recordings were pooled.

Criteria for verification of recording sites

In most cases the recording sites were identified by physiological criteria including ITD, ILD, frequency tuning, and latencies (Adolphs 1993; Cohen and Knudsen 1994; Fujita and Konishi 1991; Knudsen and Konishi 1978c; Moiseff and Konishi 1983; Takahashi and Konishi 1986; Takahashi et al. 1989; Wagner et al. 1987). The electrolytic lesions, usually made during the last recording session, confirmed these judgments.

After the last session and an appropriate waiting period, the owl was deeply anesthetized with an overdose of pentobarbital sodium (Nembutal, 30 mg) and perfused with 4% paraformaldehyde. Brains were blocked stereotaxically and placed in 30% sucrose until they sank. Thirty-micrometer-thick frozen sections were cut on a microtome. Sections were stained with cresyl violet. The position of the lesions was then reconstructed either with camera
Results

For this study we recorded from 484 single units or small cell groups (multunits) in the midbrain of the barn owl. We obtained a significant MDS response from 73 (15%) of the 484 cells (hereafter: MDS cells). About the same number (82) of cells for which responses to the CW and CCW directions did not differ significantly (hereafter: non-MDS cells) were analyzed for comparison (Table 1). No specific criteria except stability of the recordings at the beginning of drug iontophoresis were applied for the control group. The technical difficulty of obtaining stable recordings from midbrain neurons with multibarrel pipettes for long periods of time demanded that we focused on a limited number of variables that might be investigated. Thus all cells were tested with an apparent auditory motion stimulus with a speed of 310°/s as described in Methods.

The prime goal of this study was to investigate the effect of drugs on cells in the IC sensitive to auditory motion direction. Some recordings from cells in the optic tectum (OT) are also included in this study. Because inhibition was proposed to be important for the establishment of MDS (Wagner and Takahashi 1992), we tested an inhibitory neurotransmitter and its antagonist. From earlier microiontophoretic studies in the IC of the owl (Adolphs 1993; Fujita and Konishi 1991; Mori 1997), it was to be expected that GABA would reduce the response of IC cells and BMI would enhance it. The data presented below confirm this expectation, and we considered this primary drug effect as a control in our study. Of the neurons tested with drugs 70% (53 MDS, 56 non-MDS) could be further analyzed (Table 2). The reduction is due to unstable recordings during drug application (~15%), strong influence of ketamine on the response (~5%), and failure of drug effect (~10%). The cause of drug failure was unclear in most cases (see also Discussion). A case where BMI or GABA changed the MDS but not the overall responsiveness of a cell was not observed. There were no cells in which the MDS of a cell was changed with BMI or GABA application, but the overall responsiveness of the cell remained constant.

Table 1. Database

<table>
<thead>
<tr>
<th></th>
<th>MDS Cells</th>
<th>Non-MDS Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells tested: 484</td>
<td>73</td>
<td>411</td>
</tr>
<tr>
<td>Cells tested with drugs</td>
<td>73</td>
<td>82</td>
</tr>
<tr>
<td>Cells analyzed further</td>
<td>53</td>
<td>56</td>
</tr>
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MDS, motion-direction sensitivity.
The responses from single-unit recordings (Table 2) did not differ from the responses of multunit recordings (see Fig. 7) as has been reported earlier (Wagner and Takahashi 1992; Wagner et al. 1994). Therefore responses from both groups were pooled for the further analyses.

The typical responses and recording protocols can be seen in Fig. 2. In A, the cell is stimulated with paradigm 1 [CCW direction 1st (speakers 1–7 in inset)]. It responds strongly only when speaker 4 is active. In the opposite direction (here: CW) the cell is almost silent, which leads to the high directionality index of 0.92. Figure 2, B and C, shows controls: in B the cell is activated with paradigm 2 (CW motion 1st). Again, the cell responds more strongly for motion in the CCW direction. Figure 2C shows that the cell responds equally strong to two successive stimulations from a stationary sound source (just speaker 4 in this case). The latter two stimulus paradigms exclude the possibility of adaptation as the main factor in generating the MDS response.

Effects of BMI on overall response

The main effect of BMI was to increase the driven response of the cells. The increase ranged from 0% (no effect; 1 cell) up to 1,600%. In neurons where two or more intensities of ejecting currents were tested, BMI ejected with higher currents caused greater effects with a shorter delay (Fujita and Konishi 1991) (see also Fig. 5). BMI did not elevate spontaneous firing in most cells. Several units in the OT, however, were excluded from further analysis because their spontaneous activity increased substantially by even small doses of BMI. Thus the general effects of BMI were very similar to those presented by Fujita and Konishi (1991). We repeated several of their control experiments (spontaneous leakage of BMI from the pipette after the retaining current was stopped; effects of current ejection through the vehicle-containing barrels) ensuring that artifacts could not explain the effects described in this study.

In most cells, the effects of BMI were noticeable almost immediately. For the majority of cells, however, activity increased even after prolonged periods of ejection (usually 10–30 min) (see Fujita and Konishi 1991). Figure 3, A–D, shows examples of the time course of BMI effects on IC and OT neurons. For these recordings, relatively low ejection currents were used. As BMI is applied at $t = 15$ min in Fig. 3A and at $t = 5$ min in Fig. 3C, the cell’s response starts to increase. In Fig. 3A, the response reaches a plateau after 25–30 min of continuous application of BMI.

<p>| Table 2. Cells exhibiting stable changes in response to drug application |
|-----------------------------|---------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Total</th>
<th>GABA</th>
<th>BMI</th>
<th>Both*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS cells</td>
<td>53 (40)</td>
<td>17 (11)</td>
<td>51 (38)</td>
</tr>
<tr>
<td>Non-MDS cells</td>
<td>56 (22)</td>
<td>36 (14)</td>
<td>46 (18)</td>
</tr>
</tbody>
</table>

Single-unit recordings are in parentheses. GABA, $\gamma$-aminobutyric acid; BMI, bicuculline methiodide; MDS, motion-direction sensitivity. * Cells that were tested with both drugs.

Effects of BMI on MDS

As can be seen in Fig. 3, B and D, not only does the overall response of a cell increase due to the loss of inhibition during the administration of BMI, the MDS changes, too. Table 3 shows the influence of BMI on MDS and non-MDS cells. In MDS cells, BMI caused a significant decrease in the DI of $>0.2$ in the sensitivity for motion direction in 22 of the 51 MDS cells (43%) effectively tested with BMI. We chose a DI change of $>0.2$ because this is above the level of fluctuations of the DI for a given cell of the sample of cells we recorded from (Fig. 3B) (Wagner and Takahashi 1992). Fifteen of 51 (29%) of the MDS cells treated with BMI became non-MDS; i.e., the ratio of the response in the preferred direction and the response in the null direction was no longer statistically significant. An example of this effect of BMI can be seen from the peristimulus time histograms (PSTHs) before (A) and after (C) the administration of drugs in Fig. 4 (left). Like the cell shown in Fig. 2, this cell shows a strong MDS response (SDI = $-1.0$, i.e., no response in null direction). The response at the beginning of the round-trip was excluded from the calculation of the SDI. Thus in Fig. 4A, the response of the cell in the null direction is taken as 0 spikes, although 16 spikes occur in the first 40 ms after onset of the sound. This procedure was adopted because at the onset of the motion stimulus, the cell does not have any motion information. The response at the turning point (here: speaker 7) and the offset response at the end of the round-trip was not taken into account as well. In this cell, BMI affected only the response during motion but did not affect the onset response of the cell (17 spikes in the BMI condition). There were, however, cases where the onset response was affected by BMI application as well.

Another cell where BMI abolished MDS (Fig. 4, D–F) did not show an onset effect in the control conditions. While BMI was administered, the cell responded to stimulation from every speaker during both stimulation in the preferred and the null directions. The appearance of a response at all speakers suggested that inhibition was removed by BMI. The effect was that the cell became non-MDS ($\chi^2 = 1.0$, $P = 0.32$) during drug application.

Different time courses of the effects of BMI can be ob-
FIG. 4. Effects of BMI on MDS in a cell with onset response (A–C; 10 stimulus repetitions). Both cells are highly motion-direction sensitive before (A and D) and after (C, after 5 min; F, after 10 min) application of BMI. During continuous iontophoretic of BMI (B, 13 min with a current of 60 nA; E, 10 min with a current of 15 nA and subsequently 4 min with a current of 40 nA; continuous application), MDS is completely abolished. Onset response was excluded for the calculation of the directionality index (DI); only the response to speakers 2–6 was considered (see text).

served in Fig. 3, A and C. For the cell shown in Fig. 3A, the bias toward the CW direction of motion decreased first fast and then slowly over a time of 30 min of application of BMI. The cell in Fig. 3C on the other hand, lost its bias soon after the application started. However, in both cells, the bias (for the cell in Fig. 3C even briefly in the opposite direction at 14 min) was nonsignificant already at the first measurement (3 min after the application started ($\chi^2 = 2.4, P = 0.12$ for the cell shown in A) and was even less so when the effects had reached a plateau ($\chi^2 = 0.9, P = 0.34$). Both cells lost their MDS completely during the application of BMI.

In a few cells, different ejection currents were tested systematically. The MDS cell in Fig. 5 shows that an application current of 20 nA did not change the DI in this cell, whereas bigger amounts of BMI gradually decreased the DI until the cell was no longer MDS.

More than one-half of the cells did not exhibit this kind of drastic change in their sensitivity to motion direction. Figure 6 shows a highly MDS cell that stays MDS even after the overall response of the cell was about fivefold after measurement $\times 2.4, P = 0.12$ for the cell shown in A) and was even less so when the effects had reached a plateau ($\chi^2 = 0.9, P = 0.34$). Both cells lost their MDS completely during the application of BMI.

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The preferred direction (SI < 0) more than the response in the null direction (Table 4).

Effects of GABA on overall response

In most neurons GABA had an immediate clear inhibitory effect. Application of GABA decreased overall activity in MDS cells (Fig. 9B) as well as in non-MDS cells (Fig. 9D). We confirmed observations by Fujita and Konishi (1991) that less ejecting current for GABA was necessary in comparison with BMI to achieve significant changes in response and that the latency to maximum effects of GABA and its recovery were faster than those of BMI.

We encountered several cells where GABA had a weak or no effect although BMI was effective (see DISCUSSION). In cells where we applied GABA and BMI together, we were able to adjust ejection currents in a way that the two drugs antagonized each other.

In a few cases where spontaneous activity was substantial, suppression of spontaneous activity by application of GABA was observed.

Effects of GABA on MDS

Generally, GABA did not cause significant changes in the null direction. However, the change in response did not yield a significant DI in the drug situation.

The data are summarized in Fig. 8, which shows the mean DI during the drug applications in relation to the DI before application of the drugs. The percentage of change of the DI during application of BMI is highly significant (P < 0.01, Wilcoxon test).

The DI of MDS neurons has been shown to decrease exponentially with increasing neural activity (Fig. 9 in Wagner and Takahashi 1992). Figure 9A shows this observation for data of the current study as a linear relation in a semilogarithmic plot (thick line with steeper slope; \( r_s = -0.55; P < 0.001 \)). After applying BMI, there was no such correlation between the DI and the spike rate (thick horizontal line; \( r_s = -0.03 \); not significant).

We observed that cells with a high response rate tended to change their MDS less than cells with a low response rate: the slopes of the lines connecting predrug and drug response shown in Fig. 9A are correlated with the neural activity (\( r_s = 0.70; P < 0.001 \)).

Effects of BMI on the preferred- and null-direction responses

In the responses of the cells to BMI, three different classes of effects might occur. There might be a greater effect of BMI on the response in the null direction than on the response in the preferred direction, or a greater effect of BMI on the response in the preferred direction than on the response in the null direction, or the effects on both directions may be equivalent. To quantitatively assess these different effects, we calculated a specificity index, SI (see METHODS), and tested for significance of the SI. The statistical analysis demonstrated that the SI of most cells was not statistically different from 0 (71% in the MDS cells and 90% in the non-MDS cells; Table 4). All MDS cells that became non-MDS after BMI application fell into this class. In a second class (29%) of MDS cells, BMI increased the response in the preferred direction (SI < 0) more than the response in the null direction (Table 4).

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We observed that cells with a high response rate tended to change their MDS less than cells with a low response rate: the slopes of the lines connecting predrug and drug response shown in Fig. 9A are correlated with the neural activity (\( r_s = 0.70; P < 0.001 \)).

Effects of BMI on the preferred- and null-direction responses

In the responses of the cells to BMI, three different classes of effects might occur. There might be a greater effect of BMI on the response in the null direction than on the response in the preferred direction, or a greater effect of BMI on the response in the preferred direction than on the response in the null direction, or the effects on both directions may be equivalent. To quantitatively assess these different effects, we calculated a specificity index, SI (see METHODS), and tested for significance of the SI. The statistical analysis demonstrated that the SI of most cells was not statistically different from 0 (71% in the MDS cells and 90% in the non-MDS cells; Table 4). All MDS cells that became non-MDS after BMI application fell into this class. In a second class (29%) of MDS cells, BMI increased the response in the preferred direction (SI < 0) more than the response in the null direction (Table 4).

Effects of GABA on overall response

In most neurons GABA had an immediate clear inhibitory effect. Application of GABA decreased overall activity in MDS cells (Fig. 9B) as well as in non-MDS cells (Fig. 9D). We confirmed observations by Fujita and Konishi (1991) that less ejecting current for GABA was necessary in comparison with BMI to achieve significant changes in response and that the latency to maximum effects of GABA and its recovery were faster than those of BMI.

We encountered several cells where GABA had a weak or no effect although BMI was effective (see DISCUSSION). In cells where we applied GABA and BMI together, we were able to adjust ejection currents in a way that the two drugs antagonized each other.

In a few cases where spontaneous activity was substantial, suppression of spontaneous activity by application of GABA was observed.

Effects of GABA on MDS

Generally, GABA did not cause significant changes in the null direction. However, the change in response did not yield a significant DI in the drug situation.

The data are summarized in Fig. 8, which shows the mean DI during the drug applications in relation to the DI before application of the drugs. The percentage of change of the DI during application of BMI is highly significant (P < 0.01, Wilcoxon test).

The DI of MDS neurons has been shown to decrease exponentially with increasing neural activity (Fig. 9 in Wagner and Takahashi 1992). Figure 9A shows this observation for data of the current study as a linear relation in a semilogarithmic plot (thick line with steeper slope; \( r_s = -0.55; P < 0.001 \)). After applying BMI, there was no such correlation between the DI and the spike rate (thick horizontal line; \( r_s = -0.03 \); not significant).

We observed that cells with a high response rate tended to change their MDS less than cells with a low response rate: the slopes of the lines connecting predrug and drug response shown in Fig. 9A are correlated with the neural activity (\( r_s = 0.70; P < 0.001 \)).
marked by the arrow in Fig. 7B could not improve its DI further, because it was already maximally selective to the CW direction. This was, however, not the case for most of the cells tested. For example, the response of the ICx cell shown in Fig. 10 could be reduced by application of GABA with an ejection current of 30 nA from 26 spikes per repetition to \( \sim 9 \) spikes per repetition. Nevertheless, the SDI did not change significantly after 10 min of continuous application. This was true for most of the MDS cells in our sample so that the mean change of the DI overall turns out to be statistically insignificant (Fig. 8A; \( P > 0.1 \), Wilcoxon test).

The two MDS cells in the top left and the bottom right quadrant of Fig. 7B showed a significant DI decrease. As with BMI, the reversal of preferred direction is not a reversal of motion-direction preference because the difference of response during GABA application was not significant.

**Effects of drugs on non-MDS cells**

All the effects we have described so far were effects of BMI and GABA on MDS cells. It is also interesting, however, to investigate the influence of these drugs on non-MDS cells.

We found six non-MDS cells that showed a DI increase of \( \sim 0.2 \) under the influence of GABA (Table 3). In three of them, this change in DI caused by the administration of GABA led to a significant (see METHODS) motion-direction-sensitive response. The response of the IC cell in Fig. 11 shows no difference between the responses to CW and CCW motion directions in either of the control conditions (before and after the continuous application of GABA for 13 min with an ejection current of 50 nA). GABA inhibited this cell strongly leading to an overall reduction from \( \sim 19 \) spikes per stimulus repetition to \( \sim 7 \) spikes per repetition. Because the reduction was more pronounced for the CCW direction than for the CW direction, this meant a significant (\( P < 0.001 \)) MDS response with a SDI of \( 0.52 \).

Only 1 of the 46 non-MDS cells where BMI had an effect on the overall response became MDS in the \( \chi^2 \) test during drug application (Table 3).

With GABA we made a similar observation as with BMI: in a considerable number of cases, the response in the preferred direction was more susceptible to drug application than the response in the null direction.
Anatomic location of units

Anatomic locations were determined by physiological criteria during the recording session (see METHODS). In some cases lesions were made, confirming the physiological judgments.

The majority of the MDS responses (26/53; 40%) were recorded from cells in the ICx. Eleven (21%) recordings were located in the lateral shell of the IC, which directly projects to ICx; another 10 recordings were in the IC but could not clearly be assigned to a particular subnucleus. We also included recordings from six MDS cells from the optic tectum in our analysis. These cells had higher spontaneous activity as well as higher driven activity than cells encountered in the IC.

For the non-MDS cells, the situation was similar: 18 of 56 cells (32%) were judged to be in ICx, 19 (34%) in the lateral shell. Thus our sample does not show a different distribution of MDS and non-MDS cells in the IC.

There was no apparent difference in either the distribution of the directionality index or the effects of application of BMI and GABA between the different nuclei. However, the number of cells outside of ICx was too small to make a statistical comparison.

Influence of anesthesia

The anesthetic ketamine used in this study, a N-methyl-D-aspartate receptor antagonist, has been shown to reduce learned responses to auditory stimuli in the phase of plasticity during development (Feldman et al. 1996). Thus anesthesia could have influenced the results reported here. In general, however, we did not observe major changes in cell activity due to varying levels of ketamine during the recordings. In some cases where a refreshment of the anesthesia became necessary during the recordings of a cell, we saw a pronounced reduction of responses under the influence
reported in this study, because, in the cells we tested with different ejection currents, a higher ejection current usually led to a higher response (Fig. 5).

The iontophoretically applied BMI may have blocked inhibition not only to the recorded cell but also to adjacent cells. In the IC of the barn owl, cells with similar physiological properties are organized in maps (Knudsen and Konishi 1978b; Wagner et al. 1987). There is evidence that this is also the case for auditory motion (Wagner 1992a). Our

**DISCUSSION**

In the present study we examined the role of GABAergic input on auditory MDS in the IC and the OT of the barn owl. The reduction of the DI of MDS cells with enhanced responses during BMI application and the loss of selectivity for the direction of auditory motion in ~29% of those cells clearly shows that GABAergic inhibition influences MDS. In the following, we shall first consider some technical limitations. The findings will then be discussed in the context of the model of the auditory-motion detector (Fig. 1) and of earlier physiological studies on auditory motion selectivity in owls and other animals. Finally, we compare the results with studies on motion detection in other systems.

**Technical considerations**

There are several technical limitations of the iontophoresis method (e.g., Bloom 1974; Fujita and Konishi 1991; Sato et al. 1995). One concern in studies with BMI is that due to the loss of inhibition, the excitatory response of a cell could saturate. This is unlikely to be the case for the cells of ketamine (Fig. 12). Although we did not observe changes of the receptive field or of the SDI (Fig. 12), we introduced a delay of ~20 min after an injection of ketamine to assure that conditions remained as constant as possible.

**FIG. 10.** Example of a MDS cell for which GABA application did not increase MDS. A: before application of GABA. B: during GABA application (20–30 nA; 10 min). Ten stimulus repetitions.

**FIG. 11.** Example of a cell that was not MDS before (A) and after (C) application of GABA. After the application of GABA (50 nA; continuous for 13 min), the tonic part of the response was suppressed more for one direction than for the other, leading to a MDS response ($\chi^2 = 12.6, P < 0.001; B$). Note that this cell increased its activity by 50% in response to application of BMI so that the observed effect is probably not due to leakage of BMI before the application of GABA. Fifteen stimulus repetitions.
that too low doses of BMI did not block all GABA receptors on distant regions of a large dendritic tree.

The failure of GABA to evoke significant changes in the activity of some of the neurons tested has also been found in similar studies (see, e.g., Brotz and Borst 1996; Sillito 1977) and is presumably due to the distance of the neurons to the electrode tip. Note that the three to five barrel electrodes used in this study have a fairly big diameter (see METHODS). Proximity to the cell matters more for GABA than for BMI because no uptake mechanisms for BMI exist. Lack of effectiveness could also be due to blockade of iontophoresis barrels (electrodes had to pass through ~15 mm of tissue) or chemically noneffective drugs. We found that, in general, if a drug failed to increase/decrease overall activity in one unit, it also failed for other units in the same penetration.

In this study ~15% of the cells that were tested turned out to be MDS judged by our statistical tests. In previous reports (Wagner and Takahashi 1990, 1992; Wagner et al. 1994) the percentage of MDS cells was between 25 and 34%. It is possible that the different type of electrode used in this study, a carbon fiber electrode in the middle of a multibarrel assembly, was the reason for the lower number of MDS cells. Other differences between the MDS cells in this study compared with earlier studies (Wagner and Takahashi 1990, 1992; Wagner et al. 1994) were not observed.

**Mechanism of auditory MDS**

The data presented in this study demonstrate on a cellular level that GABAergic inhibition influences auditory MDS in the barn owl’s IC. About one-third of the MDS cells lost significantly different responses to leftward versus rightward motion during blockade of GABAergic inputs by BMI. As summarized in Fig. 8, the change of the DI during BMI administration for the whole sample of MDS cells is significant. GABA application did not change the DI significantly.

The responses of MDS cells in the barn owl’s IC have been described by a computational model of a two-stage motion detector (Wagner and Takahashi 1992). The direction-sensitive inhibition in the first stage of neural processing induces a motion direction bias that is enhanced by the direction-insensitive inhibition in the second stage.

The assumption of an inhibitory rather than an excitatory (multiplicative) process for the generation of a motion direction bias was based on the finding that for the majority of the MDS cells, the response in the preferred direction remained equal to the stationary response (Wagner and Takahashi 1992).

Our experiments allow us to test the validity of the computational model. Whereas Table 3 summarizes the phenomenological effects of the application of the drugs, Table 4 provides data that may be interpreted in the mechanistic framework of the motion detector. Note that the data in the two tables cannot be compared directly, because in Table 3 we use responses to calculate DI, whereas in Table 4 we use differences of responses to calculate SI.

The specificity index is a means to separate the effects of the drugs on the null and the preferred directions. Because
we found that the responses in all but one cell increased during BMI application, SI may be discussed within the framework of the motion detector (Fig. 1) as follows: a SI greater than zero suggests more influence on the null direction response than on the preferred direction response and thus indicates influence on the generation of MDS, because this would involve a direction-specific inhibition effective in the null-direction response. On the other hand, SIs below zero would suggest more influence of BMI on the preferred direction response. SIs not statistically different from zero would suggest an equal effect on both the null- and preferred-direction responses and may thus represent the second stage of the motion detector. Our results (Table 4) suggest that most of the cells tested represented the second stage of the motion detector and none represented the first stage. We thus conclude that in the cells of our sample, GABAergic inhibition was not directly involved in the generation but in the enhancement of MDS. A plausible source of the motion-direction-sensitive inhibition observed in this study is lateral inhibition in the IC and in the OT. Lateral inhibition has been shown to be responsible for the inhibitory surround of the receptive field observed in free-field experiments (Fujita and Konishi 1991; Knudsen and Konishi 1978a; Mori 1997).

Where and how might MDS be generated? One possibility is that the site of generation of MDS lies before the lateral shell of the central nucleus of the IC in the auditory pathway. MDS had been observed in neurons in the lateral lemniscal complex (LL) and in the core of the central nucleus of the IC (this study; Wagner and Takahashi 1992), but we could not collect enough data to make a statement about the effect of GABA on core cells. Another possibility is that MDS may be generated by either a different inhibitory transmitter or by directionally biased excitatory inputs (Jagadeesh et al. 1993; Sato et al. 1995) (see also Comparison with mechanisms of MDS in other systems).

Our data demonstrate that BMI and GABA influence MDS in a fair number of IC cells. Thus GABA is involved in processing of MDS information in the midbrain. This conclusion should not distract from other functions of GABA in the auditory midbrain, like conditioned enhancement or conditioned suppression (Sanes et al. 1998) or an influence on nonmonotonicity, response pause, and offset inhibition (Faingold et al. 1991).

Surprisingly, the response in the preferred direction was more susceptible to drug application than the response in the null direction in a fair number of MDS cells. This observation is similar to observations made in visual motion detection (Sato et al. 1995; Schmidt et al. 1994). It cannot be explained within the motion-detector scheme of Fig. 1. Thus this scheme needs an addition that reflects the higher susceptibility to BMI of the response in the preferred direction in these 29% of the cells. Where this additional inhibition would be effective and how it might interact with the mechanisms generating MDS is unclear.

As in earlier studies (Wagner and Takahashi 1990, 1992; Wagner et al. 1994) most of the MDS neurons in the barn owl were not exclusively excited by moving stimuli but responded also to stationary sound sources in their receptive fields. This is similar to MDS neurons in the monkey auditory cortex (Ahissar et al. 1992). Motion-sensitive neurons that did not respond to stationary stimuli would reflect a higher level of motion abstraction than are represented by the detector model analyzed here. Reports on “specific movement units” in the cat (Sovijärvi and Hyvärinen 1974) may reflect differences in the mechanisms for detection of sound movement in different species or in the level of motion computation (Ahissar et al. 1992).

Comparison with mechanisms of MDS in other systems

To our knowledge, this is the first study addressing cellular mechanisms underlying MDS in the auditory domain. It is therefore interesting to ask how these results compare with studies on motion detection in other modalities. Borst and Egelhaaf (1989) have argued that “mechanisms underlying motion detection can be attributed to only a few, essentially equivalent computational principles.” The “common principles” for visual motion detection they find “at the level of computations performed by small neural networks” (Borst and Egelhaaf 1989) may also apply to auditory motion detection and phenomena like frequency-modulated sweep selectivity (Fuzessery and Hall 1996).

Microiontophoretic studies on visual motion sensitivity have been performed in several species. Direction selectivity of movement-sensitive neurons has been shown to be severely impaired by administration of GABA antagonists in the rabbit retina (Wyatt and Duv 1976), in the fly lobula plate (Schmid 1992; Schmid and Bülthoff 1988), in the nucleus of the optic tract of the rat (Schmidt et al. 1994), and in the visual cortex of cats (Sillito 1977) and monkeys (Sato et al. 1995). BMI also destroys selectivity for the sweep direction of frequency-modulated sweeps in the central nucleus of IC of bats (Fuzessery and Hall 1996). Thus GABAergic inhibition plays an important role in shaping responses to motion in different systems and domains.

A two-stage process for motion detection has been suggested for visual motion detection in rabbits, flies, cats, and monkeys. For the generation of the MDS response, an excitatory (multiplicative) process is favored for the fly (Borst and Egelhaaf 1989), whereas an inhibitory process is proposed for the rabbit (Barlow and Levick 1965). The responses of two detector subunits, responding to opposite directions of movement, are thought to be subtracted from each other in the second stage leading to an enhanced direction selectivity in both the rabbit lateral geniculate nucleus (Levick et al. 1969) and the fly visual system (Borst and Egelhaaf 1990). GABA acts as the inhibitory transmitter at this subtraction level (Brotz and Borst 1996). In an analogue way, we demonstrated an influence of GABA on the enhancement of acoustic MDS. Intracellular recording studies on direction selectivity in the cat’s visual cortex did not detect inhibition selective to the nonpreferred direction (Douglas and Martin 1988; Douglas et al. 1991; Jagadeesh et al. 1993; Sato et al. 1991). Thus, for the visual cortex, directionally biased excitatory postsynaptic potentials (EPSPs) are suggested to be the basis of direction selectivity (Sato et al. 1995). However, nonselective intracortical inhibition plays an important role in the elaboration of direction selectivity in a second stage of information processing in V1 (Sato et al. 1995).
Sato et al. (1995) suggested that “GABAergic inhibition seems to raise the threshold of responses to the directionally biased EPSPs so that responses become directionally sensitive to a varying extent (so-called ‘ice-berg effect’).” Our results are similar to those of Sato et al. (1995). Whether the results are due to the same mechanisms involved in the generation of MDS must await intracellular studies (Moisell 1985).

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