Processing of acoustic motion in the auditory cortex of the rufous horseshoe bat, *Rhinolophus rouxi*

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1 Summary

This study investigated the representation of acoustic motion in different fields of auditory cortex of the rufous horseshoe bat, *Rhinolophus rouxi*. Motion in horizontal direction (azimuth) was simulated using successive stimuli with dynamically changing interaural intensity differences presented via earphones. The mechanisms underlying a specific sensitivity of neurons to the direction of motion were investigated using microiontophoretic application of γ-aminobutyric acid (GABA) and the $GABA_A$ receptor antagonist bicuculline methiodide (BMI).

In the first part of the study, responses of a total of 152 neurons were recorded. Seventy-one percent of sampled neurons were motion-direction sensitive. Two types of responses could be distinguished. Thirty-four percent of neurons showed a directional preference exhibiting stronger responses to one direction of motion. Fifty-seven percent of neurons responded with a shift of spatial receptive field position depending on the direction of motion. Both effects could occur in the same neuron depending on the parameters of apparent motion. Most neurons with contralateral receptive fields exhibited directional preference only with motion entering the receptive field from the opposite direction (i.e. the ipsilateral part of the azimuth). Receptive field shifts were opposite to the direction of motion. Specific combinations of spatio-temporal parameters determined the motion-direction-sensitive responses. Velocity was not encoded as a specific parameter.

Temporal parameters of motion and azimuthal position of the moving sound source were differentially encoded by neurons in different fields of auditory cortex. Neurons with a directional preference in the dorsal fields can encode motion with short interpulse intervals, whereas direction preferring neurons in the primary field can best encode motion with medium interpulse intervals. Furthermore, neurons with a directional preference in the dorsal fields are specialized for encoding motion in the midfield of azimuth, whereas direction preferring neurons in the primary field can encode motion in lateral positions**.**

In the second part of the study, responses were recorded from additional 69 neurons. Microiontophoretic application of BMI influenced the motion-direction sensitivity of 53 % of neurons. In 21 % of neurons the motion-direction sensitivity was decreased by BMI by decreasing either directional preference or receptive field shift. In neurons with a directional preference, BMI increased the spike number for the preferred direction in about the same amount as for the non-preferred direction. Thus, inhibition was not direction specific. In

contrast, BMI increased motion-direction sensitivity by either increasing directional preference or magnitude of receptive field shifts in 22 % of neurons. An additional 10 % of neurons changed their response from a receptive field shift to a directional preference under BMI. In these 32 % of neurons, the observed effects could often be better explained by adaptation of excitation than by inhibition.
The results suggest, that motion information is differentially processed in different fields of the auditory cortex of the rufous horseshoe bat. Thus, functionally organized pathways for the processing of different parameters of auditory motion seem to exist. The fact that cortex specific GABAergic inhibition contributes to motion-direction sensitivity in at least a part of cortical neurons is supportive for the notion that the auditory cortex plays an important role in further processing the neural responses to apparent motion brought up from lower levels of the auditory pathway.

Zusammenfassung

In der vorliegenden Arbeit wurde die neuronale Repräsentation von akustischer Bewegungsinformation in verschiedenen Feldern des Hörkortex der Hufeisennasen-Fledermaus *Rhinolophus rouxi* untersucht. Bewegungen einer Schallquelle in der Horizontalebene wurden durch aufeinanderfolgende Stimuli mit sich dynamisch verändernden interauralen Intensitätsdifferenzen simuliert. Die Stimuli wurden über Ohrhörer dargeboten. Die Mechanismen die der Bewegungsrichtungsselektivität von Neuronen zu Grunde liegen, wurden mit Hilfe von mikroiontophoretischer Applikation von γ-Amino-buttersäure (GABA) und dem $GABA_A$-Rezeptor Antagonisten Bicucullinmethiodid (BMI) untersucht.

Im ersten Teil der Arbeit wurden Ableitungen von insgesamt 152 Neuronen erhalten. 71 % der Zellen waren bewegungsrichtungssensitiv. Dabei konnten zwei verschiedene Typen unterschieden werden: Bei 34 % aller Neurone zeigte sich eine Richtungsabhängigkeit in der Antwortamplitude. Die Zellen antworteten bevorzugt auf nur eine Bewegungsrichtung. Bei Zellen mit einem contralateralen rezeptiven Feld war dies eine Bewegung von der entgegengesetzten Seite (d.h. der ipsilateralen Seite) in das rezeptive Feld hinein. 57 % aller Neurone zeigten als richtungsabhängige Antwort eine Verschiebung der räumlichen Position des rezeptiven Feldes. Die Verschiebung war der Bewegungsrichtung entgegengesetzt. Beide

Effekte konnten zusammen bei einer Nervenzelle beobachtet werden. Welcher der beiden Effekte auftrat, hing von den Parametern der Bewegung ab. Bestimmte Kombinationen von räumlichen und zeitlichen Bewegungsparametern bestimmten die Art der neuronalen richtungsabhängigen Antworten, die Bewegungsgeschwindigkeit wurde nicht als spezifische Größe in der Antwort kodiert.

Zeitliche Parameter und die Position der Bewegung einer Schallquelle in der Horizontalebene wurden in verschiedenen Feldern des Hörkortex spezifisch verarbeitet. Neurone in den dorsalen Feldern zeigten ihre größte Richtungspräferenz bei Bewegungen mit kurzen Interpulsintervallen, wohingegen Zellen im primären Feld mittlere Interpulsintervalle bevorzugten. Weiterhin zeigten Neurone mit Richtungspräferenz in den dorsalen Feldern ihre maximale Antwort in mittleren Bereichen der Horizontalebene, während Zellen im primären Feld stärker auf seitliche Bereiche abgestimmt waren.

Im zweiten Teil der vorliegenden Arbeit wurden die neuronalen Antworten von 69 weiteren Zellen abgeleitet. Die mikroiontophoretische Applikation von BMI beeinflußte das bewegungsrichtungssensitive Antwortverhalten von 53 % der Neurone. Bei 21 % der Zellen verringerte BMI die Bewegungsrichtungssensitivität. Es wurde entweder die Stärke der Richtungspräferenz oder die Größe der Verschiebung der räumlichen rezeptiven Felder verkleinert. Bei Zellen mit Richtungspräferenz erhöhte BMI die Antwortstärke für beide Bewegungsrichtungen in ungefähr dem gleichen Ausmaß. Es lag also keine richtungsspezifische Hemmung vor. Im Gegensatz dazu vergrößerte BMI bei 22 % der Neurone die Bewegungsrichtungssensitivität, entweder durch Vergrößerung der Richtungspräferenz oder durch Vergrößerung der Verschiebung der rezeptiven Felder. Weitere 10 % der Neurone veränderten ihre Antworteigenschaften durch BMI. Zeigten diese Zellen ohne BMI eine Verschiebung der räumlichen rezeptiven Felder, so konnte der Antworttyp mit BMI besser als Richtungspräferenz beschrieben werden. Bei diesen 32 % der Neurone konnten die beobachteten Effekte von BMI eher mit Adaptationsvorgängen erklärt werden, als durch den spezifischen Einfluß von GABAerger Hemmung.

Die Ergebnisse lassen den Schluß zu, daß akustische Bewegungsinformation spezifisch in verschiedenen Feldern des Hörkortex von *Rhinolophus rouxi* verarbeitet wird. Es scheinen funktionell organisierte Verarbeitungswege für die verschiedenen Parameter akustischer Bewegungsinformation zu existieren. Die Tatsache, daß kortexspezifische Inhibition zumindest bei einem Teil der Neurone zur Bewegungsrichtungssensitivität beiträgt, unterstützt die Annahme, daß der Hörkortex eine wichtige Rolle bei der weiteren

Verarbeitung der neuronalen Antworten auf bewegte Schallreize aus anderen Stationen der Hörbahn spielt.

2 Introduction

Animals live in an environment which is far from being stationary. Visual as well as acoustic cues permanently change due to motion of objects or self-motion of the animal. Consequently, it is crucial for an animal to detect dynamic changes in its environment. This holds especially true for microchiropteran bats, which orientate and hunt by means of echolocation. The bats should well be able to detect dynamic changes in acoustic space to get along with the challenges of hunting for moving prey in the dark.

2.1 Localization and binaural processing of sound

The cues available for sound localization in humans are interaural intensity differences (IID), interaural time differences (ITD) and spectral cues (Moore, 1982). For ITDs, two components can be distinguished: Differences in the arrival time of the first wavefront at both ears, so called onset or transient time differences, and ongoing time differences which manifest for sustained pure tones as interaural phase differences (IPD). IPDs provide unambiguous information about azimuthal position of a pure tone only for frequencies for which the period is greater than twice the maximum interaural delay. Thus, for low-frequency sounds ITDs are the main cue for sound localization in the horizontal plane (i.e the azimuth) whereas high-frequency sounds can only be localized by IIDs (Moore, 1982). Movement of a sound source in azimuth is characterized by the dynamical variation of these interaural parameters. Because of the small head size of bats and the fact that the echolocation calls are in the ultrasound range, IIDs and spectral cues only can be used by bats for localization of stationary and moving sounds.

The neural processing of binaural cues used for sound localization has been intensively studied in the past (for review see Irvine, 1992). The first site of processing of IPDs in the brainstem of mammals is the medial superior olive (MSO), whereas in the lateral superior olive (LSO) IIDs are processed. The next major binaural processing stage is the inferior colliculus (IC), where information of all lower nuclei is converged. In the auditory cortex of mammals spatial information is processed by clusters of neurons with similar binaural properties (Middlebrooks & Pettigrew, 1981; Rajan et al., 1990b; Clarey et al., 1994). No clear frequency independent point-by-point representation of acoustic space has been found in the tonotopically organized structures of the ‘classical’ auditory pathway in mammals. A

topographic organization of neurons broadly tuned to spatial positions is reported in mammals for the non-tonotopic external nucleus of IC (ICx), the nucleus of the brachium of the inferior colliculus and the superior colliculus (SC, for review see Cohen & Knudsen, 1999). However, spatial acuity and topographical organization of these 'space maps' are only weak compared to maps of acoustic space found in owls. The functional properties of space maps and the computational steps underlying their creation have best been investigated in barn owls. In barn owls, the processing of IPDs and IIDs is done in two completely separated pathways. Both pathways first converge in the midbrain in the avian homolog of the ICx, where spatial information is combined across frequency channels to yield neurons that are broadly tuned to frequency but respond only to a limited range of frontal or contralateral space (Knudsen & Konishi, 1978; see Irvine, 1992 and Cohen & Knudsen, 1999 for review). Projections from the ICx end in the optic tectum where a precise map of auditory space exists. As most neurons in the optic tectum respond to both auditory and visual stimuli, the creation of the two-dimensional map is thought to transform auditory spatial information into a spatiotopic format, which can be aligned with maps of the visual field, thus enabling neurons to integrate spatial cues of both sensory domains.

2.2 Motion processing in humans and animals

Several studies investigated the detection of a moving sound source in azimuth in humans in the past. However, results obtained from those studies are still disputed. The main question is, whether a specialized detection system for auditory motion exists or not. Grantham (1986) showed that discrimination of sound source velocities is dependent on spatial changes of the source and not on velocity *per se*. This puts forward the theory that moving and stationary sounds are not independently processed, but rather that auditory motion is computed from a series of successive static localization tasks ('snap-shot-theory'). Thus no specialized motion processing system is required. An alternate theory supports the notion that dynamic aspects of motion (e.g. time-varying IIDs or ITD) are directly processed in the central auditory system and thus velocity and acceleration are directly perceived attributes of moving stimuli. This would put forward the existence of a specialized motion processing system. Support for this comes from a study by Strybel et al. (1998), who found that velocity might be a primary dimension perceived by humans. The authors showed, that velocity estimates of subjects were only little affected by spatial separation of sound sources, contradicting the findings of Grantham (1986) and arguing against a snap-shot mechanism underlying motion perception.

In addition, an aftereffect to auditory motion in azimuth was found in humans (Grantham, 1989; Dong et al., 2000). As a motion aftereffect in the visual domain has been taken as psychophysical evidence for the existence of a specialized motion detection system (for review see Wade, 1994), the same can be inferred for the auditory system. Further evidence for a specialized motion processing system in humans comes from neuroimaging studies in which the selective activation of certain cortical regions by moving sounds was shown (Griffith et al., 1994, 1998; Baumgart et al., 1999). In addition, perception of auditory motion was selectively impaired by a patient suffering from a right hemisphere stroke (Griffith, 1996), comparable to the case of a patient suffering from motion-blindness in the visual domain (Zihl et al., 1983). Furthermore, it was shown for animals in lesion studies, that the auditory cortex is important for the detection of a moving sound source (Altman & Kalmykova, 1986).

A number of neurophysiological studies investigated the coding of auditory motion at different levels of the auditory pathway in different animals. Neurons sensitive to the direction of motion were found in the superior olivary complex (Altman, 1994), the IC of mammals (e.g. Kleiser & Schuller, 1995; Spitzer & Semple, 1998; Wilson & O'Neill, 1998; McAlpine et al., 2000) and barn owls (Wagner & Takahashi, 1992), the auditory thalamus (Altman, 1994) and the auditory cortex (e.g. Ahissar et al., 1992; Stumpf et al., 1992; Doan & Saunders, 1999; Jiang et al., 2000). The results showed, that neurons could either respond with directionally dependent shifts of the spatial receptive field (RF) position (e.g. Wilson & O'Neill, 1998) or were selectively responding to only one direction of a moving sound (e.g. Stumpf et al., 1992; Wagner & Takahashi, 1992). However, with the exception of Sovijärvi & Hyvärinen (1974) who found few neurons in the auditory cortex of cats responding exclusively to a moving sound source, no reports are published of neurons that responded to auditory motion only but not to stationary sounds. Thus, the extent to which neural responses to auditory motion indicate the existence of specialized motion processing system remains unclear.

Nevertheless, Wagner & Takahashi (1992) proposed a motion detector acting on direction selective inhibition underlying motion-direction sensitivity of neurons in the brainstem of barn owls (Fig. 1). This detector belongs to the broad class of correlation-type movement detectors and implements the general requirements proposed for directionally selective motion detectors (see Borst & Egelhaaf, 1989 for review) as there are spatially separated receptors, a temporal asymmetry achieved by a temporal delay in this case, and a non-linear interaction consisting of a direction dependent inhibition. As a last stage, a direction

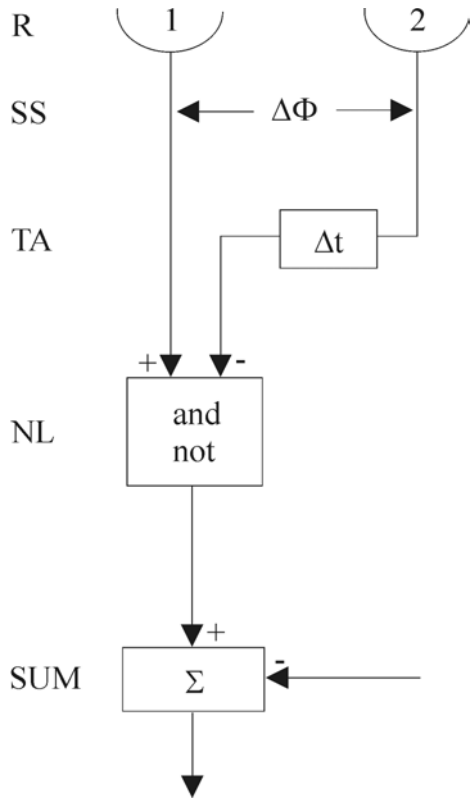

Fig. 1. Hypothetical acoustic motion detector, modified after Wagner & Takahashi (1992). R, receptor inputs 1 and 2; SS, spatial separation ΔΦ; TA, temporal asymmetry; Δt, temporal delay; NL, nonlinearity; and not, interaction as in a logical 'and not' gate; SUM, summation. See text for further details.

independent inhibition is implemented acting as a mechanism to enhance motion-direction sensitivity of neurons. In brief, the detector shown in Fig. 1 works as follows: A stimulus moving from the left to the right evokes an excitatory response from receptor 1 which arrives earlier at the nonlinear interaction stage as the inhibitory response from receptor 2, and can thus pass this stage. If a stimulus moves in the opposite direction, the inhibitory response from receptor 2 is delayed and the response from receptor 1 is blocked at the nonlinear interaction stage if receptor 1 is stimulated with the same delay as implemented in the detector. Kautz & Wagner (1998) could proof the existence of a direction independent inhibition in the IC of barn owls using microiontophoretic application of the γ-aminobutyric acid-A ($GABA_A$) receptor antagonist bicuculline methiodide (BMI). However, a GABAergic directional dependent inhibition could not be shown. Thus, a complete understanding of the cellular mechanisms underlying motion-direction sensitivity in barn owls is still not achieved. However, the notion that inhibition is involved in creating motion-direction sensitivity is further support by other studies. Sanes et al. (1998) proposed that the responses of neurons in the IC of gerbils to dynamically varying interaural level differences are controlled by synaptic inhibition. Furthermore, the motion-direction-sensitive response of neurons in the primary auditory cortex of cats was determined by inhibition (Altman & Nikitin, 1985). In contrast, using dynamic interaural phase cues and free field auditory apparent motion McAlpine et al. (2000) and Ingham et al. (2001) found no special inhibitory mechanism for the processing of auditory motion in the IC of guinea pigs. They concluded, that shifts of spatial RF position were due to adaptation of excitation, defined as the 'reduced capacity of a neuron to respond to subsequent excitatory stimuli following presentation of a stimulus that is itself excitatory' (Ingham et al., 2001, p. 24). Thus, no special auditory motion detection mechanism would exist.

2.3 Hearing in horseshoe bats

The insectivorous rufous horseshoe bat (*Rhinolophus rouxi*, Temminck 1835, old world family Rhinolophidae) belongs to the group of the so-called CF-FM-bats which emit echolocation calls consisting of a long constant frequency (CF) component preceded and followed by a short frequency modulated (FM) part (Neuweiler et al., 1987; Fig. 2). The duration of the CF component is typically 40-50 ms (range: 10-100 ms). The echolocation calls consist of a strong second harmonic (which carries the main energy of the call) of 73.5 to 79 kHz and a 10-30 dB fainter first harmonic (Neuweiler et al., 1987). Average repetition rates are about 10-12 sounds per second but can be significantly increased (with simultaneous decrease of sound duration) in so-called final buzzes when pursuing a flying insect (Schnitzler et al., 1985).

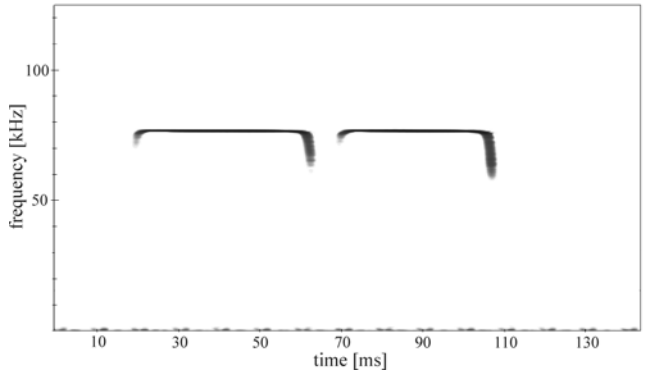

Fig. 2. Spectrogram of two echolocation calls of *R. rouxi*. The calls consist of a short upward frequency modulated (FM) part followed by a constant frequency (CF) part, and end with a downward FM part. Only the second harmonic is shown. Abscissa, time in milliseconds; ordinate, frequency in kilohertz.

Each bat has an individual resting frequency i.e. the frequency of the CF part is held constant in a range of ± 100 Hz during call emission. The long CF component serves as a carrier for frequency and amplitude modulations superimposed on the echo when it is reflected from the beating wings of flying insects. In the auditory system of horseshoe bats neurons narrowly tuned to a small frequency band up to 300 Hz higher than the resting frequency (the reference frequency) are largely overrepresented (Schuller & Pollak, 1979). In addition, in the cochlea a narrow frequency band around the frequency of the CF component is represented in expanded fashion on the basiliar membrane (Bruns, 1979). This acoustic fovea together with the sharply tuned neurons provide an effective filter resulting in low thresholds for a small range of frequencies around the CF. Many of the 'filter neurons' encode precisely the wingbeat pattern of insects (Schuller, 1984) and may thus provide a neural mechanism to detect flying prey. However, when the bat is flying, the frequency of the echoes is shifted to higher frequencies outside the acoustic fovea due to Doppler effects. Horseshoe bats overcome this problem by lowering the emitted frequency of the CF component by about the same amount as the

frequency is raised, thus holding the frequency of the echoes constant in the range of the reference frequency. This Doppler-shift compensation behavior has been studied in detail in the last decades (e.g. Schnitzler, 1968; Schuller et al., 1974; Schuller, 1977; Behrend et al., 1999).

Other aspects of auditory physiology in bats have been less intensively studied so far. Thus, only few authors have tackled the question how a moving sound is represented in the auditory system of bats. For horseshoe bats, Schlegel (1980) and Kleiser & Schuller (1995) found that neurons in the IC responded with shifts of the spatial RF location due to opposite directions of simulated acoustic motion. The same was found for IC neurons in the mustached bat (*Pteronotus parnellii*) by Wilson & O'Neill (1998). Whether the mechanism underlying these shifts is adaptation of excitation as stated by Ingham et al. (2001) for the guinea pig, or if more complex mechanisms are involved is unclear. Furthermore, how the motion information is processed at higher levels of the auditory pathway, e.g. the auditory cortex of bats is unknown. The auditory cortex of *R. rouxi* consists of at least five different fields defined by physiological and cytoarcitectural features and thalamocortical connections (Radtke-Schuller, 1997). Several specializations of the different fields have been shown in the past. For example, the dorso-dorsal field (DDF) contains neurons preferentially activated by combinations of time delayed, linearly frequency-modulated stimuli, so-called FM/FM neurons (Schuller et al., 1991). These neurons might serve as target-range encoding neurons. A special feature of the anterior-dorsal field (ADF) was the concentration of neurons responding to the bats own vocalization, whereas in the posterior dorsal field (PDF) many neurons were extremely narrow tuned to frequencies of the FM portion of echolocation calls (Radtke-Schuller & Schuller, 1995) which could be useful for exact temporal encoding of start and/or end of calls and echoes. As studies on other animals mainly investigated the coding properties for moving sounds of neurons in the primary auditory cortex (AI), the detailed knowledge about the properties of different fields additionally to the primary field in the rufous horseshoe bat provides a good basis to investigate if motion processing is uniformly in the whole auditory cortex or if specializations exist.

2.4 GABAergic inhibition in the inferior colliculus and the auditory cortex

The encoding of the position of a static sound source in azimuth is influenced by GABAergic inhibition in neurons in the IC of the mustached bat (Park & Pollak 1993, 1994). The authors showed that BMI changed the IID sensitivity and thus the shape of azimuthal RFs. The

binaural response properties of single units in the IC of the rufous horseshoe bat were also influenced by GABAergic and glycinergic inhibition (Vater et al., 1992a). As already mentioned, the influence of inhibition on motion-direction sensitivity has been shown for different species (Altman & Nikitin, 1985; Kautz & Wagner, 1998, Sanes et al., 1998). In addition, cells responding to moving sounds were almost always responding to stationary sounds, too. Thus, it was reasonable to test the influence of inhibition on the response properties of neurons to acoustic motion in the auditory cortex of the rufous horeseshoe bat, too. No immunoreactivity for glycine or glycine receptors has been found in the auditory cortex of different mammalian species (Aoki et al., 1988; Winer et al., 1995; Friauf et al., 1997). In contrast, immunoreactivity for GABA and GABA receptors was abundant in the auditory cortex of different bat species, including the rufous horseshoe bat (Vater et al., 1992b; Winer et al., 1995; Fubara et al., 1996). Consequently, GABA and the $GABA_A$ antagonist BMI were used to test the role of inhibition in motion processing in cortical neurons in the rufous horseshoe bat.

2.5 Aim of the present study

The present study was designed to investigate the processing of acoustic motion in the auditory cortex of the rufous horseshoe bat. In the first part, the cortical representation of acoustic motion in different fields of auditory cortex was investigated. This was done in order to compare the motion processing properties of the different fields as well as to investigate the differences in motion processing that might occur between the auditory midbrain (i.e. the IC) and the auditory cortex as the highest level of the auditory pathway. In a second set of experiments, the mechanisms underlying the responses to apparent motion stimuli were investigated using microiontophoretic application of GABA and the $GABA_A$ antagonist BMI while recording from neurons in the auditory cortex.

3 Methods

3.1 Experimental animals

Experimental animals were 9 rufous horseshoe bats (*Rhinolophus rouxi*, Temminck 1835) from Sri Lanka. The bats were kept in captivity under seminatural conditions for no longer than one year. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (approval 211-2531-37/98, Reg. Oberbayern).

3.2 Surgery and stereotaxic procedure

The bats were surgically prepared under Halothan (Hoechst, Frankfurt am Main, Germany) anesthesia. The skin overlying the skull was cut along the midline and the skull surface was freed from tissue. A metal tube was fixed to the skull using a light curing microglass composite (Heraeus Kulzer GmbH, Wehrheim, Germany) in order to fixate the animal in the stereotaxic device. After the animals were allowed to recover postsurgically for one day, the accurate skull position in stereotaxic coordinates was determined as described in detail elsewhere (Schuller et al., 1986). In brief, parasagittal and frontal profile lines of the skull surface were measured. The measured profile lines were compared with a standard skull profile and the coordinates of the stereotaxic device could be directly related to a standard brain atlas of *R. rouxi* (Radtke-Schuller, unpublished data).

Before an experiment started, wound margins were treated with the surface active local anesthetic Legecain® (lege artis Pharma GmbH & Co KG, Dettenhausen, Germany). Finally, small holes were drilled in the skull in order to get access to the brain at the desired positions. Daily recording sessions typically lasted for 3 to 4 hours and could be repeated up to 6 weeks. After experiments, an electrolytic lesion was made in the brain to verify recording sites from subsequent histological processing. The position of recording sites was reconstructed and transformed into coordinates of the brain atlas of *R. rouxi* (Radtke-Schuller, unpublished data). The method for the reconstruction-procedure is described in details elsewhere (Schuller et al., 1991).

3.3 Stimulus production and recording

All experiments were conducted in an anechoic chamber. Acoustic stimuli were computer generated, filtered, attenuated (Tucker Davis Technology, Gainesville, FL, USA; software by D. Molter) and presented via earphones (Schuller, 1997), thus eliminating the influence of pinna movements. The acoustic crosstalk attenuation of the closed-field dichotic stimulation apparatus was approximately 60 dB. A schematic diagram of the experimental setup is shown in Fig. 3. Either pure tones or sinusoidally or linearly downward frequency-modulated (SFM, FM) tones were used depending on which stimulus type could best drive the neuron. Duration of pure tones and SFM tones was 20 ms and 5 ms for linear FM-sweeps. They were presented

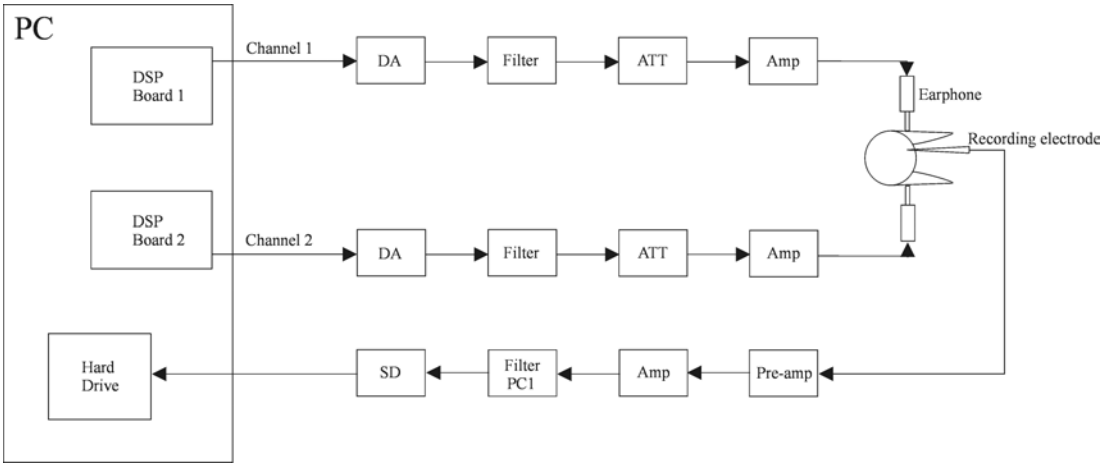

Fig. 3. Simplified schematic drawing of the experimental setup. The stimuli were generated on digital signal processing (DSP) boards and presentd via earphones. Signals from the recording electrode were stored on the hard drive. Amp, amplifier; ATT, attenuator; DA, digital/analog converter; PC1, preconditioner; SD, spike discriminator.

with 1 ms rise/fall time at 20-40 dB above threshold at the neuron's best frequency. The absolute sound pressure level at the midline was in a range of 50-70 dB SPL (re 20 μPa). These stimuli mimicked single components of the echolocation calls of *R. rouxi*. Motion of a sound source in azimuth in either direction was simulated by dynamically changing IIDs, covering a range between ± 40 dB. This represents the maximum IID occurring in this bat species, corresponding to a maximum lateral azimuthal position of approximately ± 30° under free-field conditions (Fig. 8f in Obrist et al., 1993). The IID amplitude changes were presented in successive trains of pulses to both ears (Fig. 4), mimicking the acoustical image an echolocating bat would receive from a target moving in azimuth. Using IID steps (ΔIID) of 5 and 10 dB between successive stimuli resulted in 17 and 9 virtual sound source locations in azimuth, respectively.

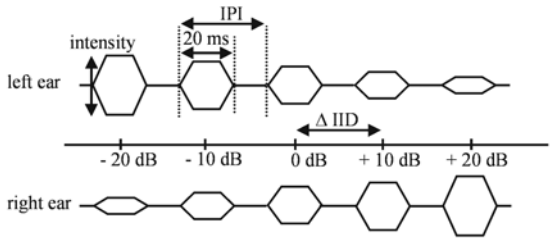

Fig. 4. Schematic drawing of the stimulus paradigm used for generation of auditory apparent motion. Successive stimuli with dynamically changing interaural intensity differences (IID) simulated apparent motion in the azimuth in either direction. Angular velocity could be manipulated by changing either interpulse interval (IPI) or IID step (ΔIID) between successive stimuli.

Angular velocity (expressed as ΔIID/t) could be manipulated by changing either interpulse interval (IPI) or IID steps. The standard combinations of parameters applied are shown in Table 1. A neuron's response to different parameter configurations and directions of motion was recorded over 30 presentations of sequential virtual locations. The direction of motion was the same in the 30 successive trails separated by a silent interval of 250 ms in order to minimize the possibility of adaptation. For the first set of experiments, the static spatial RFs of the neurons were measured by presenting pulse trains with randomized IIDs at low repetition rate (4-10 Hz). In addition, the monaural response characteristics of either ear were obtained for a subset of neurons by presenting the corresponding intensities monaurally.

Table 1. Stimulus parameters used to generate auditory apparent motion

V [dB/s]	ΔIID = 5dB		ΔIID = 10dB	
	IPI (ms)	RR (Hz)	IPI (ms)	RR (Hz)
50	100	10	200	5
100	50	20	100	10
200	25	40	50	20
400	---	---	25	40

IPI, interpluse interval; IID, interaural intensity difference; RR, repetition rate; V, velocity.

Recording the response to a static stimulus presented with a single IID at termination of data acquisition in a neuron allowed to control the stability of the neural response.

The responses of cortical neurons to acoustic stimuli were recorded extracellularly in awake animals with glass microelectrodes filled with 2 M potassium acetate and 2 % fast green or 2 M NaCl and 4 % pontamine sky blue for iontophoresis experiments. Electrode impedance was in a range of 4-10 MΩ. Neural activity was monitored audio-visually to search for neurons. Action potentials were amplified and filtered with conventional methods. Spikes were recorded relative to the onset of the acoustic stimuli and were displayed and stored as dot raster (software by D. Molter). Recordings were made from single units as well as small multiunit clusters. Multiunit clusters could be further discriminated using a window discriminator (SD1, Tucker Davis Technology, Gainesville, FL, USA).

3.4 Iontophoresis

For application of drugs while recording extracellularly from neurons, the glass microelectrodes were glued to a five-barreled glass micropipette (World Precision Instruments, Berlin, Germany). The collective tip diameter of the multibarreled electrode was 8-12 µm, the tip of the recording electrode protruded between 8-15 µm. Concentration and pH of drugs in the barrels were adjusted to 5 mM, pH 3 for BMI and 0.5 M, pH 3.5-4 for GABA (all drugs by Sigma-Aldrich, Deisenhofen, Germany). One barrel was filled with 2 M NaCl and served as balancing channel in order to eliminate current effects. To prevent the formation of salt bridges between the barrels, the upper end of the barrels were covered with vaseline. The drugs were applied by positive ejection currents ranging from 5-150 nA, mainly 15-50 nA. Negative retention currents applied to avoid uncontrolled leakage from the drug barrels were in the range of 10-15 nA. Retention and ejection currents were generated and monitored by a Neurophore BH-2 system (Medical Systems Corp., Greenvale, NY, USA). The system also allowed to monitor drug barrel resistance throughout experiments.

A neuron's response to different directions of auditory apparent motion was recorded over 30 presentations of sequential virtual locations in the predrug condition and during the application of BMI or GABA. As in the first set of experiments, the direction of motion was the same in the 30 successive trails separated by a silent interval of 250 ms in order to minimize the possibility of adaptation. To check for a possible influence of different time courses of drug application due to the order of stimulus presentation, the order in which the two directions of motion were presented was reversed between successive recordings in some neurons.

Because neurons were difficult to hold for a long time, neurons were only roughly scanned with 2 or 3 of the parameter sets listed in Table 1 to test for motion-direction sensitivity. Throughout the application of drugs only that set of parameters was used to generate auditory apparent motion, that evoked the strongest motion-direction-sensitive response. If the recording situation was stable enough occasionally a second set of parameters was tested.

3.5 Data analysis

For quantitative analysis, spikes obtained from corresponding IID positions in the 30 pulse sequences were added up in a time window corresponding to response duration and latency. Response latency and duration were determined from peristimulus time (PST) histograms

(1 ms bin-width), and were defined as the time interval from stimulus onset to the first bin which exceeded the level of spontaneous activity and the time interval from the first bin to the last bin which exceeded the level of spontaneous activity, respectively. Neurons with maximum spike count less than 10 spikes were excluded from the data analysis. In order to compare the responses of a neuron obtained with different parameters of motion, the spike count was normalized to the maximum spike count at a single IID obtained from a neuron throughout all recordings, for the first set of experiments. For the microiontophoresis experiments no normalization was done but the total number of spikes evoked from a particular IID position was plotted against IID for motion in both directions.

Neurons often responded with a strong onset response to the first stimulus of a pulse train (e.g. Fig. 10a and b), which was not included in further data analysis.

Two types of motion-direction-sensitive effects were observed in this study: Directional preferences and shifts of the RF locations. These effects were quantified as follows: Neurons showed a directional preference, if the maximum spike number from a single IID obtained by motion in one direction (the preferred direction) was stronger than the peak value from any IID obtained by motion in the other direction (non-preferred or null direction). A directionality index [$DI = 1 - (R_{null}/R_{pref})$] was used to quantify directional preference. R_{pref} and R_{null} denote the maximum response (maximum spike number) obtained from any IID (onset responses excluded) by motion in the preferred direction and the null direction, respectively. A neuron was considered to display directional preference if the ratio of spike numbers for the preferred to the non-preferred direction was equal or greater than two, which corresponds to a $DI \geq 0.5$.

If neurons showed shifts of the RF location as a response to different directions of motion, the IID values of the 50 % cut-off of the RF slopes were compared. A shift of the medial RF borders measured at the 50 % cut-off IIDs equal or greater than 5 dB IID was classified as motion-direction-sensitive. Medial RF borders were defined as the border closer to 0 dB IID i. e. the midfield of azimuth, irrespective to the direction of motion. Medial RF border slopes only were analyzed because many neurons had ‘open’ RFs, which lacked a lateral border.

For further analysis and classification the maximum DI or maximum RF shift obtained from a neuron was used.

The drug effects in the microiontophoresis experiments were quantified as follows. BMI was considered to have an effect on neurons if the overall response to acoustic stimulation was increased of at least 30 % relative to the predrug level either for contralaterally or ipsilaterally directed motion. Direct quantification of GABA was more difficult, as in some cases GABA

could have an effect on motion-direction sensitivity although the overall spike count of the neuron was only little decreased. In general, predrug spike count levels could be reached after cessation of drug application. However, in some cases predrug level was not reached completely and cells were only included in the analysis when the spike count in the recovery situation dropped at least 30 % of maximum spike count during BMI application. Influence of BMI or GABA on the directional preference of a neuron was assumed, if the DI increased or decreased by more than 0.2. This DI-change threshold was chosen as the fluctuations of the DI in neurons during application of BMI were in this range. The RF border shift was considered to be increased or decreased by BMI or GABA, if the difference of the 50 % cut-off IIDs of RF border slopes changed by more than ± 50 % relative to the predrug value.

3.6 Statistical analysis

Quantitative data are presented as mean with the indication of standard deviation, unless otherwise indicated, and analyzed using a Kruskal-Wallis-Test. Categorical frequency data were analyzed using a Chi-square-Test or a Fisher-Exact-Probability-Test (two-tailed). For analysis of paired data a Wilcoxon signed rank test was used. For all tests, significance was set at $p < 0.05$.

4 Representation of acoustic motion in the auditory cortex of the rufous horseshoe bat

4.1 Results

Recordings were derived from a total of 152 neurons (Fig. 5). 38 neurons were located in the primary field (PF), 47 in the posterior-dorsal field (PDF) and 67 in the anterior-dorsal field (ADF). For details on the different fields of the auditory cortex of the rufous horseshoe bat see Schuller et al. (1991) and Radtke-Schuller & Schuller (1995). All neurons investigated in this study responded well to stationary stimuli.

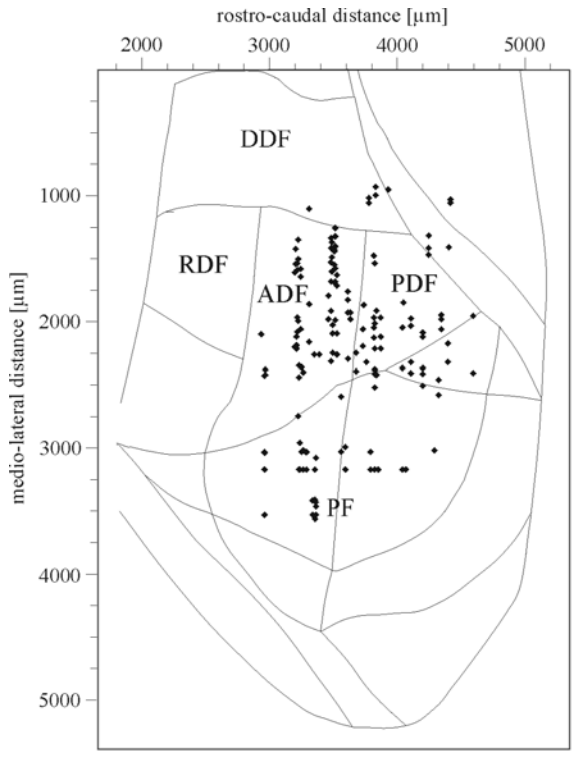

Fig. 5. Localization of recording sites in the auditory cortex of *Rhinolophus rouxi*. The position of neurons is represented in medio-lateral and rostro-caudal coordinates on a flattened surface projection of the auditory cortex. ADF, anterior-dorsal field; DDF, dorso-dorsal field; PDF, posterior-dorsal field; PF, primary field; RDF, rostro-dorsal field.

Seventy percent (106/152) of the neurons were classified to be contralateral since the static RFs had a maximum at negative IIDs (i.e. in the contralateral field) with a decrease of more than 50 % of maximum response over a range of positive or smaller negative IIDs. One fifth of the neurons (31/152) were classified to have multipeaked static RFs because the static RFs showed two or more separated response peaks between which the response decreased to more than 50 % of maximum response. Static RFs classified as ipsilateral were found in 4 % (6/152) of the neurons, since the RFs had a maximum responses at positive IIDs (i.e. in the ipsilateral field) with a decrease of more than 50 % of maximum response over a range of negative or smaller positive IIDs. Static RFs were classified to be omnidirectional if the response never decreased below 50 % of maximum over the whole range of IIDs. This kind of static RF occurred in 4 % (6/152) of the neurons. Two percent (3/152) of the neurons were classified to have midfield receptive fields since the RFs had maximum responses in the range

of 0 ± 10 dB IID with a decrease of more than 50 % in response of both sides of the maximum. The classification criteria are similar to those of Irvine et al. (1996) and Rajan et al. (1990a).

The binaural characteristics of 76 neurons were classified. Sixteen percent (12/76) of neurons were excited by monaural stimulation of one ear and showed no response to stimulation of the other ear. As the binaural response did not differ markedly from that to monaural stimulation of the excitatory ear, this type of neuron will be referred to as monaural (EO/mon). Seventy-five percent (57/76) of neurons responded to monaural stimulation of only one ear, and binaural stimulation revealed inhibitory or facilitatory influences of the other ear. These neurons were classified as EI and EO/F neurons, respectively or as EO/F&I neurons if a combination of inhibition and facilitation occurred. Finally, 9 % (7/76) of neurons could be excited by monaural stimulation of either ear and were classified as EE neurons.

4.1.1 General properties of cortical neurons

Seventy-eight percent (118/152) of neurons responded best to pure tones, 18 % (28/152) to SFM stimuli and 4 % (6/152) responded best to downward FM sweeps. Neurons responding best to downward FM sweeps were mainly located in the PDF. Neurons responding to SFM or PT stimuli were found in all three cortical fields. Responses of neurons to simulated motion showed no stimulus dependent differences.

Twenty-one percent (32/152) of the sampled neurons were either unresponsive to apparent motion or showed no differences in their responses to motion in either direction. Eight percent (12/152) of the neurons could not be classified because of an inconsistent response pattern.

Seventy-one percent (108/152) of neurons responded differentially to the direction of apparent motion and were classified as motion-direction-sensitive. Two different types of motion-direction-sensitive responses could be distinguished.

About one third (34 %, 52/152) of neurons exhibited stronger responses to motion in one direction (the preferred direction) than in the other (non-preferred or null direction) and had a directional preference with DI ≥ 0.5. Fig. 6 shows a dot-display of the response of a neuron with directional preference. Ipsilaterally directed motion (Fig. 6a) evoked only low spike activity from any IID, whereas contralaterally directed motion (Fig. 6b) evoked strong activity, especially from –30 dB IID. It is noteworthy, that the spike count at this position is very stable over all 30 passes. Fig. 7 gives four examples of neurons showing a directional preference. A common feature of all neurons with a directional preference was, that the

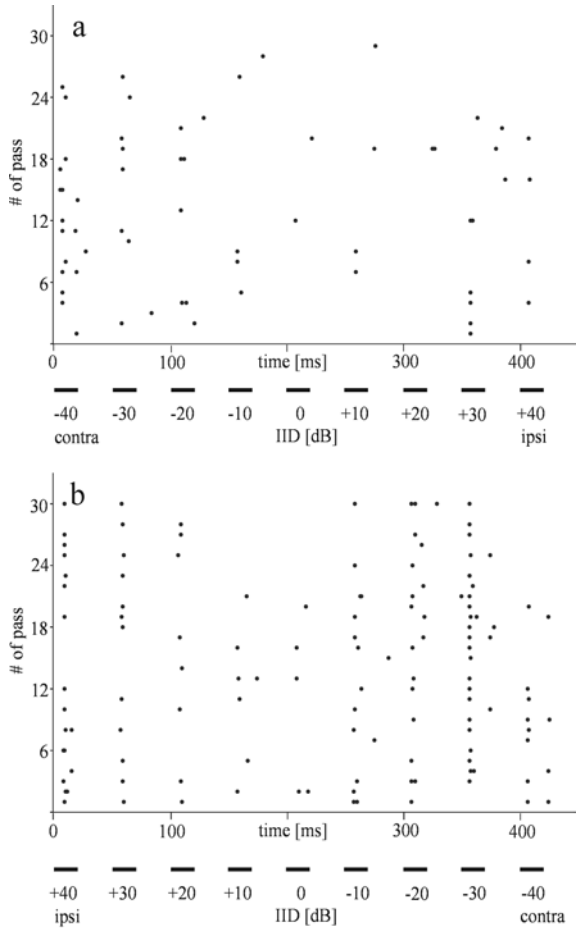

Fig. 6. Dot-display showing the raw data for the motion-direction-sensitive response of the neuron shown in Fig. 7, bottom left. The temporal occurence of spikes as a response to ipsilaterally directed motion is shown in 6a, the response to contralaterally directed motion is shown in 6b. Ordinate, number of pass; abscissa, double representation of time [ms] and the actual IID [dB]. Black bars represent the 20 ms stimuli. Note, that the sequence of IIDs is reversed in (a) and (b) because of the different motion directions.

response to the preferred direction rarely exceeded the maximum response to stationary stimuli. The response to the non-preferred direction, however, was always markedly reduced. Maximum DI's obtained from all neurons with a directional preference (DI ≥ 0.5) were in the range from 0.5 to 1.0, with a median of 0.6 and a mean of 0.63 ± 0.12 (n = 52). As can be seen from Fig. 7, the preferred direction of the neurons was contralaterally directed motion. This holds true for the majority of neurons with directional preference: In 89 % (33/37) of direction preferring neurons with contralateral or ipsilateral RFs, motion entering the RF from the opposite direction was the preferred direction. In 73 % (11/15) of direction preferring neurons with omnidirectional, midfield or multipeaked RFs contralaterally directed motion was the preferred direction. Within the class of neurons with a directional preference, the response profiles in the preferred direction reflected properties of the static RF in 75 % (39/52) of the neurons. Response peaks at approximately the same IID positions under static and dynamic conditions could be found in 77 % (30/39) of these neurons. In the remaining 23 % (9/39) of neurons, the spike count in the preferred direction of motion was elevated over a broad range of IIDs, showing a similar response as for the static RF. In one fourth (13/52) of neurons with a directional preference, response profiles for motion in the preferred direction and under static conditions could not clearly be related.

A shift of the RF location (shift ≥ 5 dB IID) due to reversal of the direction of apparent motion was found in 57 % (87/152) of neurons. Fig. 8 shows examples of neurons with RF

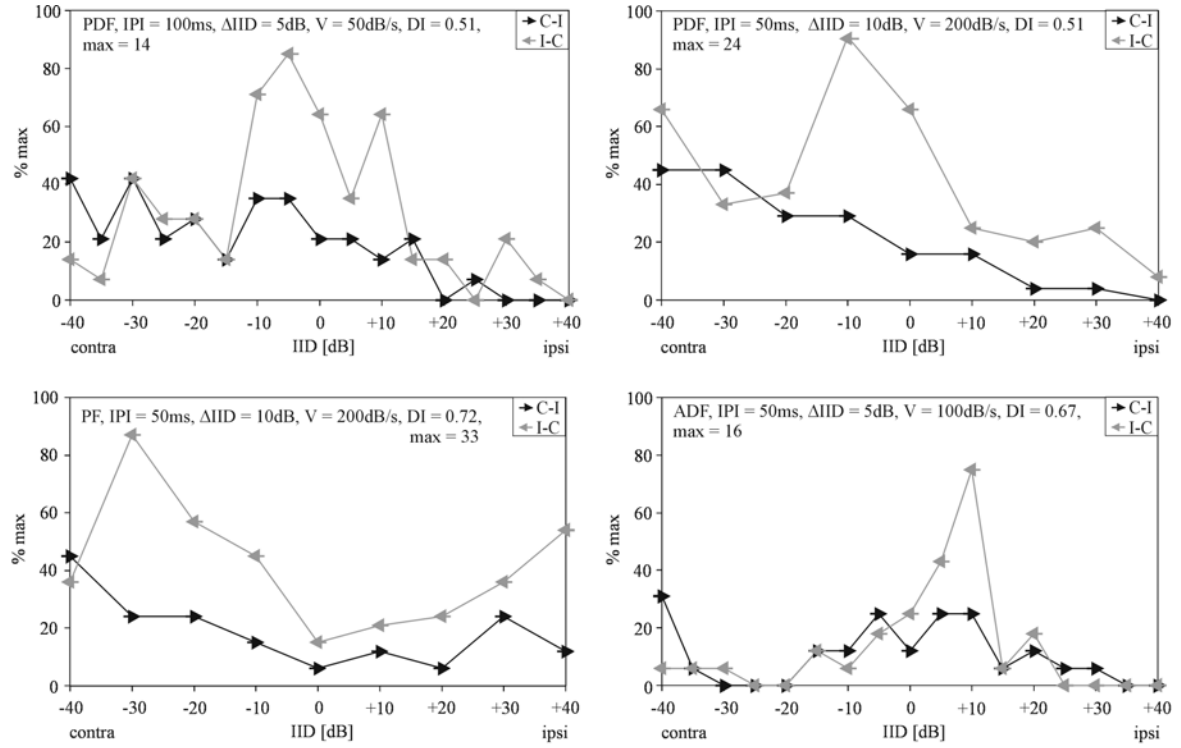

Fig. 7. Four neurons showing a directional preference. Neurons responded stronger to contralaterally than to ipsilaterally directed motion. Abscissa, IID in dB SPL. Negative values refer to the contralateral part of azimuth. Ordinate, spike-count obtained from the single IIDs over 30 stimuli presentations normalized to 100 %. Note that 100 % may not be reached because in other recordings obtained from the same neurons with other parameters of motion stronger responses might have been evoked from particular azimuthal positions. C-I, ipsilaterally directed motion; I-C, contralaterally directed motion. Velocity (V) expressed as IID [dB] per second. ΔIID, stepwidth; DI, directionality index; IPI, interpulse interval; max, maximum spike-count (100 %); ADF, anterior-dorsal field; PDF, posterior-dorsal field; PF, primary field.

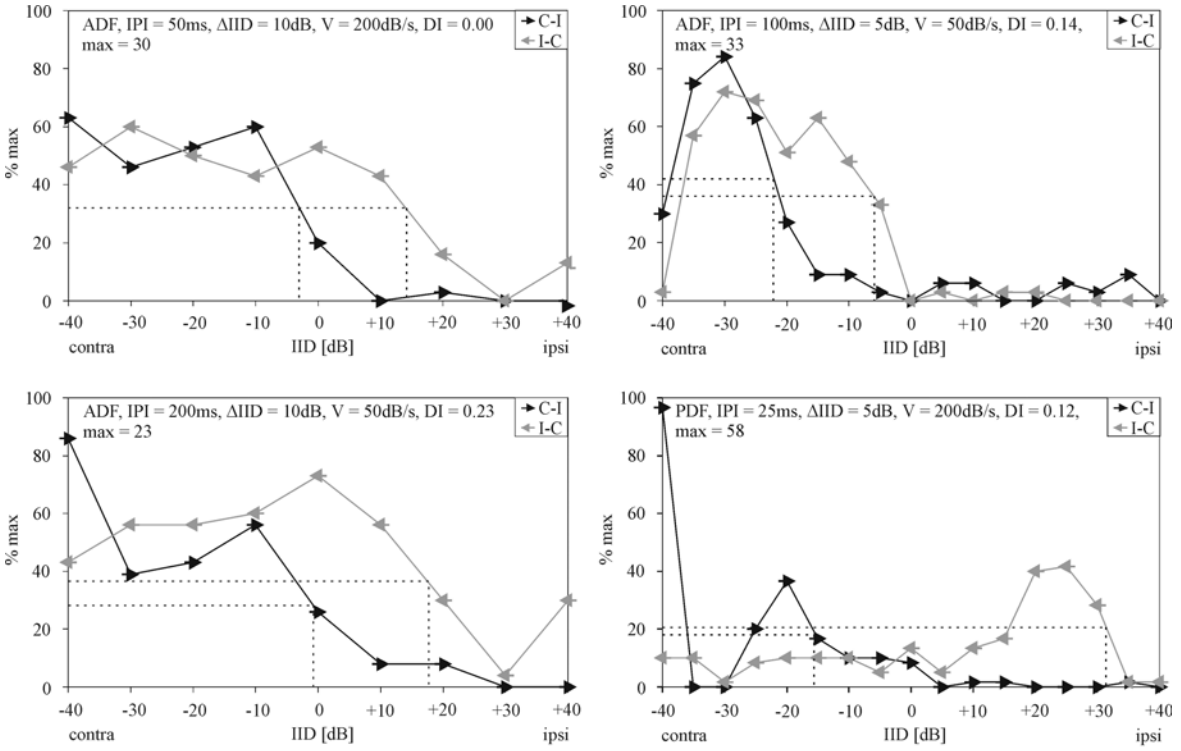

Fig. 8. Examples of four neurons with contralateral receptive fields (RF) showing a shift of RF borders. Shifts were measured using the displacement of the 50 % cut-off of the medial RF border, as indicated by dotted lines. Shifts were opposite the direction of motion. Symbols as in Fig. 7.

border shifts. The direction of shifts was not random: In 91 % (79/87) of the neurons with a RF border shift, the shift was opposite to the direction of motion (Fig. 8). Thus, the border of the RF of a neuron evoked by contralaterally directed motion was shifted to a more ipsilateral location than the border of the RF evoked by motion in the opposite direction. In most cases (91 %, 79/87), the slope of static RF borders was either in between or coincided with the borders obtained by motion in either direction. In the remaining neurons (9 %, 8/87), the position of the static RF slope was outside the range of those obtained by motion in both directions.

Maximum RF shifts were in the range of 5.2 to 50.91 dB IID, with a median of 15.07 and a mean of 16.78 ± 8.41 dB IID (n = 87). To estimate how RF shifts correspond to shifts in azimuthal position under free field conditions, the transfer function of IID to azimuthal angle of was calculated using the data presented by Obrist et al., (1993) for *R. rouxi*. Fig. 9 shows this transfer function for 75 kHz, i.e. the approximate frequency of the constant frequency part of the echolocation calls of *R. rouxi* (Neuweiler et al., 1987). Most neurons recorded in this study had best frequencies in this range. As can be seen from Fig. 9, the function is not strictly linear. The slope over the full IID range is 0.6 °/dB. However, the slope of the function is smaller in the IID range of ± 30 dB, with a value of 0.4 °/dB. As most RFs obtained by acoustic motion had their 50 % cut-off in the range of ± 30 dB, a factor of 0.4 was used to convert IID values into azimuthal degrees. Thus, maximum RF shifts were in the range of 2.08° to 20.36° azimuth with a median of 6.03° and a mean of 6.71° ± 3.36° azimuth (n = 87).

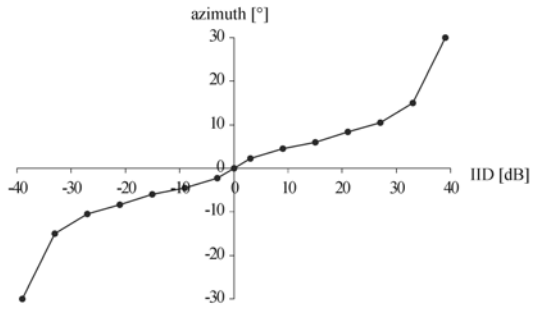

Fig. 9. Transfer function of IID to azimuthal angle in *R. rouxi* obtained for 75 kHz. Replotted after data from Obrist et al., 1993, their fig. 8f. Ordinate, interaural intensity difference (IID) in dB; abscissa, azimuthal degrees.

A directional preference or a RF shift could occur together in the same neuron. It depended strongly on the parameters of apparent motion which of the two effects was observed. For example, a neuron could respond with a RF shift to motion with long IPI, whereas it showed a directional preference to motion with shorter IPI (Fig. 10). In general, 29 % (31/108) of motion sensitive neurons showed a combination of directional preference and RF shifts depending on the parameters of motion i.e. the combination of IID step and IPI. The specific

combination of IPI with IID step determined in virtually all cases the response of a neuron rather than velocity. This is demonstrated for a particular case in Fig. 11. Apparent motion with an IPI of 25 ms and 5 dB IID step evoked a shift of RFs in the neuron shown in Fig. 11a,

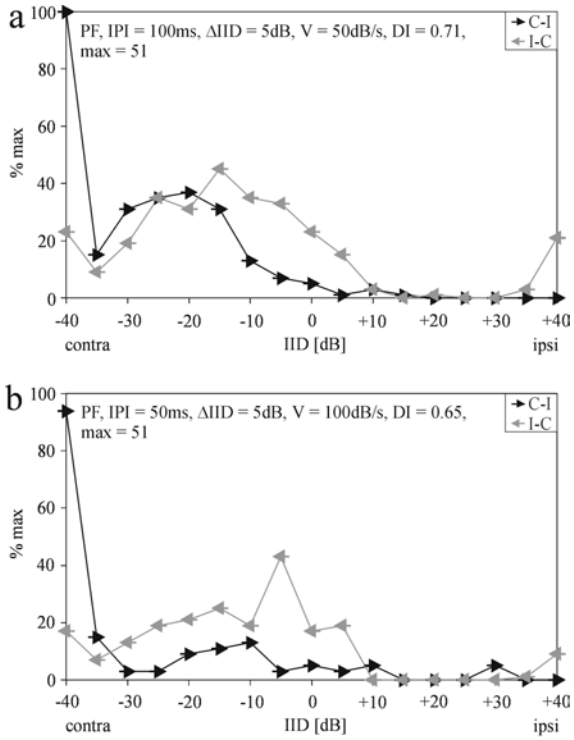

Fig. 10. A neuron showing a shift (a) or directional preference (b), depending on the parameters of apparent motion. With long IPI (100 ms), the neuron responds with a receptive field shift to motion in different directions (a), whereas with shorter IPI (50 ms) it shows a directional preference to contralaterally directed motion (b). Symbols as in Fig. 7.

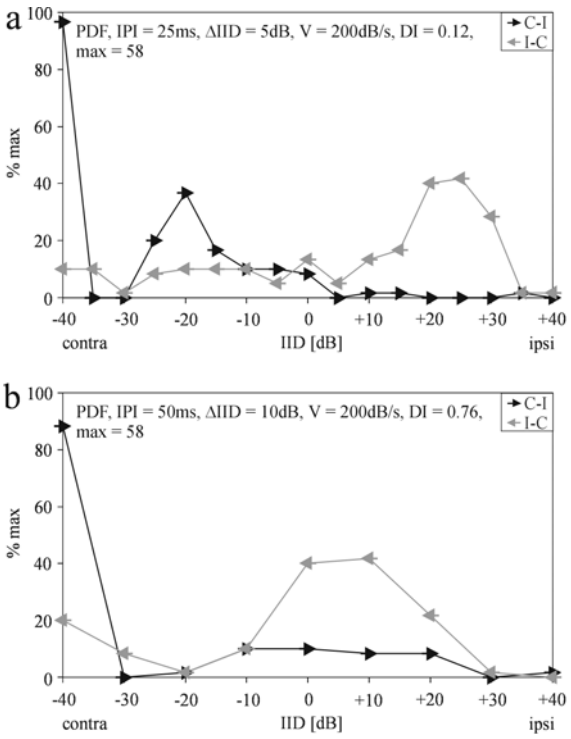

Fig. 11. Motion velocity was not the specifically encoded parameter by cortical neurons. As shown for the neuron in this figure, motion with same velocity (200 dB/s) but different spatio-temporal parameter evoked different responses. (a) ΔIID = 5 dB, IPI = 25 ms, V = 200 dB/s. (b) ΔIID = 10 dB, IPI = 50 ms, V = 200 dB/s. Symbols as in Fig. 7.

whereas with an IPI of 50 ms and 10 dB IID step only contralaterally directed motion evoked a response peak (Fig. 11b). The velocity of azimuthal motion, however, was the same in both cases, namely 200 dB/s.

In general, 95 % (103/108) of all motion-direction-sensitive neurons exhibited their specific response to apparent motion of the acoustic stimulus only for a special set of motion parameters.

According to their binaural properties, neurons were attributed two broad classes: Neurons, where the inputs from both ears differed (EI; EO/F&I; EO/F) and neurons where the input was functionally equal from both ears (EE) or input was received from only one ear (EO/mon). Of the neurons with either EI, EO/F or EO/F&I binaural response types, 75 %

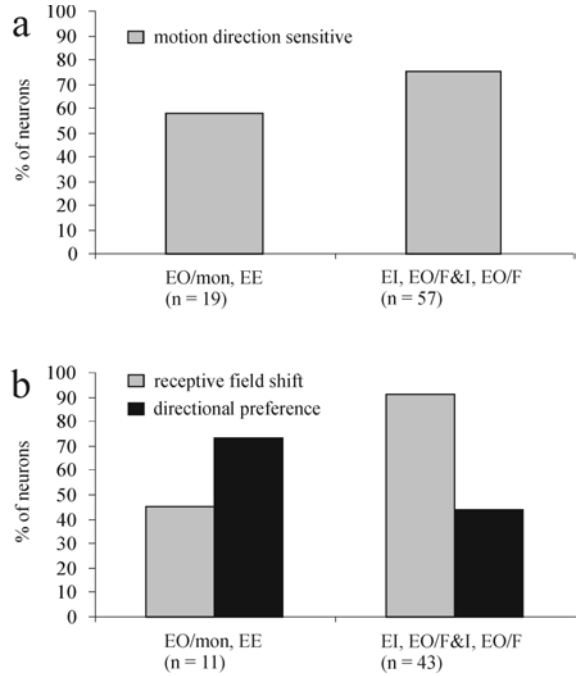

Fig. 12. Influence of binaural interactions on motion-direction sensitivity. (a) Comparison of motion-direction sensitivity in neurons of the two binaural interaction classes. (b) Percentage of motion-direction-sensitive neurons showing directional preference or receptive field (RF) shifts. Differences in the percentage of neurons showing a RF shift in the two classes were statistically significant (two-tailed Fisher-Test, $p < 0.01$). Note in (b), that a directional preference or a RF shift can occur together in the same neuron so that the percentages may not add to 100 %. EE, neurons excited by monaural stimulation of either ear; EO, neurons excited by monaural stimulation of one ear and no apparent response to stimulation of the other ear; E, excitation; F, facilitation; I, inhibition; mon, monaural.

(43/57) were sensitive to the direction of motion whereas only 58 % (11/19) of neurons with EO/mon or EE binaural interaction were motion-direction-sensitive (Fig. 12a). However, differences between the two classes of neurons were not statistically significant ($p > 0.05$, χ^2 Test). Differences, however, could be revealed when looking at the two types of motion-direction-sensitive responses. A shift of RF location was significantly more frequent ($p < 0.01$, two-tailed Fisher-Test) in motion-direction-sensitive neurons with EI, EO/F or EO/F&I binaural response types (91 %, 39/43) than in motion-direction-sensitive EO/mon and EE neurons (45 %, 5/11, Fig. 12b). In contrast, the frequency of occurrence of a directional preference ($DI \geq 0.5$) in motion-direction-sensitive neurons with EI, EO/F or EO/F&I binaural response types (44 %, 19/43) and EO/mon and EE neurons (73 %, 8/11, Fig. 12b) was not significantly different ($p > 0.05$, two-tailed Fisher-Test).

4.1.2 Comparison of motion-direction sensitivity in different cortical fields

The comparison of the total number of motion-direction-sensitive neurons revealed no clear differences between the three cortical fields investigated in this study (Fig. 13). In the PF 76 % (29/38) of neurons were motion-direction-sensitive, 72 % (48/67) in the ADF and 66 % (31/47) in the PDF. Thus, the highest number of motion-direction-sensitive neurons was found in the PF, the lowest in the PDF. However, differences were not statistically significant ($p > 0.05$, χ^2 Test). The total number of neurons with RF shifts (regardless of a directional preference) was 55 % (21/38) in the PF, 60 % (40/67) in the ADF and 55 % (26/47) in the

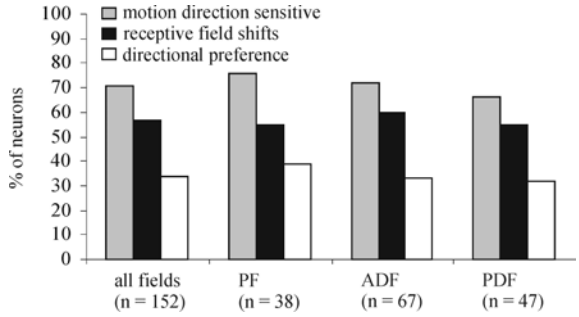

Fig. 13. Comparison of motion direction effects in the auditory cortex of *R. rouxi*. ADF, anterior-dorsal field; PDF, posterior-dorsal field; PF, primary field. Note, that a directional preference or a receptive field shift can occur together in the same neuron so that the percentages may not add to the total percentage of motion-direction-sensitive neurons.

PDF. A directional preference (regardless of a RF shift) was found in 39 % (15/38) of the neurons in the PF, 33 % (22/67) of neurons in the ADF and 32 % (15/47) of neurons in the PDF (Fig. 13). Again, differences between the different cortical fields were not statistically significant (χ^2 Test, $p > 0.05$ both for RF shifts directional preferences).

To check the possibility of a quantitative difference in the strength of motion-direction-sensitive responses of neurons, the magnitude of the DIs and the RF shifts were compared in the three cortical fields. Therefore, the maximum DI and the maximum RF shift of a neuron was measured, and the mean and median were compared in the cortical fields. Only neurons with a maximum DI ≥ 0.5 and a maximum RF shift of ≥ 5 dB IID were included in this analysis. The results are shown in Fig. 14. A Kruskal-Wallis-Test revealed no significant differences between the three cortical fields in respect to the magnitude of the mean of maximum DIs or maximum RF shifts [DI: ADF: 0.6 ± 0.1 (n = 22), PDF: 0.62 ± 0.12 (n = 15), PF: 0.67 ± 0.14 (n = 15), $p > 0.05$; shift: ADF: 17.35 ± 7.88 (n = 40), PDF: 15.67 ± 9.93 (n = 26), PF: 17.05 ± 7.58 (n = 21), $p > 0.05$].

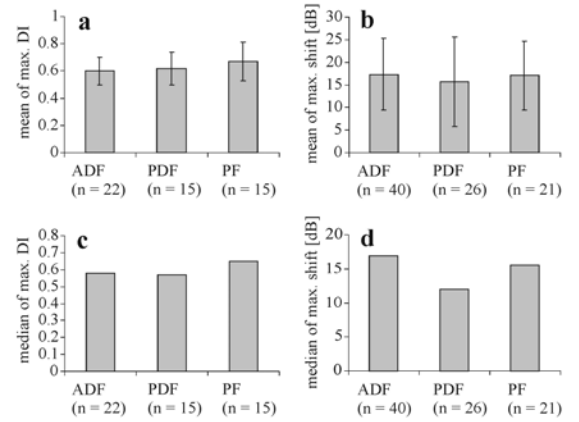

Fig. 14. Mean (a) and median (c) of the maximum DI (DI ≥ 0.5) and mean (b) and median (d) of the maximum receptive field (RF) shift (shift ≥ 5 dB IID) in the three cortical fields. The mean of the maximum DI or maximum RF shifts did not differ significantly in different cortical fields. See text for details. ADF, anterior-dorsal field; DI, directionality index; PDF, posterior-dorsal field; PF, primary field.

However, differences between cortical fields were revealed when analyzing the processing of temporal parameters of motion. For this purpose, the IPI was determined at which a neuron showed the greatest directional preference or largest RF shift. Only data from motion-direction-sensitive neurons tested with a complete set of motion parameters (as listed in Table 1) were included in this analysis (n = 82). Fig. 15a shows, that at the shortest IPI tested (25 ms), mainly neurons in the ADF and PDF reached their maximum DI. Furthermore, the largest

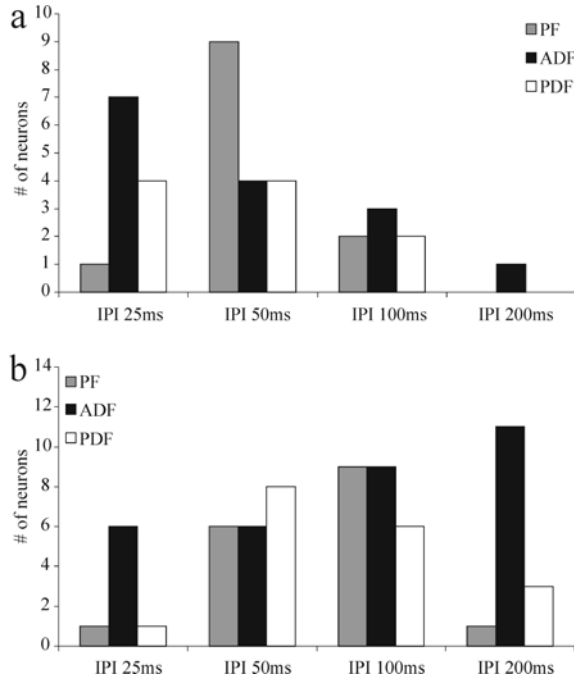

Fig. 15. Distribution of neurons with maximum DI (a) and maximum receptive field shift (b) in the three cortical fields at the single IPIs used to generate acoustic motion. See text for details. ADF, anterior-dorsal field; DI, directionality index; IPI, interpulse interval; PDF, posterior-dorsal field; PF, primary field.

proportion of direction preferring neurons in the ADF reached their maximum DI with the shortest IPI. Neurons with a directional preference in the ADF are thus preferring short IPIs. In contrast, neurons in the PF showed their maximum DI mainly at medium IPIs. The distribution of neurons with RF shifts is somewhat more complex (Fig. 15b). Again, only neurons in the ADF showed their maximum RF shifts with the shortest IPI but long IPIs evoked large RF shifts in ADF neurons, too. As with the directional preference, the largest proportion of neurons in the PF showed their maximum shift at medium IPIs, but the same can be seen for neurons in the PDF. To summarize, direction preferring neurons in the dorsal fields (with main emphasis on the ADF) encode auditory motion direction over the entire IPI range with a bias for short IPIs. Neurons in the PF show less directional preference for short IPIs, and are preferring medium IPIs. Motion direction can be encoded by neurons in all three cortical fields for medium IPIs. The distribution of motion direction preferring responses as a function of IPI shows only a trend. Statistical significance could not been demonstrated because of the small sample size. No clear trend regarding a preference of single IPIs could be seen for neurons in the different cortical fields showing a RF shift.

Differences in the properties of cortical fields can also be seen in the processing of azimuthal position of motion. As shown in Fig. 16, two different response types could be observed with neurons having a directional preference. The first type (Fig. 16a, c) included neurons that had their response maximum for motion at medial azimuthal positions, the second type responded best from lateral azimuthal positions (Fig. 16b, d). Neurons were classified in the first category when the maximum response was evoked at a position at least 10 dB IID away from the most contralateral or ipsilateral position and when the response strength on both sides of the maximum decreased to at least 50 % of the maximum response. Eighty-six percent (32/37) of the neurons with a directional preference (DI $\geq$ 0.5) in the dorsal fields (ADF and PDF)

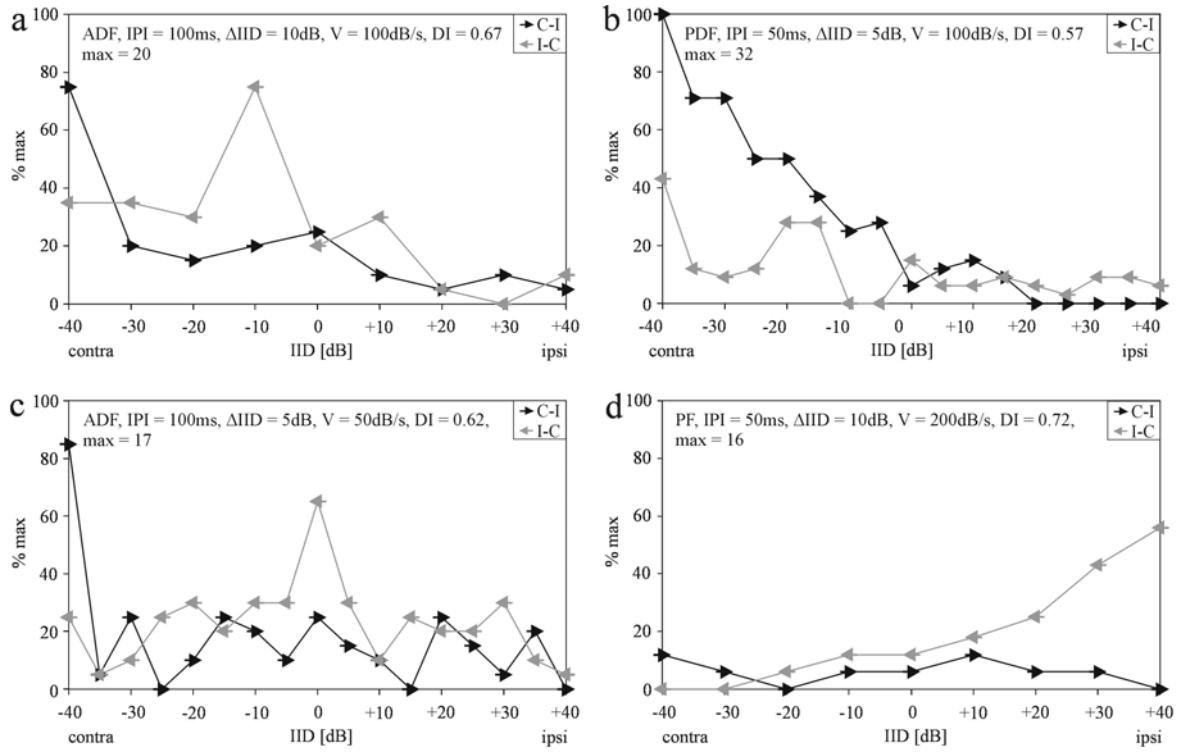

Fig. 16. Two types of neurons with motion-direction preference: The first type shows an enhanced response to motion in medial azimuthal positions (a, c), the second type responds stronger to motion in lateral azimuthal positions (b, d). Symbols as in Fig. 7.

coded for motion in medial azimuthal positions, whereas in the PF only 40 % (6/15) of neurons with a directional preference responded strongest from medial azimuthal positions (Fig. 17). Differences between the dorsal fields (ADF and PDF) and the PF were statistically significant ($p = 0.001$, two-tailed Fisher-test). Thus, direction preferring neurons in the dorsal fields encode motion in medial azimuthal positions whereas motion in more lateral regions of the azimuth could be encoded by direction preferring neurons in the primary field.

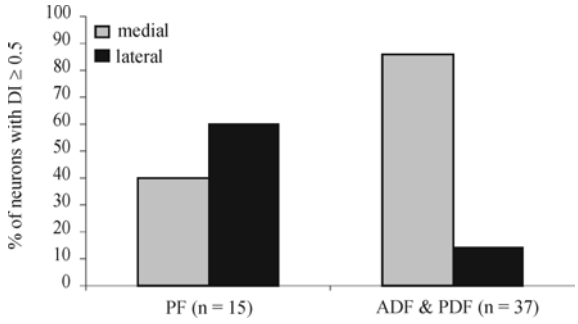

Fig. 17. Percentage of motion-direction preferring neurons coding for medial or lateral azimuthal positions in the three cortical fields. Differences between the PF and the dorsal fields (ADF and PDF) were statistically significant (two-tailed Fisher-Test, $p = 0.001$). ADF, anterior-dorsal field; PDF, posterior-dorsal field; PF, primary field.

In the dorsal fields, most neurons (76 %, 28/37) showed distinct response peaks in their static response profiles, and those peaks where often located in medial azimuthal positions. In the PF, only 40 % (6/15) of neurons showed distinct peaks in their static response profile in medial azimuthal positions. Since the responses of neurons with

directional preference often reflected properties of the static response profiles, differences in coding for different azimuthal positions seem to be related to differences in the properties of static RFs in the PF and the dorsal fields.

4.2 Discussion

The results show that neurons in the auditory cortex of *R. rouxi* respond differentially to the direction of apparent motion. The spatio-temporal parameter constellation of motion, i.e the combination of interpulse interval and spatial separation of sequentially presented virtual sound sources was an important parameter influencing a neuron's response. Velocity was not encoded as a distinct parameter. The primary field and the dorsal fields of auditory cortex have different processing properties for temporal parameters of apparent motion as well as for encoding azimuthal position of motion.

4.2.1 Technical considerations

In the present study dynamically changing IIDs were used to simulate acoustic motion in azimuth in a first approximation. This raises the question how realistically this artificial paradigm compares to free field motion. The use of an IID span of ± 40 dB corresponds to approximately ± 30° azimuth in *R. ro*uxi (Fig. 8f, Obrist et al., 1993; see Fig. 9). The range of azimuth covered in this study is relatively limited compared to other studies using free field motion (e.g. ± 70°, Ahissar et al., 1992; ± 90°, Kleiser & Schuller, 1995; ± 60°, Wilson & O'Neill, 1998). The azimuthal half-width of the acoustic beam emitted by rhinolophid bats is about ± 20° (Schnitzler, 1968). Thus, the ± 30° range covers well the azimuthal span of an echolocating horseshoe bat in a natural situation. In a passive hearing situation, however, acoustic stimuli can also come from azimuthal positions larger than ± 30°. Based on IIDs solely, high frequency pure tones could not be unambiguously perceived by *R. rouxi* from those positions. However, in a natural situation, pinna movements and frequency cues from frequency modulated parts of the calls contribute to resolve such ambiguities. Lohuis & Fuzessery (2000) showed that cortical neurons in the pallid bat (*Antrozous pallidus*) were also sensitive to interaural time differences of the envelope of a sound. Bats could use such cues for localization of complex sounds.

Another point has to be considered concerning the stimulus paradigm. As the transfer function of IID to azimuthal angle is not linear over the whole IID range, the angular velocity is not

uniform over the entire angular range. A constant IPI and IID step combination used to simulate motion, produces a rather constant velocity in the frontal range of azimuth of about ± 15° around the midline, and velocity increases to more lateral angles. The range of velocities used in this study was from 20 °/s to 160 °/s in the forward azimuthal range and covers the range of velocities used in other studies (e.g. 27 °/s and 50 °/s, Ahissar et al., 1992; 15 °/s to 225 °/s, Kleiser & Schuller, 1995; 50 °/s to 150 °/s, Wilson & O'Neill, 1998; 28,5 °/s and 57 °/s, Doan & Saunders, 1999).

4.2.2 Motion-direction sensitivity in different animals

The 71 % of neurons in the three cortical fields considered to be motion-direction-sensitive are comparable to the number of motion-direction-sensitive neurons in the AI of cats (68 %), reported by Stumpf et al. (1992) and the number found in the anterior ectosylvian cortex (88 %) of cats (Jiang et al., 2000). However, other authors reported much smaller numbers. Thus, Poirier et al. (1997) considered only 26 % of neurons in the primary auditory cortex of the cat to be motion-direction-sensitive. In the auditory cortex of monkeys 35 % (Ahissar et al., 1992) and in the AI of the rat 39 % (Doan & Saunders, 1999) of the sampled neurons showed a directional preference to auditory motion. Despite of species-specific differences, it seems more likely that the different percentages of motion-direction-sensitive neurons in the different studies can be related to the considerable procedural differences, i. e. using tonal stimuli as well as noise bursts to produce auditory motion under free-field or dichotic conditions. Differences of the chosen criterion for motion-direction sensitivity contribute also to the varying number of motion-direction-sensitive neurons in different studies.

Except for differences in number of motion-direction-sensitive neurons, other aspects of the results presented here are in accordance with the results of other studies. RF shifts opposite to the direction of motion were also shown in studies investigating motion sensitivity in the IC of bats (Kleiser & Schuller, 1995; Wilson & O'Neill, 1998) and gerbils (Spitzer & Semple, 1998). The range and magnitude of RF shift in this study (median: 6.03°, mean: 6.71°, range: 2.08° to 20.36°) correspond to the medial border displacements found in the IC of the mustached bat (Wilson & O'Neill, 1998; median: 4.32°, mean: 5.35°, range: 22.7°). The finding, that in neurons showing a directional preference the preferred direction of motion was that entering the RF from the opposite side, is supported by other studies in the auditory cortex (Ahissar et al., 1992; Toronchuk et al., 1992) and was also shown for neurons in the IC showing a directional bias (Wilson & O'Neill, 1998). In contrast, Poirier et al. (1997) reported

that neurons in the auditory cortex of the cat responded stronger when motion started in the static RF. Thus, despite a few exceptions, the results derived from different animals with different stimulus paradigms show a great similarity.

4.2.3 Functional role of different response types

The functional role of RF shifts as a response to different directions of auditory motion is not completely understood. One possibility is that RF shifts produce a pattern of activation in a population of neurons, that is more typical to an activity pattern normally produced by sound sources located further along the motion trajectory, as proposed by Wilson & O'Neill (1998) for neurons in the IC of the mustached bat. Thus, RF shifts could be considered as a neuronal correlate of a well known psychophysical phenomenon. That is, that humans perceive the location of a moving sound source displaced in the direction of motion (Perrott & Musicant, 1977). These localization errors might also occur in bats and might help the bats to predict the trajectory of a moving target. Indeed, it has been shown for the new world bat *Noctilio leporinus*, that these bats can estimate velocity and direction of a moving target and are able to extrapolate the future position of the target (Campbell & Suthers, 1988). For *R. rouxi* which forage mainly using the flycatcher style (Neuweiler et al., 1987) it might also be of advantage to predict the further trajectory of its prey to optimize its catching flights.

In contrast to neurons with RF shifts, neurons with directional preference often were tuned to a specific range of azimuth. This holds especially true for neurons in the dorsal fields which encoded primarily medial azimuthal positions. The azimuthal positions giving best responses in these neurons were in the range of azimuth where the peak energy is emitted in the calls of rhinolophid bats (Schnitzler, 1968). Thus, neurons with a directional preference in the dorsal fields might be involved in localization of moving prey in an active echolocation situation. Neurons that showed a directional preference to moving sounds in lateral parts of the azimuth might be used for the detection of objects entering the bat's auditory space from lateral positions. Such neurons might be part of an alerting system directing the attention of the bat to an approaching flying object, whereas neurons tuned to medial azimuthal positions might be used in tracking moving prey in front of the bat.

4.2.4 Origin of cortical field properties

A question, which arises from the results presented here is on which level of the auditory pathway the different properties of the different cortical fields in respect to motion processing are created. The cortical fields receive differential afferent connections from different parts of the auditory thalamus. The PF gets its main input from the ventral division of the medial geniculate body (vMGB), whereas the PDF and the ADF get strong input from associated nuclei of auditory thalamus, mainly from anterior dorsal and medial parts of the medial geniculate body (Radtke-Schuller, 1997). Different motion processing features of the different cortical fields might reflect different properties of different areas of the auditory thalamus. Consequently, the tuning to different azimuth locations of neurons in the dorsal fields and the primary field might reflect some properties already created at lower levels and brought up to the cortex over separated pathways. This is supported by the results of Aitkin & Jones (1992), who showed that neurons in the nucleus of the brachium of the inferior colliculus as well as in the vMGB were azimuth selective, suggesting that sound localization may be processed in different channels between IC and cortex. Thus, there might be a hierarchical organization in the pathway of auditory motion processing. Sensitivity to motion-direction is already created at the level of the IC or even earlier as motion-direction-sensitive responses have been reported for neurons in the nuclei of the lateral lemniscus of the barn owl (Wagner & Takahashi, 1992) and the superior olivary complex of cats (Altman, 1994). Projections from the IC might be reorganized in the auditory thalamus and then finally end in different fields of the auditory cortex where additional processing (‘fine-tuning’) occurs. Such an organization of the auditory motion pathway would resemble the pathway for processing of complex sounds (i.e FM-FM sounds) in the mustached bat, where the output of neurons in the central nucleus of the inferior colliculus tuned to the FM-sonar signals of the bat is rewired in the medial geniculate body before finally projecting to different fields of the auditory cortex (Wenstrup, 1999).

It is also of importance, that in most cases the responses to apparent motion were not independent from those obtained by static stimuli. Therefore, processing of motion and spatial information seems not to happen in separate channels. Motion of a sound source and spatial location would thus be processed together in accordance with the concept of a common pathway involved in ‘where’ processing as proposed by Rauschecker (1998).

4.2.5 Mechanisms of motion-direction sensitivity

The mechanisms underlying motion-direction sensitivity in the auditory cortex could not be settled in this first part of the study. McAlpine et al. (2000) and Ingham et al. (2001) suggested that binaural 'adaptation of excitation' is the mechanism underlying motion-direction sensitivity in neurons in the IC of guinea pig whereas other author favored inhibitory mechanisms for motion-direction sensitivity (Wagner & Takahashi, 1992; Kautz & Wagner, 1998) A directional preference could be observed in motion-direction-sensitive neurons with monaural (EO/mon) or binaural excitatory (EE) inputs as well as in neurons with mixed binaural interactions (EO/F&I) or inhibitory or facilitatory inputs from the non-excitatory ear (EO/F; E/I). No significant differences could be observed between the two groups. However, a RF shift occurred significantly more often in motion-direction-sensitive E/I, EO/F&I or EO/F neurons than in EO/mon or EE neurons. Thus, a simple adaptation mechanism might be sufficient to create a direction preferring response, whereas inhibitory/facilitatory binaural interactions seem to be important for creating RF shifts. Inhibitory/facilitatory binaural interactions might favor the occurrence of RF shifts if the time constants for inhibition/facilitation differ from those for excitation, leading to different levels of excitation depending on the direction of a stimulus crossing a neuron's RF border. Thus, in contrast to the mechanism proposed by McAlpine et al. (2000), in the auditory cortex of *R. rouxi* inhibition as well as facilitation seem to play an important role in creating motion-direction sensitivity, particularly RF shifts. However, inhibition seems not to work in the rigid framework of a motion detector as proposed by Wagner & Takahashi (1992). This is because such a motion detector is not fitted to produce a shift of RFs and a direction preferring response for different parameters of motion in the same neuron as observed in our study. In conclusion, both adaptation and inhibition/facilitation seem to contribute to motion-direction sensitivity in auditory cortex of the rufous horseshoe bat. The detailed role of inhibition in creating motion-direction sensitivity in the auditory cortex is subject of the experiments described in the following section.

5 Motion processing in the auditory cortex of the rufous horseshoe bat: role of GABAergic inhibition

5.1 Results

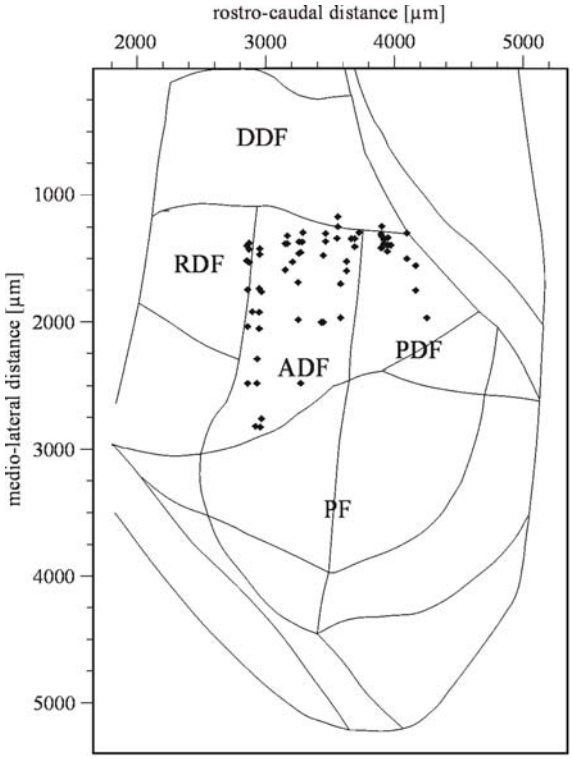

Fig. 18. Localization of recording sites in the auditory cortex of *Rhinolophus rouxi*. The position of neurons is represented in medio-lateral and rostro-caudal coordinates on a flattened surface projection of the auditory cortex. Symbols as in Fig. 5.

Recordings were derived from a total of 69 neurons (Fig. 18) most of which were located in the ADF (49/69). The remaining neurons were located in the PDF (20/69). Classification of cortical fields was according to Radtke-Schuller (1997). BMI was applied in 68 of the 69 neurons, GABA in 49/69 neurons (Table 2). Forty-eight of 69 neurons were tested with both drugs. In 67/68 neurons tested with BMI the drug was effectively applied. The increase of spike count ranged from 44 to 3300 % (mean: 604 ± 561 %). In 47/49 neurons tested with GABA, the drug had an effect on the overall response to acoustic stimulation. In these neurons GABA decreased the spike count (range: 12-100 %, mean decrease: 67 ± 20 % of predrug spike count). Among the 48 neurons tested with GABA and BMI, both drugs were effectively tested in 45 neurons.

From the 67 neurons effectively tested with BMI 42 % (28/67) showed a directional preference in the predrug condition, responding stronger to motion in one direction than in the other (mean DI:

Table 2. Number of neurons tested with bicuculline methiodide (BMI) and γ-aminobutyric acid (GABA).

	Total of neurons	Drug effect
Tested with BMI	68	67
Tested with GABA	49	47
Tested with both	48	45
Tested only with BMI	20	20
Tested only with GABA	1	1

0.73 ± 0.13, range: 0.5-1.0, n = 28). Thirty-seven percent (25/67) of the neurons showed a shift of medial RF border ≥ 5 dB IID due to reversal of the direction of apparent motion in the predrug condition (mean shift: 13.37 ± 6.02 dB IID, range: 5.64-30.77 dB IID. In accordance with results of the first part of this study the preferred direction was contralaterally directed motion, RF shifts were opposite the direction of motion. Twenty-one percent (14/67) of the neurons were not sensitive to the direction of motion in the predrug condition. The differences observed between the numbers of motion-direction-sensitive neurons in the two cortical fields were not statistically significant (directional preference: ADF: 47 % (22/47), PDF: 30 % (6/20); RF shift: ADF: 30 % (14/47), PDF: 55 % (11/20); not sensitive: ADF: 23 % (11/47), PDF: 15 % (3/20), $p > 0.05$, χ^2 test, n = 67).

5.1.1 Effects of BMI on motion-direction sensitivity

In 32 % (9/28) of neurons with a directional preference BMI decreased motion-direction sensitivity (decrease of DI > 0.2), and the DI dropped below 0.5 in 4/9 neurons (mean DI predrug: 0.75 ± 0.11, mean DI BMI: 0.45 ± 0.18, mean ΔDI: -0.30 ± 0.09; n = 9). Fig. 19 shows an example of DI decrease following BMI application. In the control conditions before and after BMI application (a, c) the neuron shows a strong directional preference to contralaterally directed motion. However, in the BMI condition (b) the neuron is also

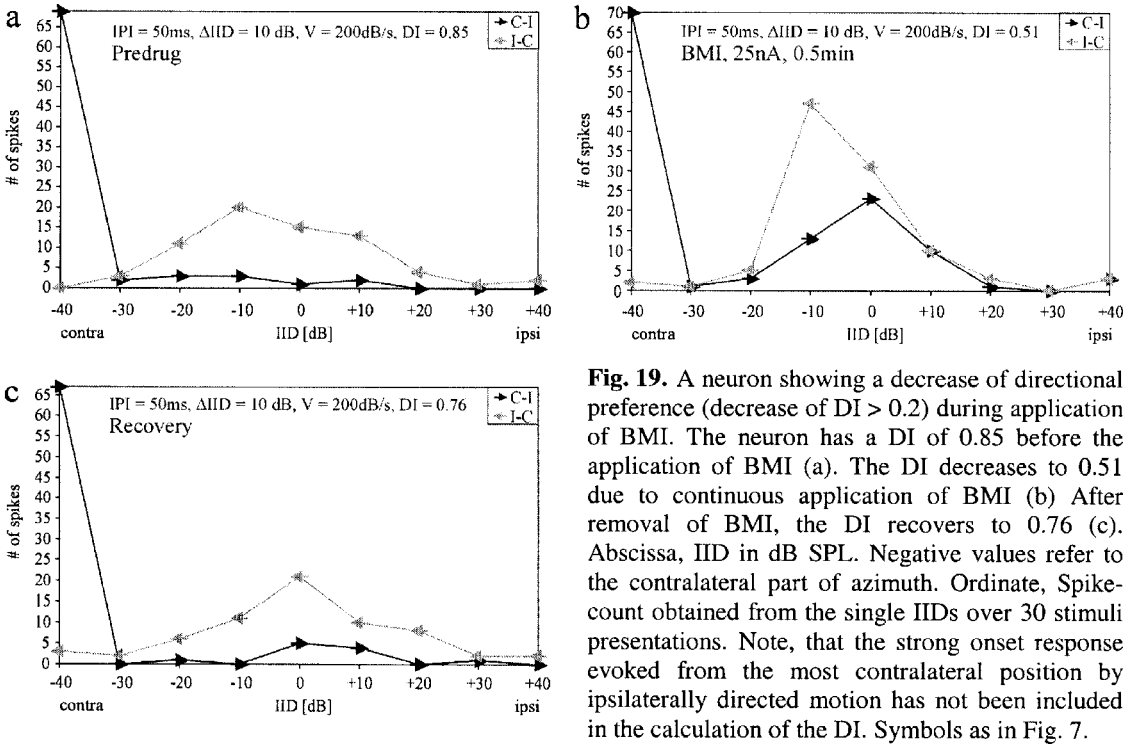

Fig. 19. A neuron showing a decrease of directional preference (decrease of DI > 0.2) during application of BMI. The neuron has a DI of 0.85 before the application of BMI (a). The DI decreases to 0.51 due to continuous application of BMI (b) After removal of BMI, the DI recovers to 0.76 (c). Abscissa, IID in dB SPL. Negative values refer to the contralateral part of azimuth. Ordinate, Spike-count obtained from the single IIDs over 30 stimuli presentations. Note, that the strong onset response evoked from the most contralateral position by ipsilaterally directed motion has not been included in the calculation of the DI. Symbols as in Fig. 7.

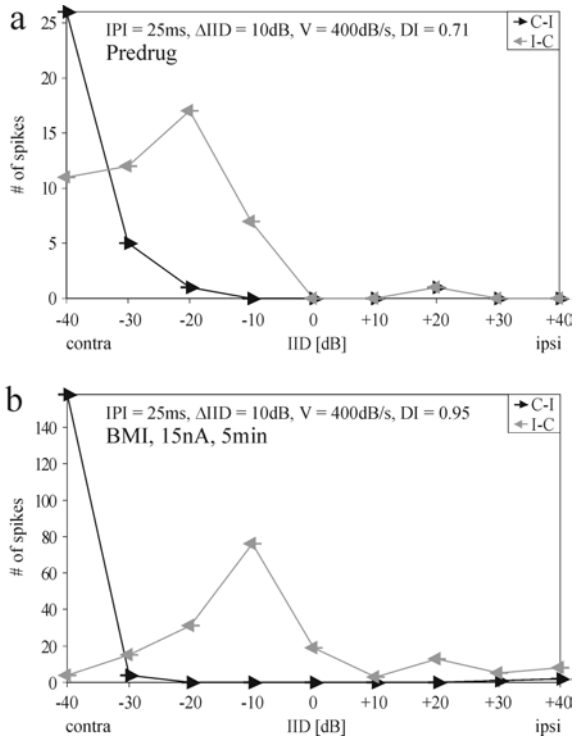

Fig. 20. A neuron showing an increase of directional preference due to application of BMI. The DI of 0.71 in the predrug condition (a) is increased to 0.95 during application of BMI (b). Note the different scaling of the ordinate in a and b. Symbols as in Fig. 7.

responding to ipsilaterally directed motion, with a decrease of DI from 0.85 to 0.51. In contrast, BMI increased the DI in 11 % (3/28) of neurons with a directional preference (mean DI predrug: 0.63 ± 0.09, mean DI BMI: 0.90 ± 0.03, mean ΔDI: 0.28 ± 0.07; n = 3). As can be seen from Fig. 20 the neuron has a DI of 0.71 in the predrug condition (a) which is increased to 0.95 during BMI application (b). It can also be seen from Fig. 20 that the response peak evoked by contralaterally directed motion is sharpened and shifted to the ipsilateral part of the azimuth. In 54 % (15/28) of neurons with a directional preference, BMI changed the DI less than ± 0.2 and thus had no influence on motion-direction sensitivity (mean DI predrug: 0.73 ± 0.14, mean DI BMI: 0.75 ± 0.15, mean ΔDI: 0.02 ± 0.12; n = 15). An example of this is shown in Fig. 21a, c. The DI as well as the shape of the response profile is basically the same in the control and the drug condition. In one cell, the application of BMI led to an inconsistent response that could not be classified.

In 16 % (4/25) of neurons with a RF shift in the predrug condition BMI increased the shift (mean shift predrug: 13.21 ± 4.71 ΔdB IID, mean shift BMI: 25.39 ± 5.64 ΔdB IID, mean Δshift: 12.18 ± 4.56 ΔdB IID, n = 4). In contrast, in 12 % (3/25) the RF shift was reduced with BMI (mean shift predrug: 12.99 ± 5.26 ΔdB IID, mean shift BMI: 2.91 ± 3.30 ΔdB IID, mean Δshift: -10.08 ± 4.15 ΔdB IID, n = 3). This is shown in Fig. 22. The RF shift is reduced from 17.43 ΔdB IID in the predrug condition (a) to 6.67 ΔdB IID during BMI application (b). In 28 % (7/25) of neurons with a shift of RF position in the predrug condition the response properties were completely changed with BMI. Thus, the response was changed from a RF shift to a directional preference in the neuron shown in Fig. 23. In the predrug condition (a), the neuron responds to the reversal of direction of motion mainly with a shift of RF border position, the response during BMI application, however, can better be characterized as a directional preference than as a RF shift (b).

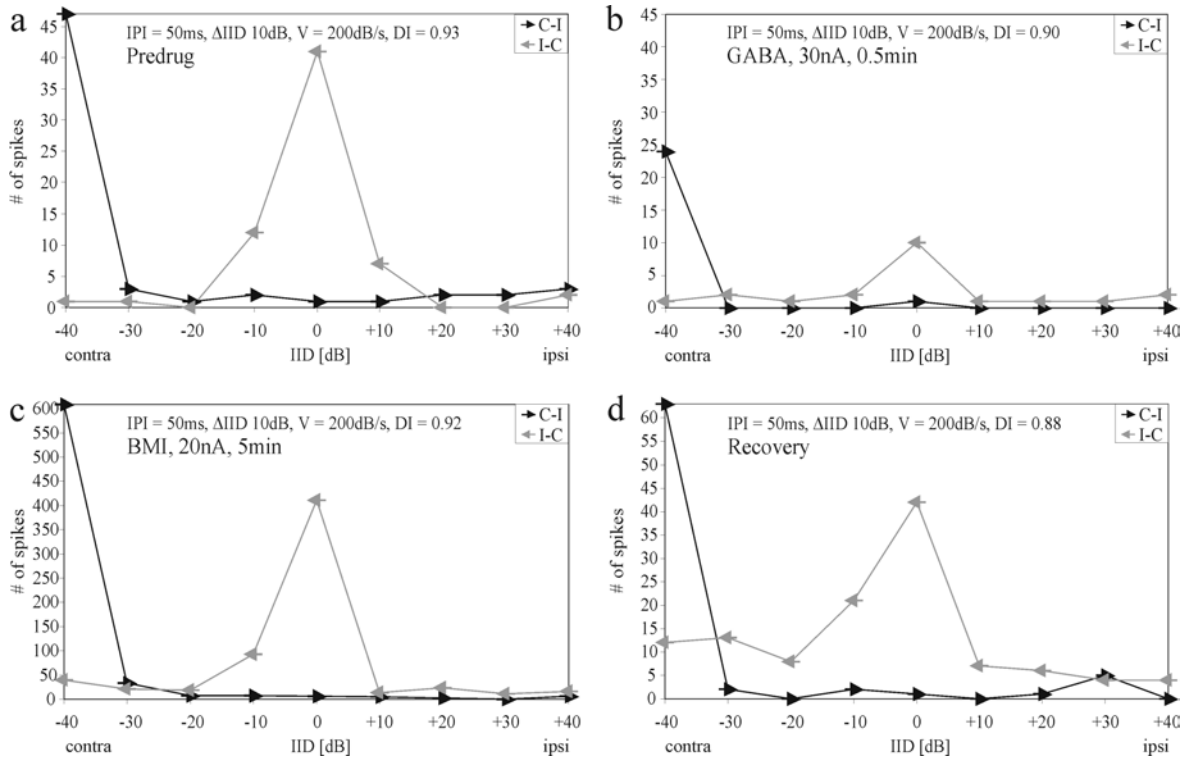

Fig. 21. Example of a neuron for which directional preference was not influenced by BMI or GABA. Neither DI nor shape of the response profiles evoked by motion in both direction in the predrug (a) and the recovery condition (d) was clearly changed during application of GABA (b) or BMI (c). Symbols as in Fig. 7.

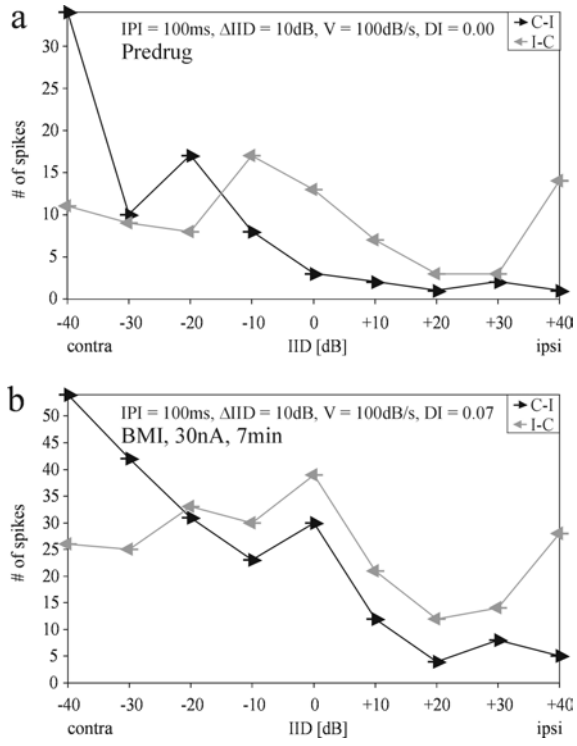

Fig. 22. Example of a cell showing a decrease of magnitude of RF shift due to BMI. The 50 % cut-off of the medial RF border evoked by ipsilaterally directed motion is shifted from the contralateral part of azimuth in the predrug condition (a) to a position in the ipsilateral hemifield during application of BMI (b), leading to a decrease of overall RF shift. The 50 % cut-off of the medial RF border evoked by contralaterally directed motion remained almost the same in (a) an (b). Symbols as in Fig. 7.

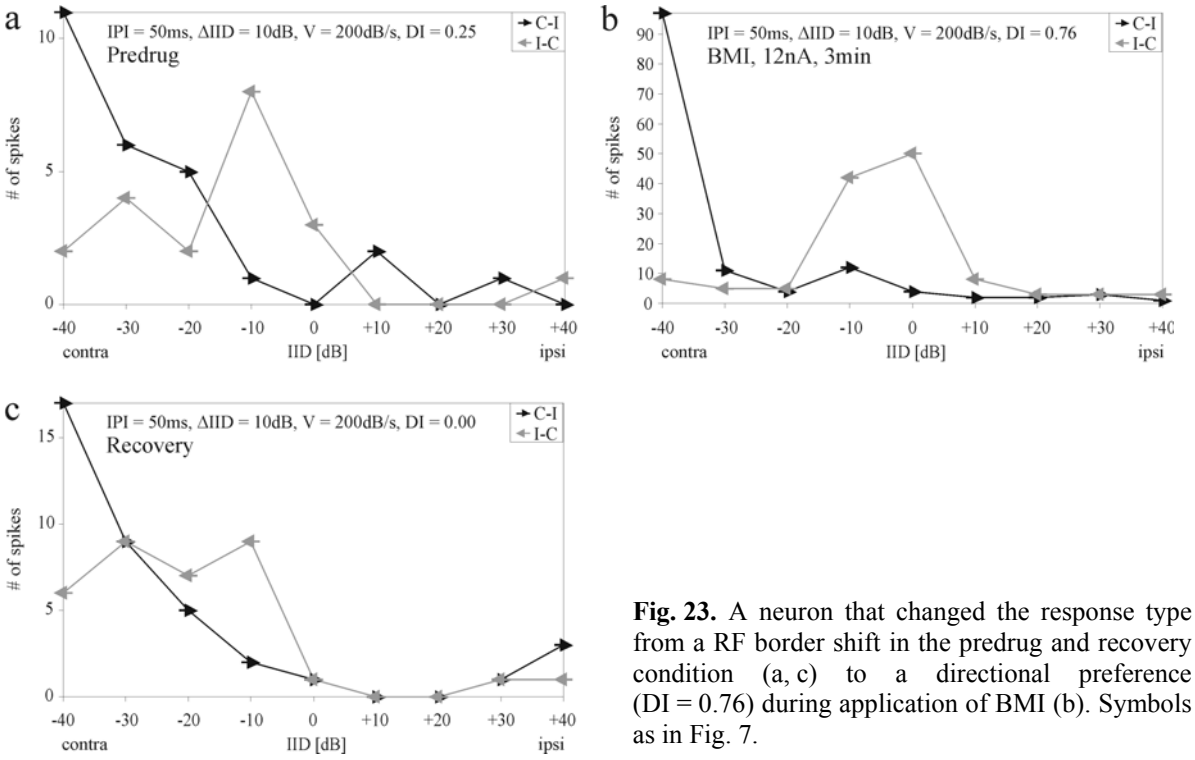

Fig. 23. A neuron that changed the response type from a RF border shift in the predrug and recovery condition (a, c) to a directional preference (DI = 0.76) during application of BMI (b). Symbols as in Fig. 7.

In 16 % (4/25) of neurons with a RF shift, BMI did not affect the response characteristic at all (mean shift predrug: 12.31 ± 5.96 ΔdB IID, mean shift BMI: 12.19 ± 7.48 ΔdB IID, Δshift: -0.12 ± 3.44 ΔdB IID, n = 4). An example for this is shown in Fig. 24a,c. Note that although the RF borders for motion in both directions are shifted to more ipsilateral positions, the relative position of the borders remains the same. In 28 % (7/25) of neurons the response during BMI application was unsystematic and could not be further characterized.

From the 14 neurons not sensitive to direction of motion in the predrug condition (DI < 0.5, shift < 5dB IID), 36 % (5/14) showed a directional preference with BM and thus increased motion-direction sensitivity (mean DI predrug: 0.29 ± 0.12, mean DI BMI: 0.82 ± 0.10, mean ΔDI: 0.53 ± 0.11, n = 5). An example is shown in Fig. 25. The neuron's DI of 0.47 in the predrug condition (a) is increased to 0.89 during application of BMI (b). Note, that the spike count for the response peak evoked by contralaterally directed motion is increased whereas the small peak evoked by ipsilaterally directed motion in the predrug condition is further decreased by BMI.

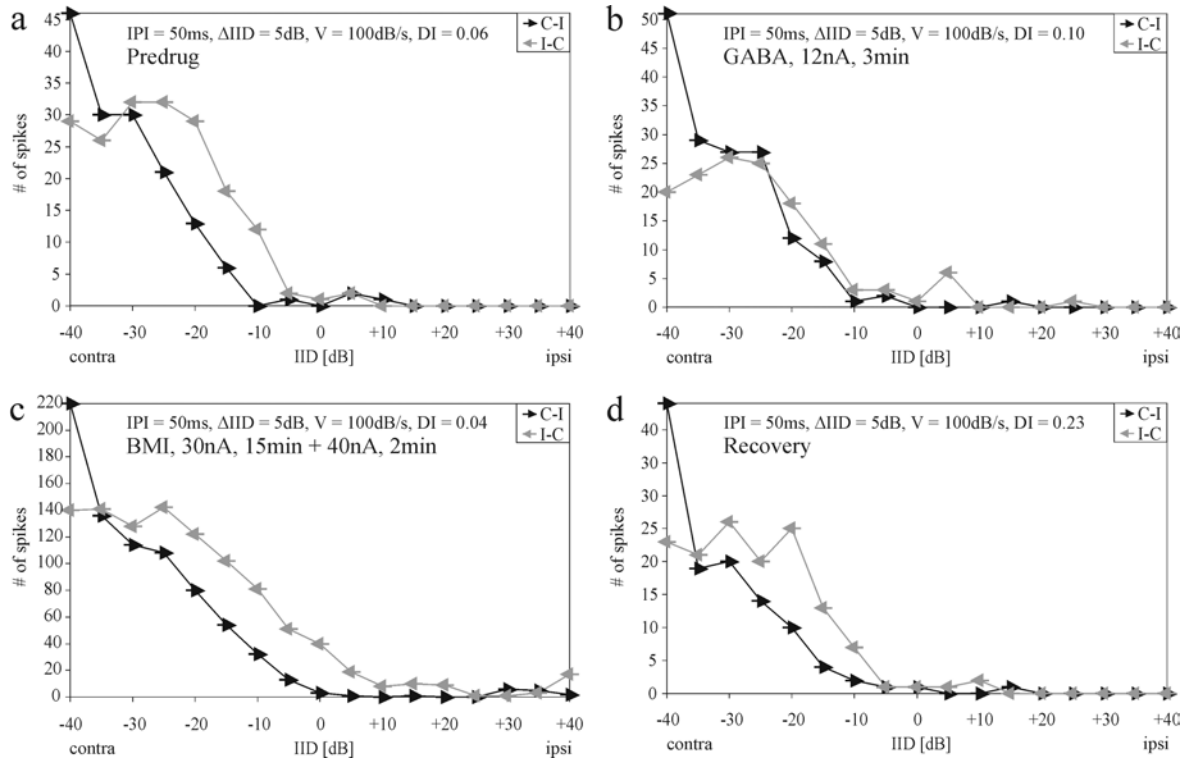

Fig. 24. Example of a neuron showing a shift of RF border location in the predrug (a) and recovery condition (d) that was not influenced by BMI (c) but decreased by GABA (b). Note in (c), that despite the RF evoked by motion in both directions is widened compared with the predrug and recovery condition, the relation between the 50 % cut-offs and thus the magnitude of RF shift remains the same during BMI application. Symbols as in Fig. 7.

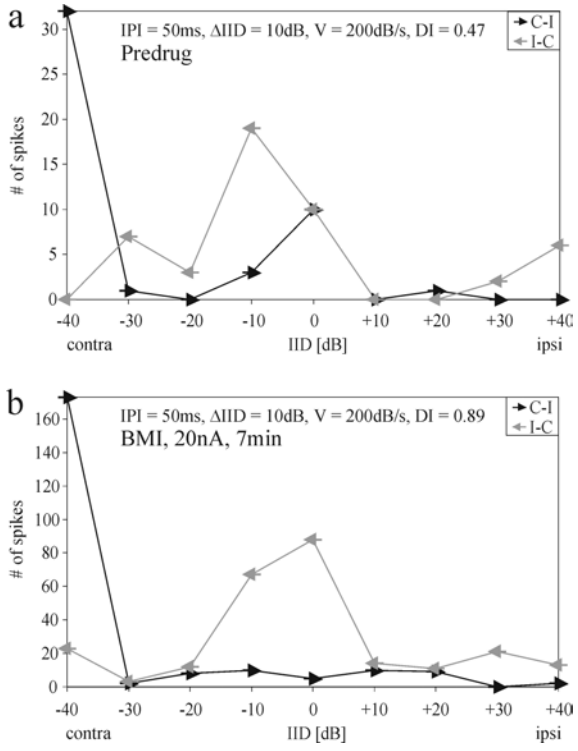

Fig. 25. A neuron that is not motion-direction-sensitive (DI ≤ 0.5) in the predrug condition (a), but showing a directional preference to contralaterally directed motion with BMI (b). Note, that the small response peak evoke by ipsilaterally directed motion in the predrug condition is decreased during BMI application. Symbols as in Fig. 7.

An increase of motion-direction sensitivity could also be seen in three neurons (3/14, 21 %) showing a shift of RF border as a response to motion in both directions during BMI application (mean shift predrug: 2.57 ± 1.78 ΔdB IID, mean shift BMI: 12.99 ± 1.19 ΔdB IID, mean Δshift: 10.42 ± 2.59 ΔdB IID, n = 3). Fig. 26 shows an example of a neuron with virtually no RF shift in the predrug condition (a). However, under the influence of BMI the RF evoked by ipsilaterally and contralaterally directed motion dissociate, creating a pronounced shift of the medial RF border (b). Thus, in 57 % (8/14) neurons that were not sensitive to the direction of apparent motion BMI increased the sensitivity either by creating directional preference or RF shifts. In 14 % (2/14) of the non motion-direction-sensitive neurons BMI evoked a further decrease of sensitivity by decreasing the DI as shown in the example in Fig. 27 a,b. The DI of 0.43 in the predrug condition decreased to 0.1 during BMI application, corresponding to an almost equal response to both directions of motion. Four of the neurons not sensitive to the direction of motion under predrug conditions either did not change their response with BMI (3/14, 21 %; mean DI predrug: 0.33 ± 0.08, mean DI BMI: 0.23 ± 0.09, mean ΔDI: -0.10 ± 0.09, n = 3) or showed an inconsistent response pattern during the application of BMI (1/14, 7 %).

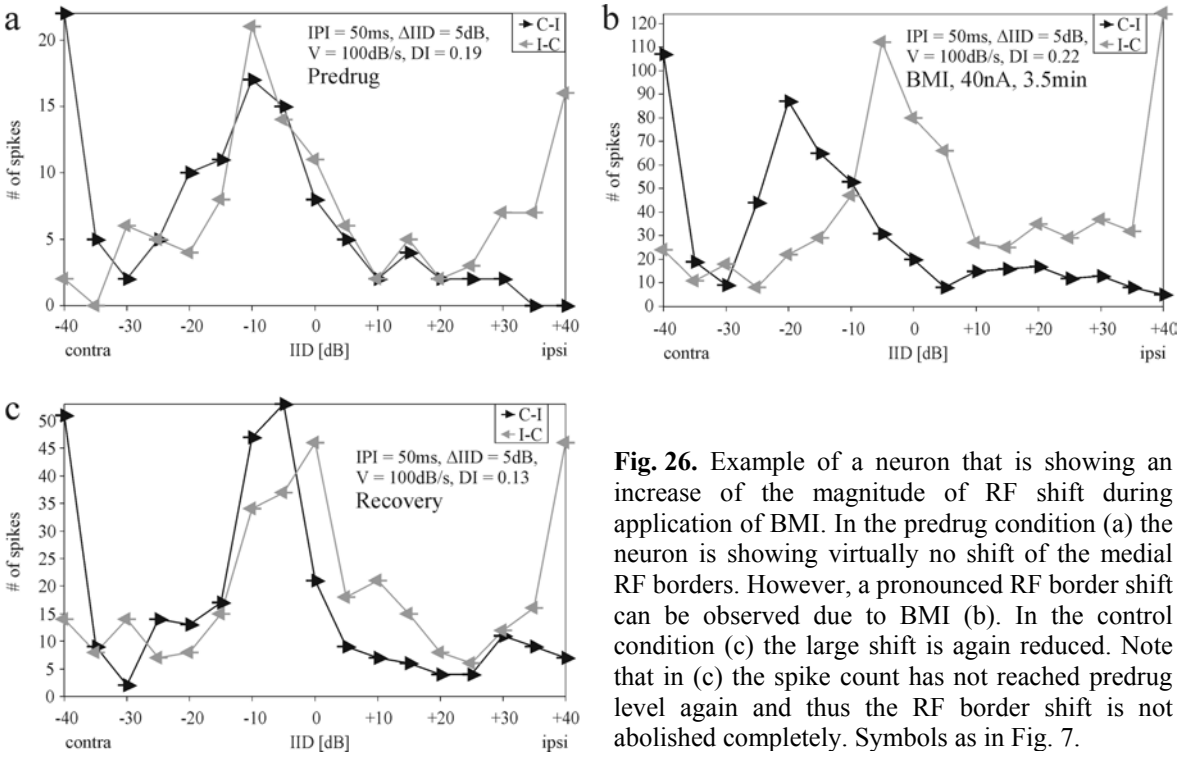

Fig. 26. Example of a neuron that is showing an increase of the magnitude of RF shift during application of BMI. In the predrug condition (a) the neuron is showing virtually no shift of the medial RF borders. However, a pronounced RF border shift can be observed due to BMI (b). In the control condition (c) the large shift is again reduced. Note that in (c) the spike count has not reached predrug level again and thus the RF border shift is not abolished completely. Symbols as in Fig. 7.

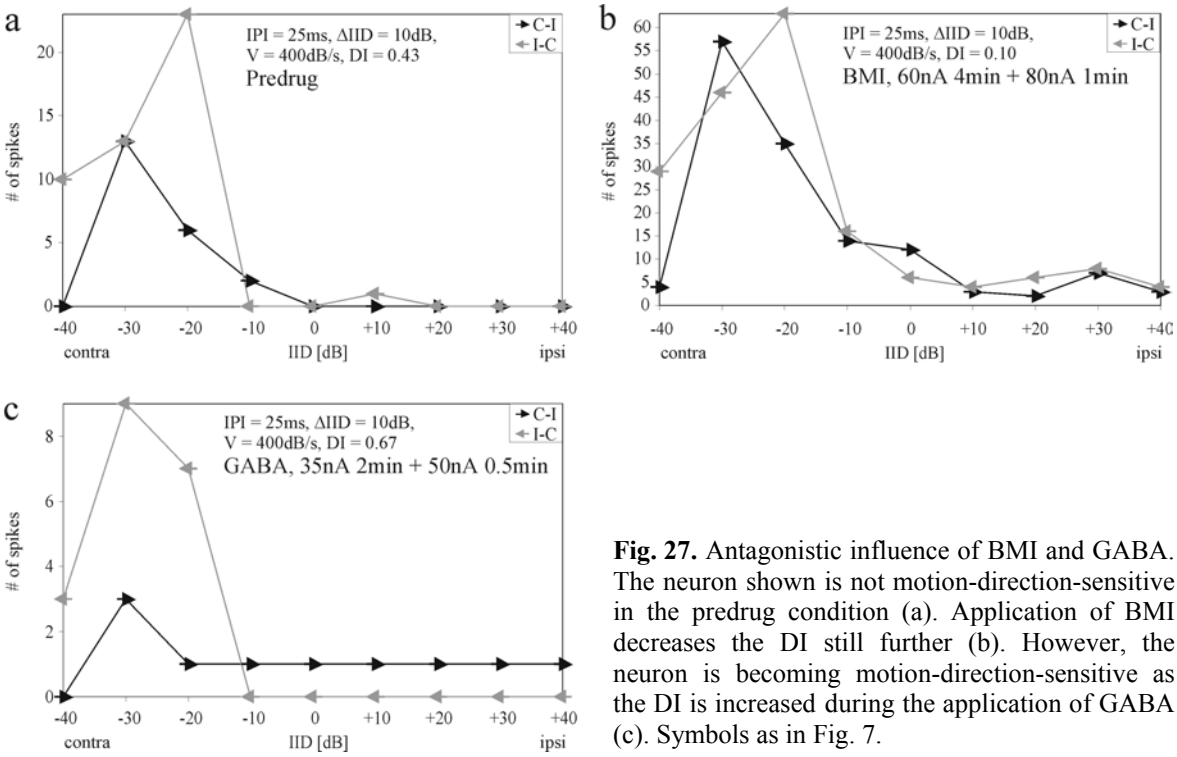

Fig. 27. Antagonistic influence of BMI and GABA. The neuron shown is not motion-direction-sensitive in the predrug condition (a). Application of BMI decreases the DI still further (b). However, the neuron is becoming motion-direction-sensitive as the DI is increased during the application of GABA (c). Symbols as in Fig. 7.

To summarize the effects of BMI, a decrease of motion-direction sensitivity could be observed in a total of 21 % (14/67; Table 3), either due to a decrease of magnitude of DI or RF shifts. In about the same percentage of cells (22 %, 15/67), however, the sensitivity to motion direction was increased by BMI (increase of DI or magnitude of RF shift). The response type per se was changed during application of BMI in 10 % (7/67) of neurons in that a shift of RF border was changed to a directional preference. In 33 % (22/67) of neurons the responses to auditory apparent motion were unaffected by BMI, an inconsistent pattern was observed in 9/67 (13 %) of neurons.

To test for differences between the two dorsal fields the numbers of neurons belonging to the above mentioned classes were compared in the ADF and the PDF (Table 4). Seventeen percent of neurons (8/47) in the ADF decreased the DI or magnitude of RF shifts (PDF: 30 %, 6/20). In the ADF an increase of DI or magnitude of RF shifts was found in 30 % (14/47) of neurons (PDF: 5 %, 1/20). A complete change of the response type was observed in 6 % (3/47) of neurons in the ADF and in 20 % (4/20) of neurons in the PDF. BMI was not effective in 34 % (16/47) of neurons in the ADF (PDF: 30 %, 6/20) and 13 % (6/47) of neurons in the ADF showed an inconsistent response pattern (PDF: 15 %, 3/20). Although there was a bias between the numbers of neurons that increased or decreased their motion-direction sensitivity under BMI in the ADF and PDF, the differences between the two fields for all classes were not significant ($p > 0.05$, two-tailed Fisher test, $n = 67$).

Table 3. Effects of bicuculline methiodide (BMI) on motion-direction sensitivity (MDS).

BMI (n=67)	decrease of MDS	increase of MDS	change of response type	no change of MDS	inconsistent
directional preference (n = 28)	9/28 (32 %)	3/28 (11 %)	---	15/28 (54 %)	1/28 (4 %)
RF shift (n = 25)	3/25 (12 %)	4/25 (16 %)	7/25 (28 %)	4/25 (16 %)	7/25 (28 %)
not sensitive (n = 14)	2/14 (14 %)	8/14 (57 %)	---	3/14 (21 %)	1/14 (7 %)
total	14/67 (21 %)	15/67 (22 %)	7/67 (10 %)	22/67 (33 %)	9/67 (13 %)

RF, receptive field.

Table 4. Comparison of bicuculline methiodide (BMI) effects in the anterior-dorsal field (ADF) and the posterior-dorsal field (PDF). Differences between the two cortical fields were not statistically significant (two-tailed Fisher test, $p > 0.05$).

BMI (n = 67)	decrease of MDS	increase of MDS	change of response type	no change of MDS	inconsistent
ADF (n = 47)	8/47 (17 %)	14/47 (30 %)	3/47 (6 %)	16/47 (34 %)	6/47 (13 %)
PDF (n = 20)	6/20 (30 %)	1/20 (5 %)	4/20 (20 %)	6/20 (30 %)	3/20 (15 %)

MDS: motion-direction sensitivity.

In four neurons tested with BMI an additional parameter set of IPI or IID steps for simulating apparent auditory motion was applied so that the influence of BMI on the response to motion with different spatio-temporal parameters could be tested. All of the four neurons were sensitive to the direction of motion (three showed directional preference, one showed a RF shift). Three neurons lost their motion-direction sensitivity when motion was simulated with shorter IPI or IID step. In these neurons motion-direction sensitivity was restored during BMI application. The neuron shown in Fig. 28 responded with a RF shift to motion in opposite directions with an IPI of 200 ms and a 10 dB IID step (a). The direction-sensitive response of the neuron is totally abolished (except for the onset response) as the IPI is decreased to 50 ms (b). However, the response to contralaterally directed motion is recovered during application of BMI leading to a directional preference of the neuron (c). Removing BMI abolished the motion-direction sensitivity again (d). The results show that the decrease of response strength, i.e. the disappearance of the shift in the neuron shown in Fig. 28b is not merely a consequence of increased stimulus repetition rate but the release of inhibition through BMI can restore the responsiveness of the neuron, even though the type of response has changed.

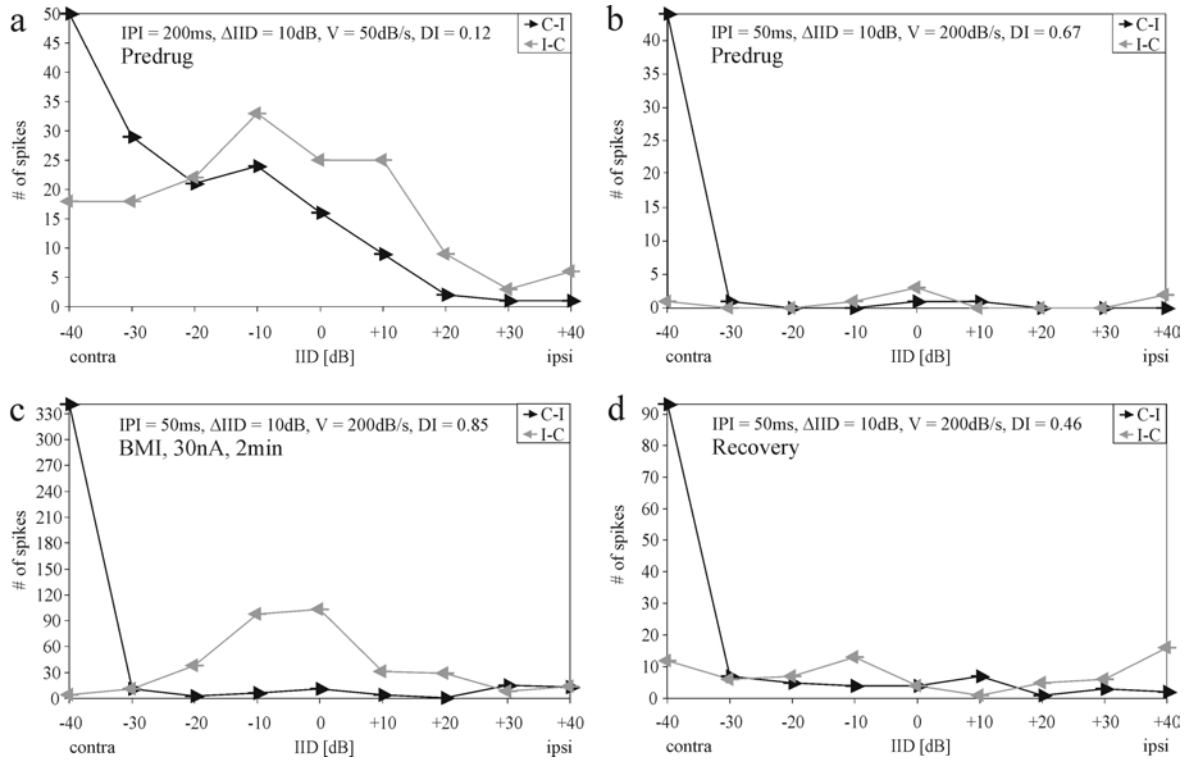

Fig. 28. A neuron tested with different parameters of motion. The neuron is showing a shift of RF borders in the predrug condition (a) with long interpulse interval (IPI, 200 ms), whereas the neuron is becoming non-motion-direction-sensitive as the IPI is decreased (b, d, IPI = 50 ms). Note that in (b) no clear response peak is visible. Due to the very low number of spikes (< 5, onset-response excluded), the DI of 0.67 is not reliable. During the application of BMI (c), despite of the short IPI (50 ms) contralaterally directed motion evokes a strong response peak, increasing the DI to 0.85. Symbols as in Fig. 7.

5.1.2 Effects of GABA on motion-direction sensitivity

In 45 of the 67 neurons effectively tested with BMI, GABA was effective, too. However, the application of GABA often completely abolished a neuron's response even when GABA was applied with low ejection currents. A differential effect of GABA was observed only in 14 of the 45 neurons tested with GABA.

In only one case a clear antagonistic effect of GABA and BMI was observed. As shown in Fig. 27c, GABA increased the DI of the neuron leading to a directional preference (DI predrug: 0.43, DI GABA: 0.67, ΔDI: 0.24), whereas BMI decreased the DI (Fig. 27b, DI BMI: 0.10, ΔDI: -0.33). There was also one neuron in which GABA had the same effect as BMI, namely increasing the directional preference (DI predrug: 0.71, DI GABA: 1.0, ΔDI GABA: 0.29, DI BMI: 0.95, ΔDI BMI: 0.24). The RF shift was decreased during GABA application in 3 neurons (e.g. Fig. 24b, mean shift predrug: 11.97 ± 3.41 ΔdB IID, mean shift GABA: 4.14 ± 1.06 ΔdB IID, mean Δshift: -7.83 ± 3.95 ΔdB IID, n = 3) whereas BMI had no

effect on the magnitude of the shift in two of these cells (mean shift predrug: 11.54 ± 4.71 ΔdB IID, mean shift BMI: 9.75 ± 0.73 ΔdB IID, mean Δshift: -1.80 ± 3.98 ΔdB IID, n = 2) or evoked an inconsistent response pattern (in one neuron). In 9 neurons GABA did not change the response to different directions of apparent motion. In these neurons either a directional preference was unaffected by GABA (6/9, mean DI predrug: 0.79 ± 0.19, mean DI GABA: 0.77 ± 0.21, mean ΔDI: -0.02 ± 0.03, n = 6, e.g. Fig. 21b) or the neurons were not sensitive to the direction of motion in the predrug condition (3/9, mean DI predrug: 0.26 ± 0.08, mean DI GABA: 0.22 ± 0.13, mean ΔDI: -0.05 ± 0.08, n = 3). Five of the nine neurons were also unaffected by BMI. The effects of GABA on motion-direction sensitivity are summarized in Table 5.

Table 5. Effects of γ-aminobutyric acid (GABA) on motion-direction sensitivity (MDS).

GABA (n = 14)	decrease of MDS	increase of MDS	no change of MDS
directional preference (n = 7)	---	1/7 (14 %)	6/7 (86 %)
RF shift (n = 3)	3/3 (100 %)	---	---
not sensitive (n = 4)	---	1/4 (25 %)	3/4 (75 %)
total	3/14 (21 %)	2/14 (14 %)	9/14 (64 %)

RF, receptive field.

5.1.3 Analysis of directional preference

To assess the mechanisms underlying the effects of BMI and GABA on motion-direction sensitivity in more detail, the increase or decrease of the strength of response to the preferred and non-preferred direction was compared. Thus, the increase of the maximum number of spikes evoked by motion in the preferred and non-preferred direction during BMI application was calculated by subtracting the number of spikes of control responses from the number of spikes with BMI, and compared for neurons in which the DI decreased (predrug directional preference or non motion-direction-sensitive, n = 11), increased (predrug directional preference or non motion-direction-sensitive, n = 8) or did not change more than 0.2 (predrug directional preference or non motion-direction-sensitive, n = 18). For neurons which changed the response type from a shift of RF borders to a directional preference (n = 7), the increase of the maximum spike number during BMI application was also analyzed. For neurons included

in this analysis that were not motion-direction-preferring (DI < 0.5) neither before nor during BMI application (n = 5), the motion direction that evoked the higher number of spikes from any IID position was taken as 'preferred direction'. The influence of GABA on the decrease of spike number of responses to both directions of motion was also analyzed by subtracting the control response from the response during GABA application in neurons in which the DI increased (predrug directional preference or non motion-direction-sensitive, n = 2) or did not change more than 0.2 (predrug directional preference or non motion-direction-sensitive, n = 9). The results are shown in Fig. 29. In neurons in which the DI increased during BMI application or did not change, or BMI changed the response from a shift to a directional preference, the increase of the response strength in the preferred direction was significantly greater than for the non-preferred direction (DI increase: mean Δspike non-preferred direction: 4.25 ± 11.23, mean Δspike preferred direction: 74.75 ± 42.95, Wilcoxon test, $p < 0.05$, n = 8; no change of DI: mean Δspike non-preferred direction: 20.44 ± 37.13, mean Δspike preferred direction: 68.83 ± 85.27, Wilcoxon test, $p < 0.05$, n = 18; change of response type: mean Δspike non-preferred direction: 14.43 ± 13.82, mean Δspike preferred direction: 56.71 ± 34.75, Wilcoxon test, $p < 0.05$, n = 7). In contrast, in neurons in which the DI was decreased with BMI, the increase of response strength was not significantly greater for the preferred or the non-preferred direction (mean Δspike non-preferred direction: 31.27 ± 17.21, mean Δspike preferred direction: 34.73 ± 19.16, Wilcoxon test, $p > 0.05$, n = 11). However, a stronger increase for the non-preferred direction would have been expected if inhibition was stronger for the non-preferred direction than for the preferred direction. Thus, the amount of inhibition is not directionally biased in these neurons.

In neurons where GABA did not change the DI, the decrease of the response strength was significantly greater for the preferred direction (mean Δspike non-preferred direction: -4.56 ± 7.06; mean Δspike preferred direction: -13.67 ± 12.37, Wilcoxon test, $p < 0.05$, n = 9). The decrease of the response strength was only slightly greater for the preferred direction in neurons which increased the DI under influence of GABA. However, the significance could not be tested because of the small number of neurons belonging to this class (mean Δspike non-preferred direction: -7.50 ± 3.54, mean Δspike preferred direction: -11.00 ± 4.24, n = 2).

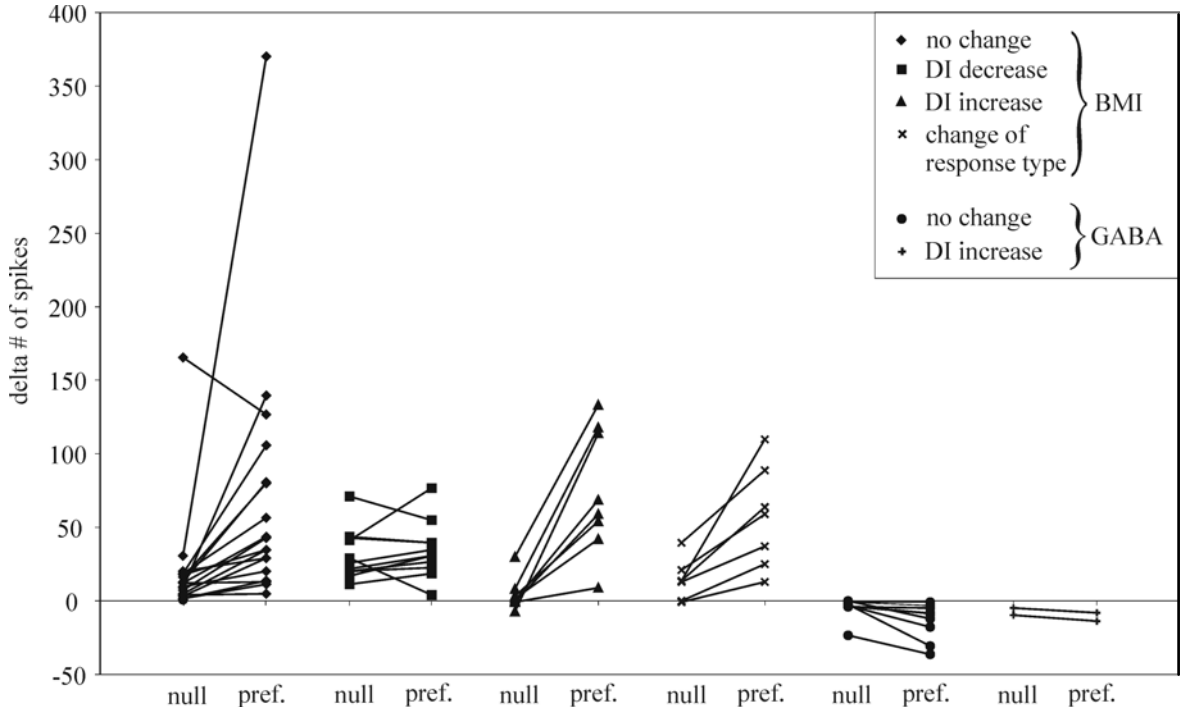

Fig. 29. Comparison of increase and decrease of spike-count evoked by motion in the preferred (pref.) and non-preferred direction (null) during administration of BMI and GABA for neurons in which the DI decreased, increased or did not change due to drug application. Neurons which changed the response type from a RF shift to a directional preference during BMI application are also included. The maximum number of spikes evoked in the control condition by motion in both directions was subtracted from the number of spikes evoked in the drug condition (BMI or GABA) and plotted as delta number of spikes (ordinate). Negative values refer to a decrease of spike-count. See text for further details.

5.1.4 Analysis of RF shifts

Mechanisms underlying RF shifts were analyzed in detail comparing the change in the position of the 50 % cut-off of medial RF borders for both directions of motion. The delta 50 % cut-off was calculated by subtracting the position of the 50 % cut-off in the predrug condition from the 50 % cut-off position during BMI application for contralaterally and ipsilaterally directed motion. Positive values for delta 50 % cut-off refer to a shift to more ipsilateral positions of azimuth. Included in the analysis were neurons in which BMI decreased (predrug shift, n = 3), increased or evoked a RF shift (predrug shift and predrug no shift, n = 7) or did not change the magnitude of RF shifts (predrug shift, n = 4). In addition, the effect of GABA on the position of the 50 % cut-off of RF borders was analyzed in neurons in which GABA decreased the magnitude of RF shifts (predrug shift, n = 3). Because of the small numbers of neurons belonging to most of the above mentioned response classes, statistical significance could not be demonstrated in this analysis.

In neurons in which the shift became smaller with BMI the 50 % cut-off of the RF border evoked by ipsilaterally directed motion shifted more to ipsilateral positions than the 50 % cut-off of the RF border evoked by contralaterally directed motion (Fig. 30, see also Fig. 22; mean Δshift ipsilaterally directed motion: 11.45 ± 4.26 dB IID, mean Δshift contralaterally directed motion: 1.37 ± 2.58 dB IID, n = 3). As expected, for neurons in which BMI did not change the magnitude of the RF shifts the 50 % cut-off values evoked by either contralaterally or ipsilaterally directed motion during BMI application were either shifted about the same amount in the same direction or were not markedly different (in the range of 5 dB IID) from those in the predrug condition (mean Δshift ipsilaterally directed motion: 2.05 ± 1.70 dB IID, mean Δshift contralaterally directed motion: 1.93 ± 4.44 dB IID, n = 4; Fig. 30 and Fig. 24a, c). In contrast, the 50 % cut-off of the response profile of contralaterally directed motion was in neurons in which the magnitude of RF shift was increased by BMI shifted to more ipsilateral positions, whereas the 50 % cut-off of the response profile evoked by ipsilaterally directed motion remained almost unaffected by BMI (Fig. 30; mean Δshift ipsilaterally directed motion: -2.05 ± 2.52 dB IID, mean Δshift contralaterally directed motion: 9.37 ± 3.91 dB IID, n = 7). However, in one of the seven neurons the 50 % cut-offs for ipsilaterally and contralaterally directed motion were displaced in opposite directions, thus increasing the RF shift during BMI application (Δshift ipsilaterally directed motion: -7.18 dB IID, mean Δshift contralaterally directed motion: 5.64 dB IID, Fig. 30 and Fig. 26). In neurons in which the RF shift decreased with GABA, the 50 % cut-off of the response profile evoked by contralaterally directed motion is shifted to more contralateral positions during GABA application than the 50 % cut-off of the profile line evoked by ipsilaterally directed motion (mean Δshift ipsilaterally directed motion: -1.92 ± 3.85 dB IID, mean Δshift contralaterally directed motion: -9.74 ± 6.23 dB IID, n = 3, Fig. 30 and Fig. 24a, b).

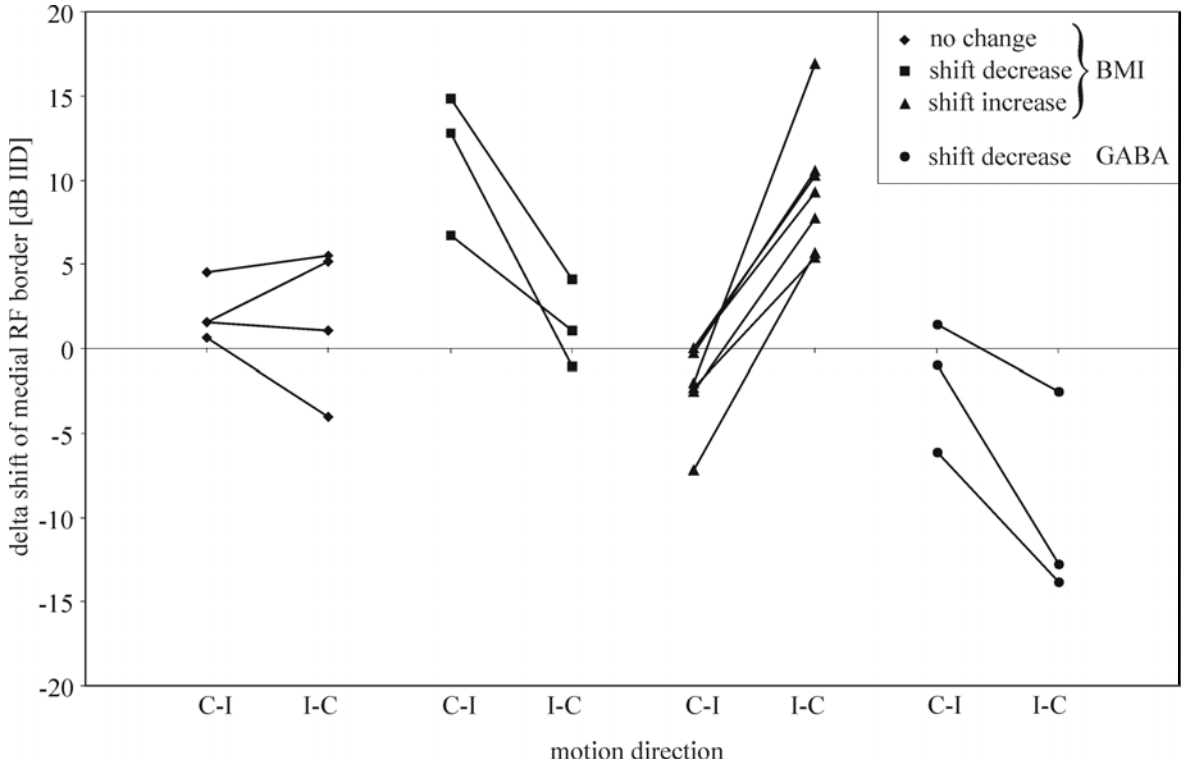

Fig. 30. Comparison of the position of the 50 % cut-off of medial RF borders evoked by motion in both directions during the application of BMI and GABA for neurons in which a RF border shift increased (or was evoked), decreased or did not change during drug application. Abscissa, motion direction; C-I, ipsilaterally directed motion; I-C, contralaterally directed motion. Ordinate, delta shift of medial RF border (dB IID) calculated by subtracting the positions of the 50 % cut-off in the predrug condition from that in the drug condition (BMI, GABA) for both directions of motion. Positive values refer to a shift of RF borders to more ipsilaterally located azimuthal positions. See text for further details.

5.2 Discussion

In the second part of the present study the influence of GABAergic inhibition on motion-direction sensitivity of neurons in the auditory cortex of the rufous horseshoe bat was investigated. Microiontophoretic application of the GABA antagonist BMI reduced motion-direction sensitivity in 21 % of neurons by decreasing either the magnitude of DI or RF shifts. However, motion-direction sensitivity was increased in about the same number of neurons (22 %).

5.2.1 Inhibition as a mechanism of motion-direction sensitivity

Several mechanisms have been proposed to underlie motion-direction sensitivity of neurons in the visual and the auditory system. In the visual system of vertebrates, motion detectors are already found at the level of the retina (Maturana & Frenk, 1963; Barlow et al., 1964) and

direction specific inhibition is thought to be involved in the underlying mechanism (Barlow & Levick, 1965). For motion-direction selectivity in neurons in the visual cortex of higher mammals at least two different mechanisms are discussed. Direction selective inhibition has been reported in the cat (Goodwin et al., 1975; Sillito, 1977; Crook et al., 1996), ferret (Roerig & Kao, 1999) and in the primate (Livingstone, 1998). Other authors show, however, that inhibition acts in a non-selective manner to enhance the directional selectivity already present by directionally biased excitatory inputs by raising the response threshold ('ice-berg effect', Sato et al., 1995; Hammond & Kim, 1996).

In the auditory system, Wagner & Takahashi (1992) proposed a motion detector working on direction dependent inhibition in the first stage and direction independent inhibition in the second stage for motion-direction-sensitive neurons in the brain stem of barn owls. Using microiontophoretic application of BMI, Kautz & Wagner (1998) could show that motion-direction sensitivity in the IC of barn owls is influenced by GABAergic inhibition. This inhibition, however, proved to be directionally untuned. Several other studies in the auditory cortex showed, that neurons responded best to one direction of motion whereas the response to the opposite direction was suppressed, suggesting that inhibitory mechanisms are involved in creating motion-direction sensitivity (Ahissar et al., 1992; Stumpf et al., 1992; Poirier et al., 1997; Jiang et al., 2000). In contrast, McAlpine et al. (2000) and Ingham et al. (2001) explained shifts in the spatial positions of RFs due to different directions of motion of IC neurons in guinea pigs by an 'adaptation of excitation'. No cells were selectively responding to only one direction of motion and no inhibitory mechanism was necessary to explain the responses.

The data presented here show that 'adaptation of excitation' can be ruled out as a mechanism for motion-direction sensitivity in at least part of neurons in the auditory cortex. As shown in Fig. 28a,b the response magnitude of this neuron decreases with decreasing IPI, which could be explained by 'adaptation of excitation' as shown by Ingham et al. (2001). The increase of response magnitude for contralaterally directed motion after blocking inhibition by BMI (Fig. 28c), however, can not be explained by 'adaptation of excitation'. This is further supported by the response of neurons in which application of BMI decreases the DI (e.g Fig. 19). If the neuron's response to ipsilaterally directed motion would be reduced due to the strong onset response evoked by motion starting in the RF, then it could be expected that this 'adaptation of excitation' would persist after the magnitude of the onset response is further increased by BMI. However, the neuron starts to respond to ipsilaterally directed motion upon application of BMI for IID positions which were unresponsive without BMI. Therefore

inhibition must be contributing to the motion-direction sensitivity of cortical neurons of *R. rouxi*.

The same holds true for neurons in which the magnitude of the medial RF border shift is decreased by BMI (Fig. 22). In these neurons the shift would be expected to increase if shifts were due to 'adaptation of excitation' as stated by Ingham et al. (2001). However, with BMI the medial RF borders evoked by ipsilaterally directed motion were shifted more ipsilaterally whereas the medial RF borders evoked by motion in the opposite direction remained largely unaffected (Fig. 30), resulting in a decrease of shift. The results show again that GABAergic inhibition contributes to RF shifts in a subset of cortical neurons rather than 'adaptation of excitation'.

As shown in Fig. 29, response strength increased by about the same amount for both directions of motion in neurons with a decrease of DI during BMI application. Thus, inhibition is not specific to the non-preferred direction. In the first part of this study it was shown that the spatio-temporal parameter combination (IPI and IID step) determined the motion-direction-sensitive responses of neurons in the auditory cortex. These results were confirmed in the second part (Fig. 28). For specific parameter combinations, specific responses appear under blockade of GABAergic inhibition that were not seen for other parameter stets of motion (Fig. 28c). Inhibition seems to work with specific time constants, and is activated by distinct spatio-temporal parameter sets.

The spatio-temporal pattern of inhibition is responsible for several important functions of neurons in different nuclei of the auditory system of bats. Park et al. (1996) showed, that the latency of inhibition is important for IID selectivity in neurons in the lateral superior olive in the free-tailed bat, and is influenced by sound intensity at the inhibitory ear, i.e the azimuth position of a sound source. Interdependence of the processing of spatial and temporal sound parameters could be shown in neurons in the medial superior olive of the free-tailed bat (Grothe et al., 1997) and the IC of the big brown bat (Koch & Grothe, 2000). Koch & Grothe (2000) demonstrated that this interdependence was modulated by glycinergic and GABAergic inhibition. Klug et al. (2000) reported, that intensity dependent latency shifts could be modified in nuclei of the central auditory system by manipulation of inhibition. Further evidence for the involvement of inhibition in processing of temporal information comes from delay-tuned FM-FM combination-sensitive neurons in bats (Olsen & Suga, 1991, Saitoh & Suga, 1995) as well as from mechanisms for duration tuning of neurons in the IC of the big brown bat (Casseday et al., 1994). Calford & Semple (1995) showed that monaural inhibition influences forward masking in neurons of the primary auditory cortex of cats. This is

important, in that motion-direction sensitivity was also observed in monaurally driven neurons in the cortex of the rufous horseshoe bat in the first part of the present study. Furthermore, coding of sequential sounds in cortical neurons has been shown to be influenced by spectral composition (Brosch & Schreiner, 1997) as well as of intensity of consecutive tones (Phillips et al., 1989) indicating that additional mechanisms different from adaptation contribute to the temporal processing of sounds.

All those interactions were shown for the processing of stationary sounds, i.e. sounds that do not move in azimuth. The dynamic responses of neurons in the auditory cortex of the horseshoe bat are not independent of the static RF properties as shown in the first part of this study, and in a first approach it can be the assumed that the stationary spatio-temporal pattern of inhibition certainly influences the processing of dynamically changing IIDs. However, the dynamic aspect of neuronal interaction in cortical microcircuits might contribute to motion-direction sensitivity. The responses of a single neuron would therefore rather reflect the effect of BMI not only on the recorded cell but also on the local circuitry, as due to diffusion the microiontophoretically applied drug exerts its effect in a limited neural space. In the auditory cortex of gerbils BMI diffusion was estimated to be about 400 µm maximum for a microiontophoresis with 40 nA current and 5 min duration (Foeller et al., 2001). As ejection currents and duration were in a comparable range in the present study, local cortical networks of this size potentially contribute to the response properties of cortical neurons observed in this study.

5.2.2 Role of adaptation

For neurons in which the DI or RF shift increased with BMI, ‘adaptation of excitation’ is a good candidate mechanism to explain the observed response properties at least in some cases. An increase of the RF shift as shown in Fig. 26 can be a consequence of widening of the static RF to both sides during the application of BMI. Responses are now evoked from position located more on the ipsilaterally and contralaterally side of the azimuth, respectively, and borders where the sound is entering the RF drift in opposite directions. This would agree with data presented by Ingham et al., (2001). A shift of the 50 % cut-off of static IID functions due to BMI was indeed found in over 50 % of neurons in the IC of the mustached bat (Park & Pollak, 1993), supporting the view that the increase of motion induced RF shifts during BMI application is due to ‘adaptation of excitation’. Adaptation would also explain the response changes from RF shift to directional preference of cortical neurons. As the strength of the

response evoked from the first position by ipsilaterally directed motion increases under BMI (e.g. Fig. 23), the responses from positions located further on the trajectory of the apparent motion stimulus might be suppressed by adaptation. The RF for ipsilaterally directed motion narrows, and the neuron's response type changes to directional preference rather than RF shift. As the response evoked by contralaterally directed motion is concurrently increased by BMI, the DI is markedly raised in such neurons. In addition, the number of spikes would increase stronger for the preferred direction than for the non-preferred direction (onset-response excluded) as shown in Fig. 29. The same mechanism can be supposed to underlie the response of most neurons in which the DI is increased by BMI. As evident in Fig. 20, the response peak evoked by contralaterally directed motion is sharpened and shifted to more ipsilateral positions during the application of BMI. The sharpening could be explained by adaptation, as the response strength raised by BMI would further suppress responses to positions beyond the response peak. The shift of the response peak could be explained by a general broadening of the RF under BMI.

5.2.3 Neurons not affected by BMI

BMI had no effect on at least 33 % of neurons in the auditory cortex. One possibility is that motion-direction sensitivity in these neurons is created at lower levels of the auditory pathway, and no mechanism underlying these responses can be deduced from cortical recordings. As BMI influences only $GABA_A$ receptors, in can not be ruled out that inhibitory processes mediated by others than $GABA_A$ receptors (e.g. $GABA_B$ receptors) are involved. A third possible explanation that BMI was not effective in changing the response evoked by apparent motion would be a too low dose of BMI to block all $GABA_A$ receptors.

5.2.4 Effects of GABA

As the microiontophoretic application of GABA often abolished a neurons response completely, especially when the predrug spike-count level was low, only few neurons could be monitored. In neurons that showed a response peak to motion in only one direction (e.g. Fig. 21) no differentiated effect of GABA could be expected as GABA either decreased the response peak in a moderately way leaving the DI largely unaffected (Fig. 21b), or silenced the neuron completely. The neuron in Fig. 27 is one of the rare cases in which both directions

of motion were responded to in the predrug condition and the antagonistic effect of GABA and BMI on motion-direction sensitivity could be shown.

In three neurons little influenced by BMI, the RF shift was markedly decreased by GABA (Fig. 24b). The remove of RF shift by GABA could be explained by ‘adaptation of excitation’ as mechanism underlying the shift observed in the predrug condition. If the 50 % cut-off of the static RF is shifted to more contralateral positions by the application of GABA, then the response to contralaterally directed motion is now evoked from positions further on the contralateral side compared to the predrug condition. The response profile and thus the 50 % cut-off evoked by ipsilaterally directed motion would be less influenced by GABA if the response strength is decreased due to ‘adaptation of excitation’, irrespective of the width of the static RF. Under this assumption, the shift evoked by motion to both directions in the predrug condition would be decreased with GABA. However, as can be seen from Fig. 24c, the 50 % cut-off of the RF borders evoked by motion in both directions is shifted to more ipsilaterally positions during application of BMI without changing the relative position of the two borders, i.e. leaving the magnitude of RF shift largely unaffected. Thus, BMI and GABA had no clear antagonistic effects on motion-direction sensitivity in this case. The reason for this may be, that due to an insufficient dose of BMI the increase of width of the static RF was not large enough, so that no clear increase of RF border shift due to motion in both directions could be observed.

6 General discussion

The results of the present study have shown, that neurons in the auditory cortex of the rufous horseshoe bat are sensitive to the direction of auditory apparent motion. The auditory cortex is generally considered to be the highest level of the auditory pathway. Thus, the question remains whether motion-direction sensitivity as seen at cortical levels is already present at lower levels of the auditory pathway and merely passed through to higher levels, or whether cortical processing essentially adds to the analysis of dynamic spatial auditory information. Schlegel (1980), Kleiser & Schuller (1995) and Wilson & O'Neill (1998) demonstrated motion-direction-sensitive neurons in the IC of different bat species. The basic features of motion-direction sensitivity (e.g. the direction of RFs shifts) were the same as in the auditory cortex of *R. rouxi*. However, there seem to be distinguishing details in that neurons in the IC of bats have not been reported to have a strong directional preference, so far. Furthermore, neurons in the auditory cortex of *R. rouxi* could change their response properties with changing parameter sets of motion, i.e. the response could change from a shift of RFs to a directionally selective response. This has not been reported for neurons in the IC of *R. rouxi* (Kleiser & Schuller, 1995). Wilson & O'Neill (1998) found that neurons in the IC of the mustached bat could respond with a shift and a directional bias at the same time but the directional bias was not as prominent as it can be seen in the auditory cortex in the present study. Thus, although subcortical features of motion processing are reflected in the response of cortical neurons, the data presented here suggest that some specific processing occurs in the auditory cortex of bats, increasing the specificity to distinct parameters of motion. This is supported by Altman (1994), who reported number and specificity of neurons responding to motion direction to increase from the level of the IC to the auditory cortex in the cat. However, features of thalamic neurons were comparable to those of cortical neurons, suggesting that parts of the additional processing already have occurred in the auditory thalamus, or that thalamocortical feedback loops are involved in motion processing.

The notion that motion processing is refined at thalamocortical levels is further supported by the finding, that cortex specific GABAergic inhibition contributed to motion sensitivity in the auditory cortex of *R. rouxi*. That is, features of responses to acoustic motion were further shaped in the auditory cortex by the means of inhibition. Studies investigating the role of inhibition in motion processing in the auditory cortex are sparse so far. Except for this study, only Altman & Nikitin (1985) addressed this question for the auditory cortex of the cat and

found, that inhibition was involved in creating motion-direction-sensitive responses. However, considering the results of Altman & Nikitin (1985) as well as the results reported here, it seems reasonable to assume that the auditory cortex might represent a high-order stage involved in motion processing.

However, it is still unclear from this study whether a special motion processing system exists in the auditory system. All neurons investigated in this study responded well to static stimuli. Furthermore, the responses to auditory motion were not independent from the static response properties, i.e the shape and azimuthal position of static RFs. The spatio-temporal parameters of motion were important for the response of neurons rather than velocity. Thus, the results are in contradiction to psychophysical studies on humans, which argue for a special motion processing system (e.g. Strybel et al., 1998; Dong et al., 2000). In addition, no specialization of any cortical field investigated in this study to auditory motion per se was found. About the same number of neurons in all fields responded to motion, showing differences only to spatial and spatio-temporal parameters of motion. Thus, the results of imaging studies on humans showing specializations of certain cortical areas for motion processing (e.g Griffith et al., 1994; Baumgart et al., 1999) are not corroborated by the data presented here. Species specific differences and the development of more abstract processing of motion in humans may be the reason for this discrepancy. In addition, it is possible that cortical areas responsive to auditory stimuli different from those described so far for the rufous horseshoe bat exist. Eiermann & Esser (2000) described an area in the frontal cortex of the short-tailed fruit bat (*Carollia perspicilliata*) sensitive to auditory stimuli. This area is supposed to be important for goal-directed behaviors guided by auditory information. Furthermore, Romanski et al. (1999) found that projections from auditory areas thought to be involved in spatial processing, target spatial domains of the frontal lobe in primates. These findings are supported by a neuroimaging study that showed the selective activation of frontal areas during sound localization tasks in humans (Bushara et al., 1999). Thus, areas involved in higher processing of spatial information of sounds might exist in the frontal portions of cortex of *R. rouxi*, too.

The mechanism underlying motion sensitivity in the auditory system is still not completely understood. Although inhibitory mechanisms have been supposed to contribute to motion-direction sensitivity (e.g. Altman & Nikitin, 1985; Wagner & Takahashi, 1992), pharmacological manipulation of neurons revealed no direction specific inhibition in the auditory system (Kautz & Wagner, 1998). This is in accordance with the results presented in this work and also favors the point of view that no specific motion processing system exists in the auditory cortex. It is more likely, that static spatial information and motion information

are integrated and processed together in the auditory cortex of the rufous horseshoe bat. Thus, GABAergic inhibition influencing the static RF properties also contributes to the response to auditory motion. Motion detectors like the one proposed by Wagner & Takahashi (1992) are not necessarily required to explain the responses to moving stimuli seen in this study.
It can not be excluded, that the stimuli used in the present study to generate auditory motion were not optimal for probing cortical neurons in respect to motion processing. The simulation of acoustic motion using linear changes in interaural level differences is only a first approximation of the real situation. Rosenblum et al. (1987) showed, that in addition to interaural intensity and time differences, Doppler effects can be used for localization of a moving sound source by humans. This is supported by Dong et al. (2000) who supposed that the strong auditory motion aftereffect seen in human subjects in their study was due to the use of free field motion that provides a larger variety of motion cues. In addition, Müller & Schnitzler (2000) proposed that horseshoe bats can make use of acoustic flow information for target localization. Thus, a more realistic simulation of acoustic motion might reveal response features of neurons or specializations of cortical fields not seen in this study.

In conclusion, the auditory cortex of the rufous horseshoe bat processes temporal and spatial aspects of auditory motion differentially in the different fields. GABAergic inhibition contributes to motion processing in at least part of neurons. Thus, the auditory cortex adds substantially to motion processing and can be seen as high-order processing stage, extracting further details from neural responses to auditory apparent motion brought up from subcortical nuclei. The use of more complex and realistic stimuli to simulate apparent auditory motion is expected to further elucidate the role of the auditory cortex in motion processing in the future.

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8 Abbreviations

AI: primary auditory cortex
ADF: anterior-dorsal field
BMI: bicuculline methiodide
CF: constant frequency
DDF: dorso-dorsal-field
DI: directionality index
EE: excitatory-excitatory binaural response
EI: excitatory-inhibitory binaural response
EO/mon: monaural response
EO/F: excitation by monaural stimulation of one ear and facilitatory influences of the other ear
EO/F&I: excitation by monaural stimulation of one ear and a mixture of inhibitory and facilitatory influences of the other ear
FM: frequency modulated
GABA: γ-aminobutyric acid
IC: inferior colliculus
ICx: external nucleus of the inferior colliculus
IID: interaural intensity difference
IPD: interaural phase difference
IPI: interpulse interval
ITD: interaural time difference
LSO: lateral superior olive
MSO: medial superior olive
PDF: posterior-dorsal field
PF: primary field
RDF: rostro-dorsal field
RF: receptive field
SC: superior colliculus
SFM: sinusoidally frequency modulated
vMGB: ventral division of the medial geniculate body

9 References

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10 Curriculum Vitae

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11 Publications

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