Aspects of Sound Localization and Spatial Attention in Barn Owls and Rats.

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1. General Introduction

1.1. Effects of attention on perception

In his famous statement, James (1890) described that attention is a specific mental state in which the mind takes possession "*in a clear and vivid form*" of one object (or thought) out of many at a given time. What "clear" and "vivid" mean in psychological terms, is, that attention somehow influences perception, i.e. that attention changes the way how stimuli of the environment (or how internal states) are perceived. Since the time of James, many researchers in experimental psychology could show quantitatively, that attention enhances sensitivity and acuity and that it decreases reaction time to environmental stimuli (e.g. Posner, 1980; Broadbent & Gregory, 1964, Eriksen & Eriksen, 1974, Müller & Findlay, 1987, Treisman & Gelade, 1980). So, attended stimuli have an important benefit: They become more easily noticed and stay longer in mind than other stimuli. Unattended stimuli, however, have to pay the cost that they are often neglected.

Psychological findings suggested three separate systems that carry on the major attentional functions: Orienting to stimuli, recognizing target events, and maintaining the alert state. This thesis will focus on orienting to sensory stimuli as described in a separate chapter where 'orienting' will be addressed to as 'spatial attention' (see 1.3.). Orienting to a point in space is typically accompanied by an overt movement e.g. of the eyes or the head. Thereby, the subject signals that it is paying attention to a certain part of the environmental scene. This is so whether the subject's attention is summoned exogenously by salient stimuli or endogenously by internal states and goals. However, orienting can also occur in the absence of overt signs, a phenomenon termed 'covert' orienting (Posner, 1980). Both, overt and covert orienting improve perception of sensory stimuli, indicating that orienting is an important attentional function.

1.2. Effects of attention on the physiology of single neurons

Attention can be understood in neurophysiological means as the modulation of an internal representation, i.e. a reversible impact on feature-specific neurons that code for a sensory or goal-related representation. So far, mainly visual pathways were investigated for attentional effects on neural activity. Two important effects were shown on the level of single neurons: First, attention modulates neural excitability (Motter, 1993, 1994a, b; Treue & Trujillo, 1999). This means that attention enhances the response to attended stimuli and reduces responses to unattended stimuli, thus influencing the neuron's sensitivity. Second, attention sharpens tuning characteristics (Desimone & Duncan, 1995). In this case, the specificity of a neuron is enhanced in the attention condition which results in a more accurate representation of a stimulus in the neuron's response profile. Both effects are reasonable in the light of psychophysical findings, however, only the first has been found consistently reproducible.

1.3. Spatial attention

Spatial attention is a specific form of attention and related to the perception of space. It can be described as a perceptual window, which allows stimuli from a certain direction of a scene to become more important than others. Thus, spatial attention exhibits all typical characteristics of attention in general: it enhances sensitivity and acuity and reduces reaction time to environmental stimuli falling into the attended area. This benefit is paralleled by a cost for stimuli that fall into the unattended area.

Internal representations of space may be generated by space-specific neurons which respond only to parts of the external space. In the visual system, the construction of internal space starts at the level of the retina: the ganglion cells are space-specific and topographically arranged insofar that neighboring ganglion cells represent neighboring parts of the external space. The retinal topography is an important characteristic of the visual system and observable throughout most of the visual pathways. In the auditory system, the construction of internal space is profoundly different (Knudsen, 1987): the information at the basilar membrane contain no spatial information per se. Spatial directions have to be calculated from differences between

the phase angles and the amplitude of a sound wave travelling to the two ears. Thus, a separate pathway of the auditory system has evolved which processes binaural cues to generate an internal coordinate system of sound source directions that is represented by space-specific neurons in the brain (barn owls: Olsen et al, 1989, Knudsen, 1995; mammals: Dräger & Hubel, 1976; Palmer & King, 1982; Middlebrooks & Knudsen, 1984; King & Hutchings, 1987). In the barn owl, two distinct internal representations of auditory space have been described: One in the midbrain and one in the forebrain (Cohen & Knudsen, 1998).

Spatial representations in different sensory systems interact on the perceptual and neurophysiological level (Stein & Meredith, 1994). Multimodal neurons that receive input from different sensory systems have been suggested as neural substrate for this phenomenon (Wallace, Meredith & Stein, 1998; Jay & Sparks, 1987 a,b; King & Palmer 1985; for review see Stein, Wallace & Meredith, 1995). Importantly, there are also well described interlinks between spatial attention at different modalities (Spence & Driver, 1996, 1997, for review see Driver & Spence, 1998).

Neurons in the midbrain of birds and mammals are intensively discussed in the light of spatial attention, because they seem to be adequate neural substrates to show attentional influences. Neurons are space-specific and form maps of auditory and visual space; they are often bimodal, and they are closely related to premotor-output processing. These characteristics evolved because of their function in organizing overt shifts of attention, i.e. orienting behavior. Investigations on effects of covert orienting of attention on midbrain structures, however, are only sparse, leaving unclear whether the midbrain is supporting attention both in overt and covert spatial attention. Two studies concentrating on visual responsive neurons (Wurtz, Goldberg & Robinson, 1982; Robinson & Kertzman, 1995) found that the activity of midbrain neurons was not different between the conditions that the receptive field (RF) was within the attended area or outside, thus indicating that these neurons may be involved in eliciting overt orienting but that they were not modulated by covert orienting of attention. Later, this result was challenged by a study that investigated neurons from midbrain sites where motor-command related information is encoded. Here, a clear difference was found between the conditions that a movement towards an attended area or an unattended area was made (Kustov & Robinson,

1996). However, this study used electrical stimulation, so that the responsiveness of motor-related midbrain sites to sensory stimuli could not be investigated.

The neural network that mediates acoustically-evoked orienting in barn owls became a model system for processing of auditory spatial information and generating orienting behavior. In the midbrain of barn owls, information about the location of a sound source is represented in the activity of space-specific neurons which are arranged in a map of space (Knudsen, 1982). In my thesis, this system was chosen to investigate influences of covert orienting of attention on activity of midbrain neurons. To be able to compare the results with the auditory system of mammals, midbrain neurons of rats were investigated in parallel.

1.4. Aims of the thesis

The aim of this study was to investigate midbrain neurons in two model systems, the barn owl and the rat. Here, three experiments dealing with different questions on perception, internal representation of space, and spatial attention are presented. Investigations concentrated on the auditory system, but were addressed also to visual-auditory interactions. Neurophysiological measurements were conducted in auditory midbrain structures. Experiments are described in detail in the chapters 3., 4., and 5. which are organized like scientific publications.

In the first experiment, I carried out measurements that were performed to investigate the influence of spatial attention on sound localization behavior in barn owls. This study helped to get insight in the abilities of barn owls to bias their perception, i.e. to engage endogenous mechanisms that alter their sensitivity, acuity, or reaction speed to future events. The behavioral paradigm was originally devised by Posner (1980), but in this experiment a design from Spence & Driver was adapted (Spence & Driver, 1996).

Secondly, an experiment in anaesthetized rats was conducted to investigate a candidate for an internal representation of auditory space in the rat's brain. The midbrain of the rat contains a structure, the superior colliculus (SC), where space-specific neurons has been found in other mammals such as mice (Dräger & Hubel, 1976), cats (Middlebrooks & Knudsen, 1984), guinea pigs (Palmer & King, 1982) and monkeys (Jay & Sparks, 1987). However, although the rat was often used in behavioral studies testing sound localization abilities, the representation of auditory

space in the rat's midbrain had not been investigated so far. This study was introductory to further investigations in spatial attention in rats (Gaese, 1999).

In the third experiment, a combined behavioral and neurophysiological investigation was performed to measure the influence of spatial attention on neurons in the midbrain of barn owls. The barn owl's midbrain contains a well studied map of auditory space (for review: Knudsen, 1995), i.e. a precise internal representation created by orderly arranged space-specific neurons. In my experiment, again shifts of spatial attention were induced by the behavioral paradigm. The question was, how spatial attention influences midbrain neurons, which contain a representation of auditory space at an early level of auditory processing that is also a premotor output stage for saccadic movements of the head (DuLac & Knudsen, 1990; Wagner, 1993).

The thesis will close with a general discussion, which summarizes the main results of the three experiments. The influence of attention on perception and internal representations of auditory space in barn owls and rats will be discussed with a special focus on the interaction between sensory and premotor processing of auditory information.

2. General Material and Methods

In this chapter, the main techniques used for the measurements are explained briefly. The separate experimental chapters (chapter 3.,4., and 5.) contain the most detailed information how the measurements were conducted.

Three separate experiments combining behavioral and neurophysiological measurements were conducted. Two barn owls were trained to perform a crossmodal-spatial cueing paradigm adapted from Spence & Driver (1996). In a next step, combined behavioral and neurophysiological data was obtained from the same owls performing a cue-directed selection paradigm. Additionally, neurophysiological measurements in the colliculus superior (SC) of anaesthetized rats were carried out.

2.1. Influence of spatial attention on sound localization in barn owls

Auditory spatial attention has almost entirely been investigated in humans (e.g. Spence & Driver, 1996). I therefore started out to investigate auditory attention in a well established animal model for sound localization, the barn owl. Most of the behavioral studies in humans used a cueing paradigm devised by Posner (1980) which provides valid or invalid information about the spatial position of upcoming target stimuli. These paradigms allow the subject to orient attention according to the information provided by the cue. Since this information is not always correct, responses to target stimuli presented within the attended area can be compared with responses to the same target stimuli presented outside the attended area.

In my experiment, a visual cue in front of the animal pointed towards the side (80% during a session, valid trials) or away from the side (20%, invalid trials) of a subsequently occurring peripheral auditory target (a noise burst presented via one of two speakers, 30° and 50° in each hemisphere). The exact position of the auditory target was not cued and had to be localized with a head turn. Head movement trajectories were obtained with a magnetic tracking system to evaluate response latency and head turning angle.

For analysis, I asked whether auditory stimuli appearing at the cued side were processed differently to stimuli appearing at the uncued side, indicating the influence of a spatial attention mechanism.

2.2. Coding for auditory space in the SC of the rat

There is a profound body of work on the involvement of different nuclei in sound localization and on binaural information processing in the rat. However, the representation of acoustic space in the rat brain has not been investigated so far. Therefore, I determined spatial response properties of SC neurons in order to investigate whether a representation of auditory space exists in this midbrain structure.

Extracellular recordings from several neurons were conducted simultaneously in the SC of anesthetized rats using an array that consisted of six varnish coated tungsten electrodes. Units recorded at the same electrode were separated according to their spike waveforms. For auditory stimulation from different directions a hoop-based stimulation system was used. Broadband noise bursts were presented via a hoop-mounted loudspeaker from different positions with a spatial resolution of 20°. At every position, 20 stimulus repetitions at 1 Hz repetition rate were given. From the spatial response profiles, best azimuthal or elevational speaker position and half-maximal response width were calculated. Recording sites were defined after reconstruction of the electrode track based on electrolytic lesions. The mediolateral position from the midline and the rostrocaudal position in relation to the expansion of the SC of every recording site were determined.

Finally, a linear correlation was made between the position of the recording site and the best azimuthal or elevational position of the sound source to look for gradients that suggest a map of auditory space in the rat's SC.

2.3. Influence of spatial attention on auditory midbrain neurons in the barn owl

Investigations how spatial attention influences midbrain neurons are very sparse and concentrated on the visual system (Wurtz, Goldberg & Robinson, 1982; Robinson & Kertzman, 1995). This lack is surprising, since midbrain structures, which are suggested to play generally an important role in orienting behavior and also in the orientation of attention (Posner, 1994), often contain auditory responsive neurons. Therefore, I carried out combined behavioral and neurophysiological measurements in barn owls to investigate the presence of attentional effects in sound localization behavior and in activity of auditory midbrain neurons.

The same two barn owls which participate in the first experiment were trained to perform a cue-directed selection paradigm: A visual cue in front of the owl pointed towards the side of the next relevant auditory target. The cue was followed by two simultaneously presented noise bursts (100 ms, one in every hemisphere) that were generated by independent waveform generators. Independent noises are represented in separate neural populations in the barn owls midbrain (Takahashi & Keller, 1994) and should therefore be distinguishable on the behavioral level. However, this had never been tested in detail. The relevant sound source, as indicated by the cue, should be localized with a head turn that was measured with a tracking system. From the head movement trajectories latencies and head turning angles were evaluated. Owls could make correct responses, incorrect responses or no-responses. The direction of the response could be rightward or leftward which was of interest for the neurophysiological measurements.

Under anesthesia, up to three varnish-coated tungsten electrodes were permanently implanted into the owl's brain. Electrodes were mounted in custom-build microdrives that allow for vertical movement of the electrode to isolate new units. During surgery, anesthetized owls were stimulated via earphones. Neural activity recorded from the electrodes was tested for auditory evoked responses and for spatial response profiles as determined by interaural time differences and interaural level differences. Electrophysiological characteristics in combination with stereotaxic coordinates allowed to decide if the position of the electrode tip was within the OT. Microdrives were fixed to the owl's skull when a preferred OT side was found. After implanting the electrodes, the opening in the skull was closed with dental cement and the skin was reattached. Implanted owls behaved completely normal after recovery from the surgery.

When the implanted owls performed the cue-directed selection paradigm, neural activity was recorded from the electrodes. At the beginning of a behavioral session, new units were isolated by lowering the electrode tips into deeper parts of the OT. Spike rates of OT units were analyzed according to response types (correct, incorrect, and no response) and to response sides (leftward or rightward).

A mechanisms based on mean spike rate was suggested which could explain the behavioral findings and reflect top-down influences from higher brain areas on auditory midbrain neurons. Influences on sensitivity of auditory evoked activity and on orienting-related activation could be distinguished, since auditory stimulation and head orienting were separated in time. Results were compared with studies that investigated attentional effects on the execution of saccades in the monkey SC (Wurtz, Golberg and Robinson, 1982; Robinson & Kertzman, 1995; Kustov & Robinson, 1996).

3. Influence of spatial attention on sound localization in barn owls

Attentional influence on sound-localization behavior of barn owls was investigated in a cross-modal spatial cueing paradigm. After being cued to the most probable target side with a visual cueing stimulus, owls localized upcoming auditory target stimuli with a head turn towards the position of the sound source. In 80% of the trials cueing stimuli pointed towards the side of the upcoming target stimulus (valid configuration), and in 20% they pointed towards the opposite side (invalid configuration). I found that owls initiated the head turns by a mean of 37.4 ms earlier in valid trials, i.e. mean response latencies of head turns were reduced by 16% after a valid cueing stimulus. Thus, auditory stimuli appearing at the cued side were processed faster than stimuli appearing at the uncued side, indicating the influence of a spatial-selective attention mechanism. Turning angles were not different when owls turned their head towards a cued or an uncued location. Other types of attention influencing sound localization, e.g. a reduction of response latency as a function of the duration of cue-target delay, could not be observed. This study is the first attempt to investigate attentional influences on sound localization in an animal model.

3.1. Introduction

Several studies have shown that spatial selective attention can modulate sound localization behavior in humans (e.g. Spence & Driver, 1994; Mondor & Zatorre, 1995). Using a paradigm adapted from Posner (Posner et al. 1980), auditory attention was guided towards desired locations by presenting cueing stimuli prior to the target stimulus in these studies. It was observed that responses to auditory events at cued locations are faster compared to responses to auditory events at uncued locations. The faster processing of auditory stimuli was related to the different attentional states at cued and uncued locations.

Comparative investigations in auditory spatial attention, however, have so far been neglected. I therefore studied auditory spatial attention at the behavioral level in a well

established animal model for sound localization, the barn owl. During hunting from a perch, barn owls initially turn the head towards faint sounds (Konishi, 1983), apparently waiting for further sounds that might indicate the presence of a potential prey. This behavior demonstrates that barn owls can draw attention to particular auditory events. Furthermore, localization behavior is elicited only by specific sounds, indicating, that barn owls use selective attention to assess the relevance of a sound source (Wagner, 1995).

3.2. Methods

Experiments were carried out with two barn owls (*Tyto alba*) obtained from the institute's breeding colony. Since barn owls are typically active during evening and night, training sessions started within two hours after dusk. All procedures were approved by the Animal Care Committee of the RWTH Aachen and the Regierungspräsidium Köln.

3.2.1. Owl handling

Before the experiments, owls were fixed in the aviary to a perch with leather bands which were permanently bound to their legs. On this perch, owls were carried to the laboratory and placed into the center of the behavioral apparatus. Otherwise they were unrestrained and could make head, body, or wing movements. However, with respect to their main hunting strategy, owls mostly sat upright on the perch and gazed to frontal directions, waiting for relevant stimuli. Two infrared-sensitive video cameras were installed to monitor the owls' general behavior during the experiments.

3.2.2. Stimulus design

The behavioral setup (Fig. 3-1a) was mounted in an anechoic chamber (IAC, base: 2 x 2 m, height: 2.6 m) which was completely darkened during the measurements. A custom-built display for the cueing stimulus was placed in front of the owl, about 45 cm from the owl's head. The display contained horizontally arranged LEDs, in total covering 5° of visual angle. The

number of LEDs was either 2 (Experiment 1) or 3 (Experiment 2, see below). Acoustic target stimuli were generated by a waveform generator (WG1; all instruments from Tucker Davis Technologies), regulated by an attenuator (PA4), and were randomly switched between 4 loudspeakers in the owl's periphery (positions: 27° and 47° to the left and right of the owl's midline at 0° elevation, switched by SS1).

3.2.3. Experimental design

In Experiment 1 (Exp. 1), the display for the cueing stimulus consisted of 2 LEDs. When the owls gazed towards a window of 10° around the center of the display, the cueing stimulus was presented: Both LEDs were switched on for 200 ms, then one LED was switched off and the stimulus hemisphere was cued with the remaining LED for another 300 ms. The target sound, a noise burst (100 ms, 50 dB SPL), followed after a variable cue-target delay (200 or 300 ms).
The cue-target delay was always determined as the time elapsing between the offset of the remaining LED and the onset of the target sound.

In Experiment 2 (Exp. 2), the display for the cueing stimulus consisted of 3 LEDs, with two lateral LEDs and a central fixation-LED. The fixation-LED extinguished only during reward delivery and served as a reference for orientation towards the display. When owls gazed forward, one lateral LED was switched on for 500 ms. This LED pointed towards the most probable stimulus hemisphere. The auditory target-stimulus was presented after a variable cue-target delay (200-500 ms, 100 ms steps).

In 80% of the trials, cueing stimuli pointed towards the side of the upcoming target stimulus, and in 20% they pointed towards the opposite side. Cueing stimuli which were spatially consistent with the target side will be referred to as 'valid cueing stimuli', the others will be referred to as 'invalid cueing stimuli'.

The required behavioral response was a head saccade towards the source of the target stimulus. Correct trials were rewarded with small portions of chick meat delivered from a custom-built feeder. Incorrect responses (see below) were followed by a manually-applied correction trial that was omitted from analysis. Correction trials consisted of the only presentation of the target sound from the same source as in the original trial. This was repeated

until the bird responded with a head turn towards the sound source, which was rewarded. In non-response trials, owls had to wait for 5 s until they could start the next trial. In a correction trial or during waiting periods the fixation-LED (only in Exp. 2) was continuously switched on.

Head saccades were measured with a magnetic motion-tracking system (miniBIRD, Ascension Technology Corporation, Burlington, U.S.A, 120 Hz sampling frequency) attached to the owl's head during the experiment. Using the motion-tracking technique, the direction of gaze could reliably be determined from the orientation of the head, as eye movements are virtually absent in owls (Steinbach & Mooney, 1972).

3.2.4. Data analysis

Only horizontal head movement trajectories, i.e. rotations around the Z-axis, were analyzed. Turning angles of head saccades were calculated as the maximum of the horizontal trajectory. Additionally, locations of the loudspeakers relative to the orientation of the owl's head were calculated by measuring the head orientation at the onset of the auditory stimulus and subtracting this angle from the absolute stimulus position. Thereby the external coordinate system was transformed into an owl-centered coordinate system. The maximum deviation of head orientation at sound onset was preset to +/- 5° by the window around the display.

As response latency, I defined the time elapsing between the onset of the auditory stimulus and the point when the head saccade exceeded a threshold of 8° minimum turning angle. Due to this threshold criterion, latencies were generally prolonged by about 12 ms with respect to the exact start of the head saccade. Response latencies were afterwards log-transformed for multivariate statistics.

Head turns were classified into 3 response types by a computer algorithm using simple classification attributes: (i) correct responses, i.e. head turns towards the sound source with a minimum angle of 8°, (ii) incorrect responses, i.e. head turns away from the sound source with a minimum angle of 8°, and (iii) non-responses, when the head-turning angle was < 8°, or the latency of the head turn > 1300 ms. For correct and incorrect responses, the maximum head-turning angle, i.e. the perceived target direction, had to be stable within 2° for at least 85 ms. Both response types generally consisted of a single head movement. Head shakings which

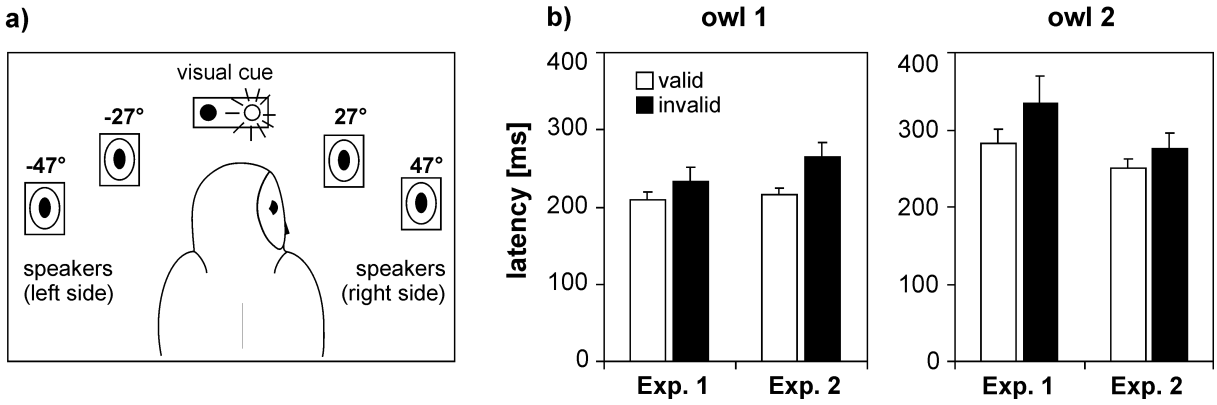

Fig. 3-1) Shifts of spatial attention selectively modulate sound localization in barn owls in an visual-auditory cueing paradigm. (a) Schematic illustration of the experimental setup. An indirect visual cue located in front of the owl pointed in 80% of the trials towards the side of an upcoming auditory stimulus (valid configuration) and in 20% towards the opposite side (invalid configuration). Owls gazed to frontal directions during presentation of the cueing stimulus and responded to the peripheral auditory target stimulus with a head saccade towards the sound source (located 27°or 47° to the side, 0° elevation). (b) Response latencies of head saccades towards peripheral auditory events are significantly shorter in valid trials than in invalid trials. Depicted are means and 95% confidence intervals of response latencies of owl 1 and owl 2 separated according to experiments (Exp.1 / Exp.2) and cue validity (valid: open bars / invalid: closed bars). Depending on owl and target side, mean response latencies were reduced by between 10% and 22% when the auditory target was presented at the attended side.

occurred in less than 6% of the trials missed the stability criterion and were omitted from analysis.

To quantify the stability of initial fixation, I calculated the magnitude and direction of the largest movement in a 200 ms-sliding window during the time prior to target presentation. Detailed analysis revealed that the owls made only small head movements while waiting for the target stimulus: In 84 % of the trajectories, the movements were smaller than 4°. Most of the early movements occurred at onset of the cueing stimulus, when the owls had gazed to frontal directions but not precisely towards the display. In Exp. 1, movement directions showed no consistent pattern with respect to the directional information of the cueing stimulus (Fisher's Exact Test; owl 1: $n = 2066$, $p = 0.33$; owl 2: $n = 912$, $p = 0.73$). In Exp. 2, movement directions differed significantly after leftward or rightward pointing cueing stimuli (Fisher's exact test; owl 1: $n = 1852$, $p < 0.01$; owl 2: $n = 1622$, $p < 0.01$). However, a separate analysis for each direction

of the cueing stimulus showed, that movement biases in only one out of 4 cases significantly pointed into the direction of the cueing stimulus (Chi^2 goodness-of-fit test; owl 1 cued rightward: $Chi^2 = 37.4$, $p < 0.01$).

3.3. Results

I obtained 4768 trials from owl 1 (2494 in Exp1, 2274 in Exp2) and 3557 trials from owl 2 (1442 in Exp1 and 2115 in Exp2). Owl 1 made correct responses in between 81% and 83% of the trials, depending on experiment, and owl 2 in between 63% and 77%. Multivariate statistics revealed, that the behavioral variability between sessions (Wagner, 1993) occurred independent of trial validity. Correct trials were therefore collapsed across sessions. Incorrect responses (see Methods) occurred, depending on owl and experiment, in between 3% and 9% of the trials, and non-responses in between 8% and 24% of the trials.

A clear effect of the validity of the cueing stimulus was visible in the response latencies (Fig. 3-1b) which indicates, that spatial attention modulates the processing of sound source location in barn owls. In valid trials, mean response latencies were significantly reduced by between 10% and 22% (mean 16%, i.e. 37.4 ms), depending on owl and experiment. A multivariate ANOVA which tested differences in response latencies between owls, experiments, or valid and invalid trials was significant ($F_{7,6452} = 33.2$, $p< 0.01$). Mean response latencies differed significantly between valid and invalid trials ($F_{1,6452} = 42.5$, $p < 0.01$) and between owls ($F_{1,6452} = 105.0$, $p < 0.01$). The effect of trial validity was robust and showed no interaction with owl ($F_{1,6452} = 0.08$, $p = 0.77$) or experiment ($F_{1,6452} = 0.12$, $p = 0.73$). Latencies generally were similar in both experiments ($F_{1,6452} = 0.02$, $p = 0.89$). Nevertheless, in owl 1 latencies increased slightly between experiments, whereas in owl 2 latencies slightly decreased, which led to a significant interaction between owl and experiment ($F_{1,6452} = 33.6$, $p < 0.01$, Fig. 3-1b). I also found, that the effect of trial validity did not depend on relative sound source position in both Exp. 1 and Exp. 2 (owl 1: $F_{1,3918} = 0.05$, $p = 0.82$, owl 2: $F_{1,2534} = 2.01$, $p = 0.16$).

Generally, the proportions of the response types (correct, incorrect, and non-responses) were similar in valid and invalid trials. In owl 1, however, I found a significant increase of

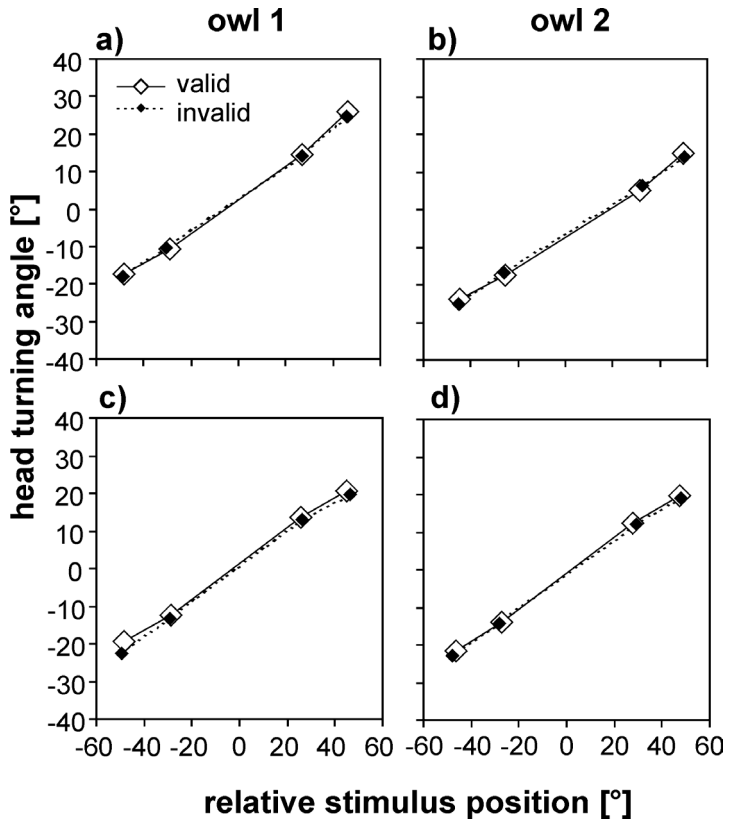

Fig. 3-2) Turning angles of head saccades showed no dependence on the validity of the cueing stimulus. After gazing to frontal directions, owls turned their head quickly towards an auditory target coming from a peripheral speaker located at 27° or 47°. Head turnings were measured with a magnetic motion-tracking system. Depicted are means of turning angles from valid (open diamonds / solid lines) or invalid trials (closed diamonds / dashed lines) in relation to the relative stimulus position. Results were separated according to owls (left column (a, c): owl 1, right column (b, d): owl 2) and experiments (top row (a, b): Exp. 1, bottom row (c, d): Exp. 2). 95% confidence intervals of relative stimulus positions and head-turning angles are smaller than the symbols (0.2° to 2.2°).

incorrect responses after an invalid cueing stimulus (Chi^2-test; Exp. 1: $n = 2143$, $Chi^2 = 13.3$, $p < 0.01$; Exp. 2: $n = 1999$, $Chi^2 = 6.8$, $p < 0.05$).

In contrast to response latencies, turning angles of head saccades in valid trials were generally similar to those in invalid trials (Fig. 3-2). In owl 2, an ANCOVA revealed that turning angles differed between relative sound source positions and between experiments ($F_{7,2533} = 2393.2$, $p < 0.01$). Interestingly, turning angles did not differ between valid and invalid trials ($F_{1,2533} = 1.5$, $p = 0.23$), which indicates, that they were not modulated by spatial attention. This result was similar between experiments ($F_{7,2533} = 0.159$, $p = 0.69$) and across relative sound source positions ($F_{7,2533} = 0.158$, $p = 0.69$). In owl 1, a more detailed analysis was performed. I observed that turning angles were not different between valid and invalid trials for most stimulus

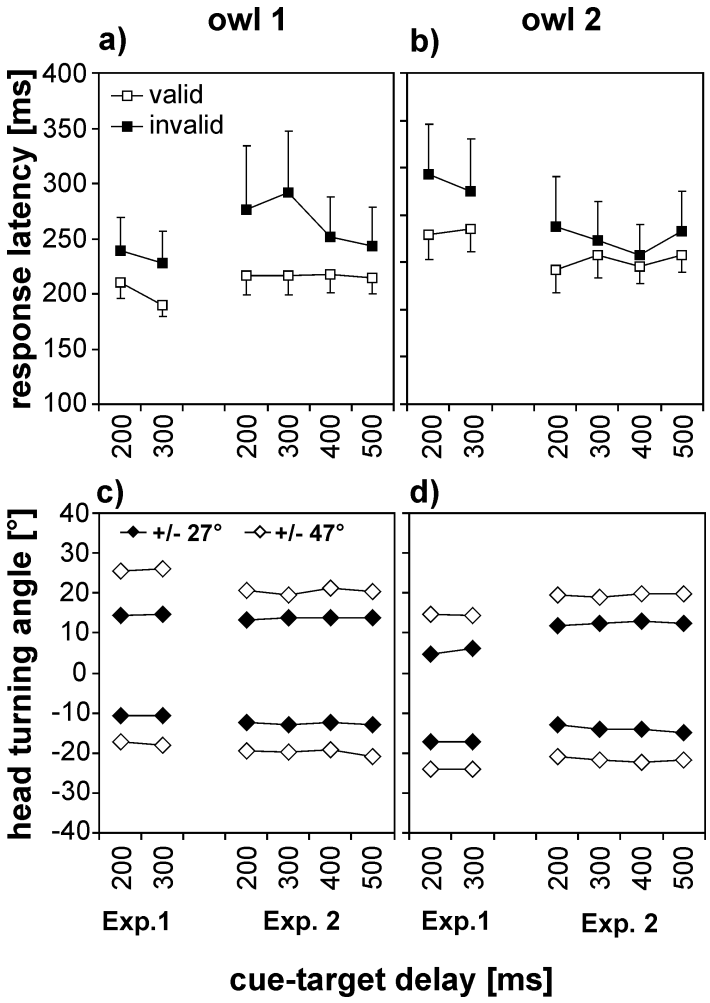

Fig. 3-3) The duration of the cue-target delay had no effect on response latencies or turning angles of head saccades in barn owls. (a, b) Response latencies (means +/- 95% confidence intervals) are depicted separately for valid trials (open squares) and invalid trials (closed squares). (c, d) Mean head-turning angles differed between owls and experiments (Exp. 1 / Exp. 2), as well as between stimulus positions (closed diamonds: 27°, open diamonds: 47°), but not between valid and invalid trials or between cue-target delays. 95% confidence intervals of head-turning angles are smaller than the symbols (0.5° to 2.3°).

positions with the exception of stimulus positions around –47° in Exp. 2 (Fig. 3-2c, ANOVA: $F_{1,496} = 13.7$, $p < 0.01$).

A general observation from previous studies carried out in other species is the decrease of mean response latency with increasing cue-target delays (rats: Bushnell, 1995; monkeys: Bowman et al., 1993; humans: Spence & Driver, 1996). I found, however, that the duration of the cue-target delay had no effect on response latencies or turning angles of head saccades (Fig. 3-3). The cue-target delay varied between 200 and 300 ms (Exp. 1) or between 200 and 500 ms (100 ms-steps, Exp. 2). A multivariate ANOVA which tested differences in response latencies between cue-target delays, owls, and valid or invalid trials was significant ($F_{15,6451} = 14.8$, $p < 0.01$). These differences occurred between owls ($F_{1,6451} = 61.8$, $p < 0.01$) and between valid and

invalid trials ($F_{1,6451} = 29.3$, $p < 0.01$), as revealed before (see above). Surprisingly, latencies did not vary significantly with the variable duration of the cue-target delay ($F_{3,6451} = 1.49$, $p = 0.21$). The lack of influence did not depend on the owl ($F_{3,6451} = 2.03$, $p = 0.11$) or on trial validity ($F_{3,6451} = 0.10$, $p = 0.94$). Multivariate ANOVAs performed for each experiment separately had the same results. Likewise, turning angles of head saccades did not vary significantly with the duration of the cue-target delay (ANOVA: $F_{3,6452} = 1.1$, $P = 0.33$).

3.4. Discussion

Effects of spatial selective attention on sound localization have so far exclusively been studied in humans. Experimental guidance of the attentional focus was achieved in these studies by peripheral auditory cueing stimuli (Spence & Driver, 1997) or indirect visual cueing stimuli (Spence & Driver, 1994, 1996). Spatially consistent cueing stimuli, i.e., valid cueing stimuli, reduced response latencies towards attended stimulus positions compared to response latencies towards unattended positions. Here I could show that in owls processing time of sound source location was shortened in a similar way as in humans by presenting a valid cueing stimulus.

The main criticism on studies carried out in the visual system was, specifically when peripheral cueing stimuli were used, that the validity effect is possibly not related to shifts of spatial attention, but can be explained by pure stimulus additivity (rats: Bushnell, 1995, humans: e.g. Posner et. al., 1984). For the data presented here, this explanation is highly unlikely. First, the validity effect was long lasting and still significant after a cue-target delay of 500 ms. Thus, a visual cueing stimulus influenced the processing of an auditory target, even when the cue had disappeared 500 ms before target onset. Direct interaction over a temporal separation in this range is highly unlikely. The second possibility could be stimulus additivity in the spatial domain based on direct interaction in bimodal neurons, e.g. in the owl's midbrain. This could only happen, if the visual and auditory spatial receptive fields of these neurons are large enough to cover both stimuli, cue and target. The knowledge about characteristics of audio-visual neurons in the owl's midbrain, however, does not support this hypothesis. Bimodal neurons in the optic tectum generally show small visual receptive fields (10° to 17°: Knudsen, 1982), and a very good alignment of the centers of the visual and auditory receptive fields (Knudsen, 1982). I

therefore used cues and targets that should be sufficiently separated in space (cue: 2.5° lateral, target: at least 27° lateral). Thus, a direct stimulation of the same cell from the visual cue and the auditory target is highly unlikely in our setup.

Importantly, in our experiment, the effect of cue validity was not influenced by the detailed structure of cueing stimulus, which differed considerably between Exp. 1 (target side was indicated by switching off a LED) and Exp. 2 (target side was indicated by switching on a LED). In both experiments, owls were able to use the cueing stimulus to estimate the most probable position of the next upcoming auditory stimulus, as observable by the validity effect. This leads us to the conclusion that the mechanisms resulting in a validity effect may be the same in owls and in humans that emerge from spatial attention.

The duration of the cue-target delay, i.e., the waiting period between the cueing stimulus and the auditory target, did not affect either latencies or turning angles of head saccades. In a paradigm providing pre-stimuli (informative or uninformative), as used here, subjects are forewarned that the target stimulus will soon be presented. A waiting period of randomized duration is often introduced to prevent the subjects from temporal learning. Nevertheless, the probability that the target stimulus will be presented increases with the duration of the waiting period. As a result, mammalian species (rats: Bushnell, 1995; monkey: Bowman et al., 1993; humans: Spence & Driver, 1996) show continuously decreasing response latencies as the cue-target delay increases. This "alerting effect" (Niemi & Naatanen, 1981) was found to be unrelated to the orientation of spatial attention, although it indicates that other types of attention may be engaged by the cueing stimulus. Barn owls, however, showed no sensitivity to the increasing probability of a stimulus appearance. The constraints why this was not an evolutionary strategy for the owl remain to be investigated.

4. Coding for auditory space in SC of the rat

Although the rat is often used to determine behavioral sound-localization capabilities or neuronal computation of binaural information, the representation of auditory space in the rat brain has not been investigated so far. I obtained extracellular recordings from auditory neurons in the superior colliculus of anesthetized rats and examined them for spatial tuning characteristics and topographical order. Many neurons (73%) showed significant tuning, with a single peak in the azimuth response profiles based on spike rates and response latencies. Best azimuth values from neurons in one SC were generally tuned to contralateral and rarely to frontal or ipsilateral directions. Tuning width was mostly broad: at supra-threshold intensities (35 dB SPL), 55% of the units had a tuning width of more than 120° in contralateral space. Additionally, tuning width increased with stimulation intensity. A significant but considerably scattered topographical order of best azimuth directions was observed in the deep layers of the superior colliculus with frontal directions being represented closer to the rostral pole. Tuned auditory units in the intermediate layers of the superior colliculus, however, showed no systematic spatial arrangement. This pattern was confirmed by analyzing best azimuth directions from simultaneously recorded units. Our results indicate that the rat superior colliculus contains a representation of auditory space which is comparable to that described for other small mammals.

4.1. Introduction

Neurons located in the intermediate and deep layers of the mammalian superior colliculus (SC) typically exhibit spatial tuning in response to auditory, visual and somatosensory stimulation and show a topographical arrangement of sensory maps. Such maps of the auditory, visual, or the somatosensory space are described for SC neurons in a variety of mammals (e.g. mouse: Dräger *&* Hubel, 1976; guinea pig: Palmer *&* King, 1982, King *&* Palmer, 1983; cat: Middlebrooks *&* Knudsen, 1984; ferret: King *&* Hutchings, 1987). The most precise map of

auditory space, however, was found in the midbrain of the barn owl (Knudsen & Konishi, 1978; Knudsen, 1982). These maps, in which different sensory representations share the same coordinates in the SC, are thought to enable the neurons to integrate stimuli from different sensory channels and allow a novel target to elicit an orientation response through the motor output pathways (Meredith & Stein, 1983; Knudsen & Brainard, 1995).

Investigations on the representation of auditory space in the SC and its importance as a neural substrate for sound localization are still continuing. For the rat, it was shown that orienting toward auditory targets was abolished after bilateral removal of the SC (Milner & Taylor, 1990). Anatomical data indicate that the SC gets strong input from the ascending auditory pathway at or near the level of the inferior colliculus (Edwards et al., 1979; King et al., 1998). Along the rat ascending auditory pathway severe sound localization deficits have been found after lesioning the superior olive (Kelly & Li, 1997), the lateral lemniscus (Kelly et al., 1996), and the inferior colliculus (Ito et al., 1996; Zrull & Coleman, 1999). Lesions at the level of the medial geniculate body (Kelly & Judge, 1985) and the auditory cortex (Kelly & Kavanagh, 1986; Kelly, 1980), on the other hand, had no effect.

Neural processing of auditory spatial information depends strongly on binaural interactions and their processing in the central auditory system. In the rat, neurons sensitive to interaural time delays have been found in the dorsal nucleus of the lateral lemniscus (Kidd & Kelly, 1996) and in the central nucleus of the inferior colliculus (Irvine et al., 1995). Sensitivity to interaural level differences is found in the inferior colliculus (Irvine et al., 1995; Kelly et al., 1991; vanAdel et al., 1999) and the auditory cortex (Kelly & Sally, 1988; Sally & Kelly, 1988).

Despite this body of work on the involvement of different nuclei in sound localization and on binaural information processing, the representation of acoustic spatial information in the rat brain has not been investigated. Therefore, I started out to determine spatial response properties of rat SC neurons in order to investigate whether a topographic representation of auditory space exists in this midbrain structure. A topographic representation of visual space that fits the general pattern in small mammals has already been described for the superficial layers (Siminoff et al., 1966; Diao et al., 1983).

4.2. Methods

4.2.1. Surgery and Anesthesia

Experiments were carried out in 13 female Sprague-Dawley rats (260g - 360g, obtained from the university breeding colony). In 11 rats multiple electrode recordings were performed. All animals were examined otoscopically and their ears were free of obstruction and disease at the time of the experiment. Surgical anesthesia was induced i.p. with a mixture of ketamine (100 mg/kg; Ketamin, Sanofi-Ceva GmbH, Düsseldorf, F.R.G.) and xylazine (4 mg/kg, Rompun, Bayer AG, Leverkusen, F.R.G.) and maintained with an injection of one-third of the initial dose as required. The depth of anesthesia was controlled by testing for the presence of reflexes. To reduce tracheal mucous secretion, animals were treated with atropine sulfate (0.3 mg/kg). Rectal temperature was maintained at 38°C by a thermostatically controlled heating blanket.

The animal was placed in a stereotaxic frame using blunt ear bars. A midline incision of the scalp was performed and a piece of cranium above the cortex overlying the left SC (3 x 3 mm) was removed. The dura mater was left intact. To fix the head during electrophysiological recording, a metal post was mounted on the skull overlying the cerebellum using screws and dental acrylic. The incision was partially closed and the scalp was reattached so that the pinnae resumed their preoperative positions. The eyes were rinsed with saline from time to time to prevent drying of the cornea.

The experiments reported in this study were carried out in accordance with the "Guide for the care and use of laboratory animals" (National Institutes of Health publication No. 86-23) and were following German federal regulations.

4.2.2. Neurophysiological recordings and data acquisition

Experiments were performed in an electrically shielded, anechoic chamber (IAC, Niederkrüchten, F.R.G., base: 2 x 2 m, height: 2.6 m). The head of the animal was adjusted to the coordinates of a hoop-based stimulation system. Varnish-coated tungsten electrodes (9-12 MΩ, F. Haer Corp., Brunswick/Maine, U.S.A.) were arranged in an electrode array and glued

together using dental acrylic. The array consisted of two rows of four electrodes, each spanning 2 mm between the four electrodes and 0.5 mm between the rows. Six electrodes were recording electrodes, two were lesion electrodes (one in every row). The rows of electrodes were always parallel to the rostrocaudal axis of the SC. A stepper motor-controlled microdrive advanced the electrode array into the brain. After recording, each track was marked with one or two lesions by passing a tip negative current of 10 μA for 10 – 15 s through the lesion electrodes.

Experiments were terminated under deep anesthesia by transcardial perfusion with phosphate buffer in saline (PBS; 0.1M) followed by 4% formalin in PBS. The brain was removed and placed in 4% formalin in PBS and 5% sucrose for 1-2 days. Afterwards it was placed in 4% formalin in PBS and 30% sucrose until it sunk. The brain was then blocked and parasagittal sections (30 μm) were cut on a freezing microtome. Sections were mounted and alternately Nissl-stained with cresyl violet or stained with gold chloride against myelin (Schmued, 1990). Electrode tracks were reconstructed from the lesions. For the electrode array used, in which the rows of electrodes were always parallel to the rostrocaudal axis of the SC, positions of electrodes inside the rows were determined from lesions through electrodes at one end of the row and the known distances inside the electrode array. Recording sites were defined by measuring the mediolateral position from the midline and the rostrocaudal position from the caudal border in percent with respect to the maximal expansion of the SC.

Neural activity was passed through a custom-built headstage, then amplified and band pass filtered (300 - 6000 Hz) with an 8-channel amplifier (Lynx8, Neuralynx, Tucson / Arizona, U.S.A), and displayed on an oscilloscope. Single units (SU) and multi units (MU) were separated according to their waveform using customized spike sorting software (Discovery, DataWave Technologies Corporation, Longmont, U.S.A.)

4.2.3. Stimulus design

Acoustic stimuli were generated by a noise generator (HP 8904 A, Hewlett-Packard, Palo Alto, U.S.A) and gated with a custom-built envelop generator (100 ms duration, 5 ms rise/fall time). Stimulus intensity was regulated by an attenuator (PA4; Tucker Davis Technologies, Gainesville, U.S.A.). Signals were amplified (PMA-S10; Denon Electronics Ltd., Parsippany,

U.S.A.) and transmitted by a loudspeaker (TW 6 NG, 8 Ohm, Visaton, Haan, Germany) which was attached to a semicircular hoop. The loudspeaker was movable along horizontal directions (azimuth) and vertical directions (elevation) covering an imaginary sphere with 190 cm in diameter. The sound field was calibrated using a microphone (1/4‘‘, type 4135, Brüel & Kjaer, Naerum, Denmark) with preamplifier (type 2660, Brüel & Kjaer) and power-supply (type 2804, Brüel & Kjaer). RMS sound pressure levels (dB SPL) are expressed relative to 20 µPa. The frequency response of the system was flat (+/- 6 dB) between 1 and 20 kHz (loudspeaker cutoff frequency).

To obtain spatial response profiles, noise bursts were presented via the hoop-mounted loudspeaker from different positions. Azimuth response profiles were measured at 0° elevation. Elevation response profiles were measured separately at the unit's maximal azimuthal response. Positions were selected in sequential or pseudorandom order with a spatial resolution of 20° (53% of the units tested sequential, 42% tested pseudorandom, 5% tested with both). A Kolmogoroff-Smirnoff test revealed that there was no significant difference in the tuning curves of units stimulated under both conditions. Mechanical constraints restricted the measurements to the range of 100° (ipsilateral) to -180° (contralateral) along the azimuthal and to the range of -60° to 60° along the elevational axis. At every position, 20 stimulus repetitions were delivered with 1 Hz repetition rate. All units were tested with 35 dB SPL noise bursts. Some of them were additionally tested with 25 dB SPL and/or 50 dB SPL noise bursts.

Auditory units were tested for visual responsiveness using a hand-held lamp (3°-5° visual angle) which produces triggered light flashes. Responses to light flashes (500 ms duration, 20 to 40 repetitions, 1 s repetition rate) were displayed in PSTHs. Only general responsiveness of auditory units to static visual stimuli was tested. Visual spatial tuning and non-auditory units were not investigated.

As recordings were performed in the left SC, azimuth on the left side of the rat corresponds to positive angles and ipsilateral acoustic space. Azimuth on the right side of the rat corresponds to negative angles and contralateral acoustic space.

4.2.4. Data analysis

Electrophysiological data were analyzed off-line based on dot-displays and PSTHs. Further analysis was performed using programs written in IDL (Research Systems Incorporated, Boulder/Colorado, U.S.A.). Response strength for every sound source direction was quantified in two different ways:

(i) Summing the neuron's response rate for the whole stimulus duration (100 ms) and subtracting spontaneous activity calculated from a 100 ms time window before stimulation. Responses after stimulus offset were not taken into account.

(ii) Calculation of the peak response rate during the stimulus with 5 ms binsize.

Summed spike rates and peak spike rates were plotted as a function of sound source azimuth or elevation to obtain tuning curves. From the spatial tuning curves I determined "best azimuth" or "best elevation" as sound direction with maximal (peak-) response rate and "tuning width" as angle between adjacent sound directions of 50 % response activity. Additional regions, where the response was again above this level were not taken into account. Auditory spatial response profiles were classified as single-peaked, double-peaked (with two peaks having the same height), and complex tuning (with more than 2 peaks having the same height). In double-peaked units and complex units, the activity in intervening regions often dropped below 75% of maximal response activity. Best azimuth tuning in double-peaked and complex units was only determined as mean response direction (see following).

Mean response direction (MRD) was calculated additionally by summing the response vectors for all sound directions i (20° spacing).

$$MRD = \arctan\left(\sum_i \text{peak rate} * \sin(i) \Big/ \sum_i \text{peak rate} * \cos(i) \right)$$

The 3 missing sound directions were computed by linear interpolation of the activity at the margins of the measuring range, i.e. 100° and -180°.

I further used circular statistics to test the significance of tuning to sound source direction for auditory units (Batschelet, 1981). Level of significance for the normalized vector strength (VS) was set to $p < 0.001$:

$$VS = \left(\left(\left(\sum_i \text{peak rate} * \sin(i) \right)^2 + \left(\sum_i \text{peak rate} * \cos(i) \right)^2 \right) \Big/ \sum_i \text{peak rate} \right)^{1/2}$$

Response latencies for latency tuning were calculated from PSTHs with 2 ms binsize. Response latency was then measured as the occurrence of the peak activity rate (i.e. the max. peak in the PSTH). For the mainly found phasic ON-activity this latency measure is reliable, although about 4 to 8 ms longer than standard latency measures. For SUs, I compared latencies at "non-preferred" sound directions (average latency from 60°, 40°, 20°, 0°) to "preferred" sound directions (average latency from -20°, -40°, -60°, -80°).

4.3. Results

4.3.1. Data set

I recorded from 234 auditory responsive units in the rat SC. Out of those, 219 units (107 multi units, 112 single units) showed significant auditory spatial tuning. They were recorded from 75 recording sites in 37 electrode penetrations in the SC, as determined by histological verification. At 65 recording sites (87%) pairs, triplets or quadruplets of auditory units positioned along the rostrocaudal axis were recorded simultaneously.

Auditory units were found at positions ranging from about 250 µm to about 3000 µm below the surface of the SC (Fig. 4-1). The distribution of recording sites with depth for auditory units showed a gap around 1500 µm. Nevertheless, non-auditory units exhibiting spontaneous acitivity or responsiveness to other modalities could frequently be found at this depth. This suggests that our data set might possibly be formed by two different populations of auditory units: one population of neurons located in the intermediate gray layer and the dorsal part of the intermediate white layer (depth < 1500 µm, N = 108, thereafter called "medium population"), and one population of neurons located in the ventral part of the intermediate white layer and in the deep gray and deep white layer of the SC (depth ≥ 1500 µm, N = 111; thereafter called "ventral population"). A transition zone between the two populations is found in the intermediate white layer (Paxinos & Watson, 1998). Several differences in the characteristics of the two populations were found (see below).

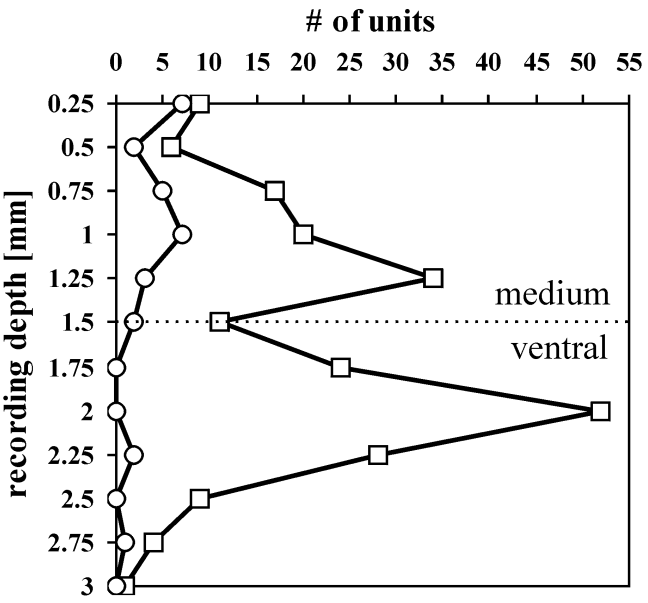

Fig. 4-1) Distribution of auditory units (squares) and visual/auditory neurons (circles) in depth of recording positions below the dorsal surface of the rat SC. A gap in the distribution at a depth of 1500 µm below dorsal surface of the SC led to the differentiation of a medium population (located in the intermediate layers) and a ventral population of auditory neurons (located in the deep layers). The two populations showed differences in visual-auditory responsiveness and inherent spatial arrangement (medium population: N = 108; ventral population: N = 111).

A subset of the auditory neurons was also responsive to visual stimulation (21 %). The recording depth for auditory-visual bimodal units differed significantly from the depth of visually unresponsive units: Visually responsive units were more often found in intermediate layers of the SC (median depth = 992 µm, Wilcoxon: $N_{non\text{-}visual} = 111$, $N_{visual} = 29$, $Z = -4.66$, $p < 0.0001$) and visual unresponsive units more often in deep layers of the SC (median depth = 2164 µm). 90 % of such visual responsive cells were located in the medium population, whereas only 45 % of the visually unresponsive cells were found here. Comparison of best azimuth and halfmaximal width of visually responsive and unresponsive units revealed no significant difference (best azimuth, Wilcoxon: $N_{non\text{-}visual} = 84$, $N_{visual} = 23$, $Z = -1.03$, $p = 0.302$; halfmaximal width, Wilcoxon: $N_{non\text{-}visual} = 99$, $N_{visual} = 26$, $Z = 1.15$, $p = 0.251$).

Most units were spontaneously active. Mean spontaneous activity of single units (SU) was 11.5 spikes/s. Spontaneous activity up to 10 spikes/s was found in 54.4 % of the units. The remaining units exhibited a spontaneous activity up to 40 spikes/s.

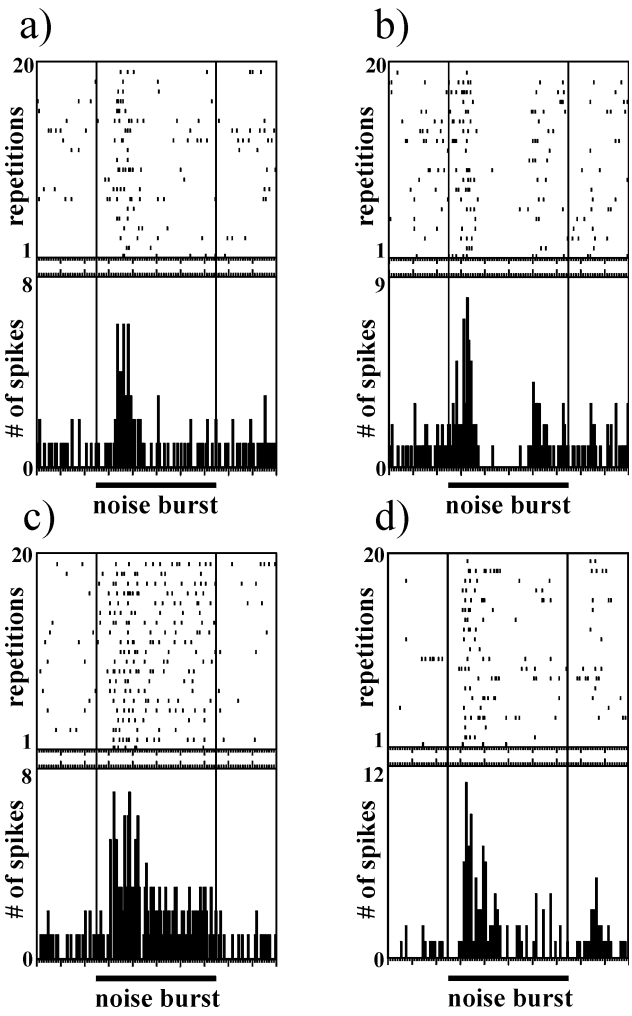

Fig. 4-2) Types of responses to noise burst stimulation observed in the rat SC. Dot displays and PSTHs were generated from 20 stimulus repetitions at best azimuth (duration = 200 ms, binsize = 1ms, noise burst duration = 100 ms). PSTHs show (a) a phasic response, (b) a phasic response with inhibition, (c) a phasic-tonic response, and (d) a phasic ON-response with additional phasic response after stimulus offset. Phasic ON-responses were observed in 61 % of the units, phasic-tonic responses in 39% of the units. Responses after stimulus offset were observed in less than 10 % of the response profiles.

4.3.2. Auditory response characteristics

Auditory response thresholds fell in the range of 15 to 25 dB SPL. Most cells showed a typical temporal response profile: large phasic ON-responses were followed by a phasic inhibition or a tonic response (Fig. 4-2a–c). Response characteristics were mainly phasic (61%), often showing an inhibitory gap during stimulation, and phasic-tonic (39 %). I observed an additional phasic response after stimulus offset in less than 10 % of the units (Fig. 4-2d).

4.3.3. Azimuth response profiles

I obtained responses from auditory SC neurons with noise stimulation from 15 different sound directions and at three sound levels: 25 dB SPL (0-10 dB above threshold), 35 dB SPL (10-20 dB above threshold) and 50 dB SPL (25-35 dB above threshold). Recordings were performed in the left SC. Measuring coordinates referring to the rats head are shown in Fig. 4-3a. From the neuronal responses I obtained spatial response profiles using 2 different methods:

(i) Tuning curves were obtained by summing the activity over the whole stimulus duration (SUM) as was the preferred method in earlier studies. Consistent results could only be obtained from neurons with purely excitatory phasic and phasic-tonic response characteristics. Neurons with an inhibitory activity gap during stimulation had to be excluded from examination.

(ii) Tuning curves and response latencies were calculated for the peak response rate of the PSTHs (PEAK) averaged from 20 stimulus repetitions at every speaker position. In the vast majority of the units the tuning was thereby determined by the height of the phasic ON-response. Since our recording sample contained up to 30 % of neurons with an inhibitory activity gap further analysis of spatial tuning was mostly based on the tuning obtained this way. This method was very reliable and took into account response characteristics of units with excitatory and inhibitory response components. Examples of PSTHs and tuning curves obtained from a SC unit with this method are shown in Fig. 4-3b and Fig. 4-3c, respectively. In general, peak spike rates fell in the range of 100 to 400 spikes/s (5 ms binsize).

Azimuth tuning measured from peak response rates was narrow only in a small set of units. An example of a narrowly tuned auditory SC unit is shown in Fig. 4-3c. In most cases, however, tuning was rather broad, covering a major part of the contralateral space. Activity in the ipsilateral space was typically reduced. Many tuning curves were skewed with steeper slopes at either the frontal or the far contralateral side (Fig. 4-4). In others, the general shape was more broad and flat, however with a prominent peak at off-center locations. At 35 dB SPL stimulus intensity, 73% of the tuning curves obtained from peak response showed a significant tuning to sound source azimuth with a single peak in the response profile (Fig. 4-4, circles, squares, large triangles). The remaining units were either double-peaked (17%; Fig. 4-4, small triangles) or showed complex tuning (10%). Inhibitory response components changed in parallel to the

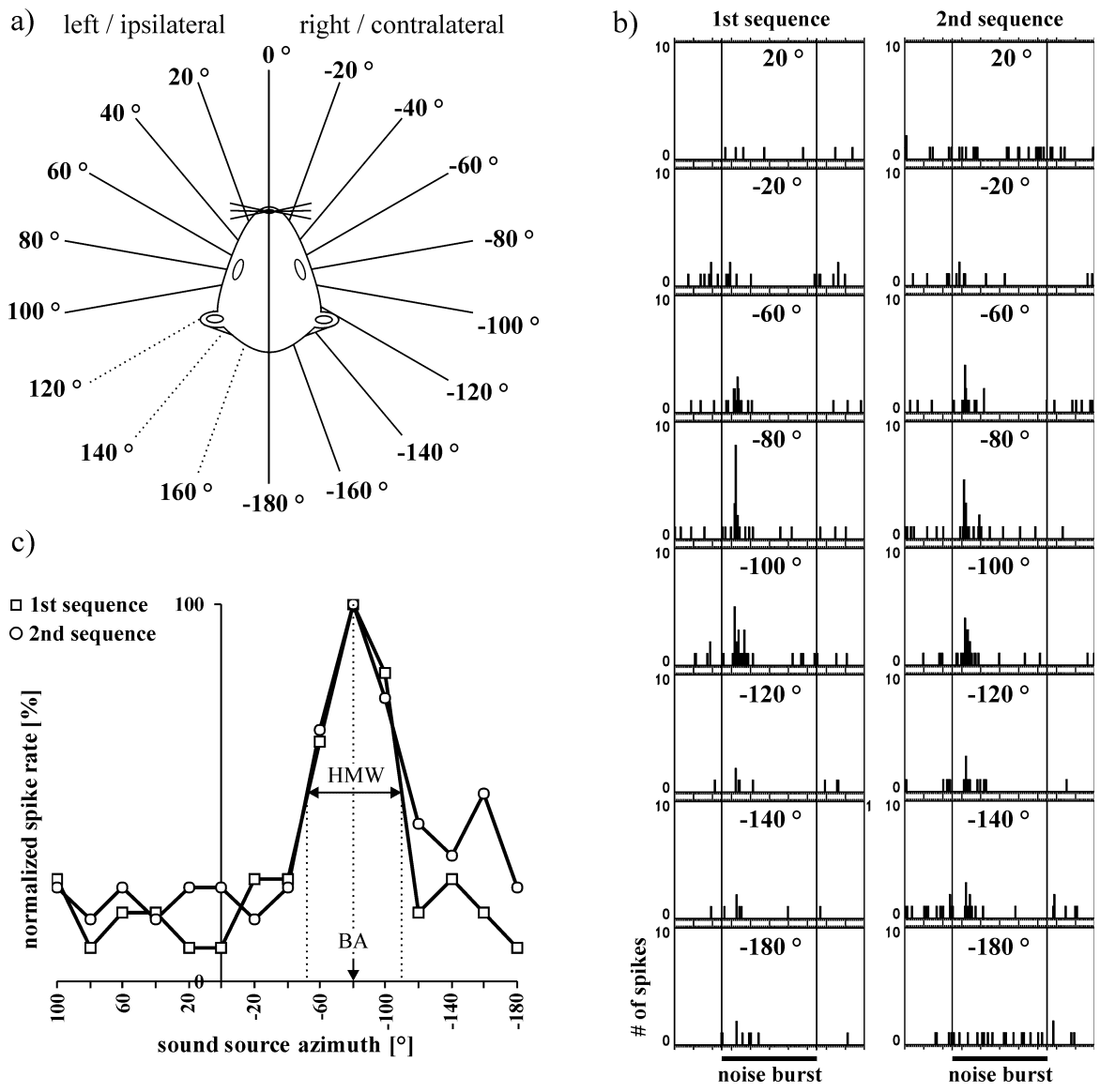

Fig. 4-3) Calculation of spatial response profiles. (a) Reference coordinates for sound source directions in the horizontal plane (azimuth) with respect to the rat's head. Recordings were performed in the left SC, therefore contralateral space is indicated by negative angles. Mechanical constraints restricted the measurements to a certain range (100° to -180°). The remaining azimuth directions were indicated by hatched lines. (b) Activity of a single unit tested with two pseudorandom sequences (left, right) of stimulus positions (10 repetitions at every sound direction, PSTH and stimulation attributes as in Fig. 2). The unit shows a stable response pattern with narrow spatial tuning to 80° azimuth. (c) Tuning curves calculated from the PSTHs. The peak response was plotted as a function of sound source azimuth. For this unit, the two sequences were averaged for further analysis. Best azimuth (BA) was determined as sound source direction with maximal peak response and halfmaximal width as the angle between adjacent sound directions with 50 % response activity (HMW).

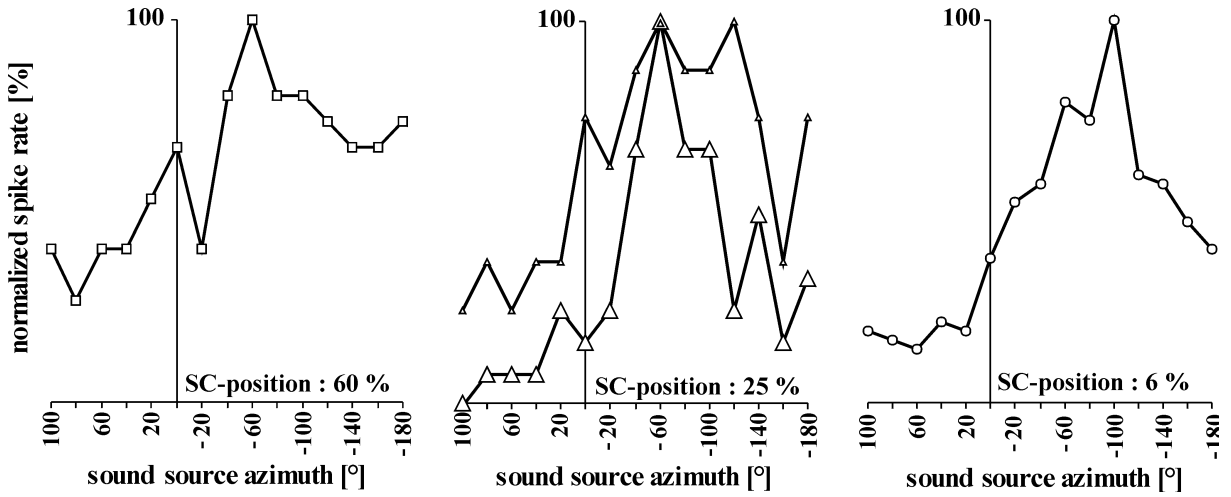

Fig. 4-4) Azimuth tuning curves based on peak activity rates. Four examples are given for the mostly broad tuning covering a major part of the contralateral space. The curves were often skewed having a steeper slope at the frontal or the far contralateral side. Three units are tuned to azimuth with a single peak (circles, squares, large triangles), one is double-peaked (small triangles). Units were recorded simultaneously from three different electrodes arranged along the rostrocaudal axis of the SC with a distance of 660 µm between adjacent electrodes. Two units were recorded with one of the electrodes (small and large triangles).

excitatory components, as determined by visual inspection of PSTHs. Their spatial response profile was not quantified in detail.

From the single-peaked response profiles I determined "best azimuth" (BA) as sound direction with maximal response rate (BA_{SUM}) or maximal peak response rate (BA_{PEAK}). Tuning to sound source azimuth was additionally calculated as "mean response direction" for single- and also for double-peaked response profiles (BA_{MRD}, see METHODS section). Further, "tuning width" (HMW) was determined for all response profiles as angle between adjacent sound directions with 50 % of the maximal response activity (HMW_{SUM} or HMW_{PEAK}). BA values and HMW values were then examined for further properties.

Spatial tuning characteristics (BA_{PEAK}, HMW_{PEAK}) obtained from SU did not differ significantly from that obtained from MU (BA_{PEAK}, Wilcoxon: $N_{SU} = 83$, $N_{MU} = 81$, $Z = -0.0017$, $p = 0.999$; HMW_{PEAK}, Wilcoxon: $N_{SU} = 100$, $N_{MU} = 92$, $Z = 0.98$, $p = 0.329$). Data from SU and MU were therefore pooled for most examinations with the exception of response latency tuning.

4.3.4. Best azimuth

As mentioned above, a large proportion of cells showed consistent tuning as determined by peak firing rate. There was no significant difference when I compared cells best azimuth calculated from the summing (BA_{SUM}) or peak (BA_{PEAK}) algorithm (25 dB SPL, paired t-test: N =16, t = -1.31, p = 0.211; 35 dB SPL, Wilcoxon MPSR: N = 98, t = -68.0, p = 0.703; 50 dB SPL, paired t-test: N = 40, t = -1.22, p = 0.230). The finding that neuronal ON-responses give the best estimate for auditory spatial tuning is consistent with the usually rapid nature of orienting responses. I therefore restricted further examination mainly to the data gathered with the peak detection algorithm.

BA_{PEAK} was generally tuned to contralateral directions and in a few cases to frontal directions. All best azimuth directions observed were well inside our azimuthal measuring range. I found a preferred region of BA_{PEAK} directions in the contralateral hemisphere between 60° and 140° lateral: Two-third of the units in our sample had a BA_{PEAK} in that area (Fig. 4-5, 63.8 % at 35 dB SPL and 66.3 % at 50 dB SPL). BA_{MRD} values showed a smaller distribution of preferred sound directions in the contralateral hemisphere than the distribution of BA_{PEAK} values (Fig. 4-5).

The skewness observed from the tuning curves obtained with peak response indicated that BA_{PEAK} was often located at off-center directions. By comparing paired BA-values from PEAK-locations with MRD-locations, which are a direct measure for the "center" of the tuning curve, I revealed that this skewness was significant (Wilcoxon MPSR-test, N = 164, p = 0.012; 35dB SPL).

4.3.5. Azimuth tuning width

Narrow azimuth tuning was found in a larger number of units when stimulation intensity was near threshold. When supra-threshold stimulation intensities were used (35 dB SPL, 50 dB SPL), azimuth tuning width was rather broad in rat SC neurons. At 35 dB SPL, 55% of the units had a tuning width of more than 120° in contralateral space. At 50 dB SPL, 74.8 % of the units showed a tuning broader than 120° and 55% a tuning broader than 180° within the contralateral

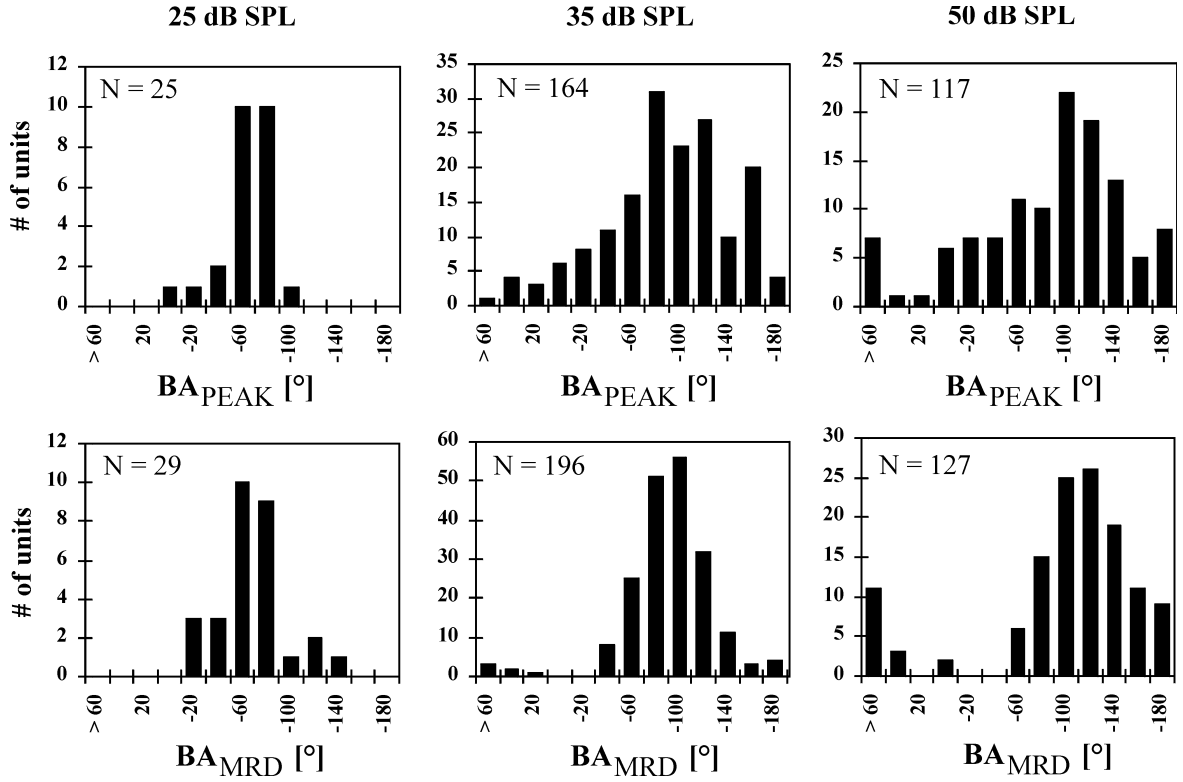

Fig. 4-5) Distribution of best azimuth directions of units calculated from peak activity (BA_{PEAK}, top row) or as "mean response direction" (BA_{MRD}, bottom row) recorded at 3 different stimulus intensities (left to right). Distributions of BA_{PEAK} are much broader as compared to BA_{MRD} due to skewed tuning curves and off-center located peaks on top of broad, flat tuning.

and parts of the ipsilateral space. In summary, tuning to sound source azimuth was typically dominated by all contralateral directions, having a 'hemifield'-like shape. Although spatial response profiles were found to be broad in rat SC neurons, they were still tuned, i.e. in the majority of tuning curves I observed a prominent peak at a single sound direction.

The width of azimuth tuning depended to some extent on the type of activity the response profile was based on. Although it gave rather reliable results, the width of tuning based on peak activity (HMW_{PEAK}) was usually broader than the width of tuning based on summed activity (HMW_{SUM}). The difference ranged from 8 % at 50 dB SPL to 14 % at 25 dB SPL (25 dB SPL paired t-test: N =19, t = -3.79, p = 0.001; 35 dB SPL paired t-test: N = 142, t = -3.75, p = 0.0003;

50 dB SPL paired t-test: N = 71, t = -2.06, p = 0.044). This was an absolute increase in tuning width of about 20° to 40°.

4.3.6. Influence of stimulus intensity

The influence of stimulus intensity on tuning characteristics of SC auditory units was a matter of discussion in several studies. An influence was found in guinea pigs (Palmer & King, 1982) but not in cats (Middlebrooks & Knudsen, 1984). In the primary auditory cortex of the cat, neuronal responses to different sound directions have also been reported to be broadly tuned and strongly intensity dependent (Middlebrooks & Pettigrew, 1981;Eggermont, 1998).

I tested the units with up to three different stimulus intensities: 25, 35 and 50 dB SPL. In a few cases BA_{PEAK} shifted with stimulus intensity, but for the whole sample there was no significant influence of stimulus intensity on BA_{PEAK} (25 dB vs. 35 dB, paired t-test: N = 18, t = -1.77, p = 0.0942; 35 dB vs. 50 dB SPL, Wilcoxon MPSR: N = 80, t = 26.5, p = 0.877). HMW_{PEAK}, on the other hand, strongly increased with stimulus intensity. At 35 dB SPL, the mean HMW_{PEAK} was on average 54.8° broader than at 25 dB SPL (paired t-test: N = 25, t = -4.91, $p < 0.0001$). At 50 dB SPL, HMW_{PEAK} was on average 40.8° broader than at 35 dB SPL (paired t-test: N = 89, t = -5.06, $p < 0.0001$). Paired values for BA_{PEAK} and HMW_{PEAK} are shown in Fig. 4-6.

4.3.7. Auditory response latencies

Response latencies were calculated for SUs in this dataset as the time of occurrence of the peak response (not the onset of activity) within the stimulation period (2 ms binsize). In general, peak rate latencies fell in the range of 10 to 80 ms. At 35 dB SPL stimulation intensity and contralateral sound directions, 80.9 % of the units showed a response latency shorter than 40 ms. At 50 dB SPL and contralateral sound directions, 91.2 % of the units showed a response latency shorter than 40 ms, and 75 % showed a latency shorter than 30 ms. On the other hand, 94% of response latencies were above 15 ms at best azimuth (35 dB SPL) which seemed to fit for this stage of auditory processing.

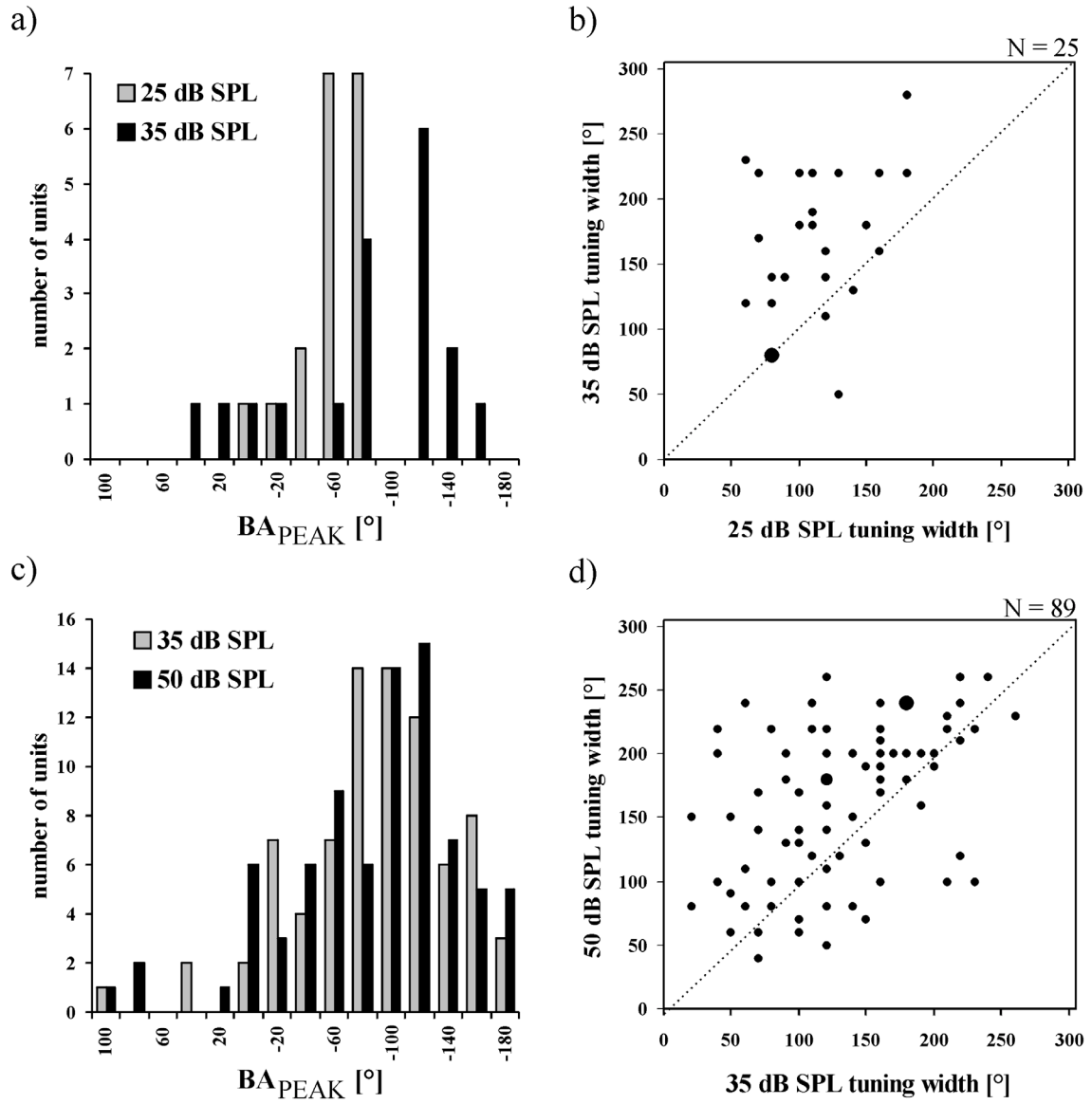

Fig. 4-6) Influence of stimulus intensity on best azimuth directions (BA_{PEAK}) and halfmaximal width (HMW_{PEAK}) of units (paired data only). (a) Distributions of best azimuth values of units stimulated with noise bursts at 25 dB SPL and at 35 dB SPL (N = 25). (b) Tuning width at 25 dB SPL vs. tuning width at 35 dB SPL for units shown in (a).Tuning width is on average 54.8° larger at 35 dB than at 25 dB SPL. (c) Distributions of best azimuth values of units stimulated with noise bursts at 35 dB SPL and at 50 dB SPL (N = 89). (d) Tuning width at 35 dB SPL vs. tuning width at 50 dB SPL of units shown in (c). Tuning width is on average 40.8° larger at 50 dB than at 35 dB SPL.

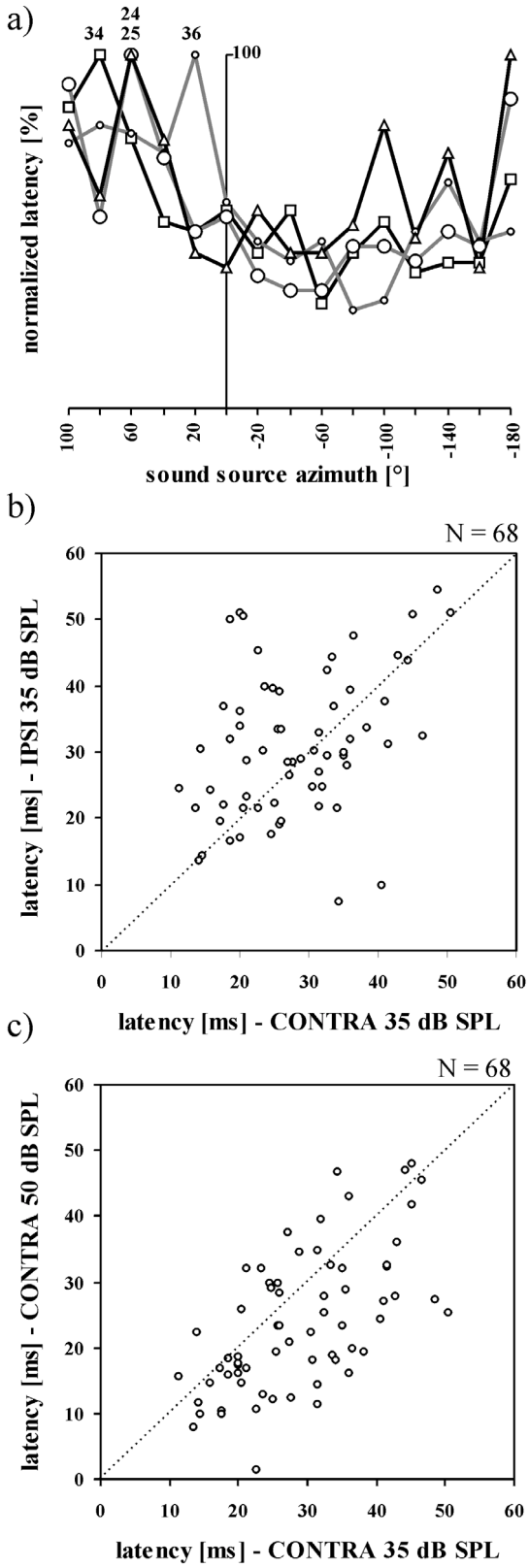

Fig. 4-7) Latency tuning to sound source azimuth in SC neurons: (a) Normalized tuning curves of 4 units calculated from response latencies. Tuning based on latencies is generally broad but gives similar results to the tuning based on spike rates. Numbers above latency peaks indicate absolute latency (in ms) at 100 % normalized latency. (b) Paired data for latencies averaged over 4 ipsilateral (60°/40°/20°/0°) vs. 4 contralateral directions (-20°/-40°/-60°/-80°). We found significant shorter response latencies to noise bursts in the contralateral hemisphere. (c) Paired data for latencies averaged over 4 contralateral directions at 2 stimulus intensities (35, 50 dB SPL). Latencies are significantly shorter at 50 dB SPL than at 35 dB SPL stimulus intensity.

The analysis of azimuth tuning was so far based on averaged neuronal activity rates. Another possibility would be a representation based on temporal encoding, e.g. response latency. Latency shifts in the cat auditory cortex were recently found to encode sound source azimuth in addition to the encoding by neuronal activity rates (Eggermont, 1998; Middlebrooks et al.., 1998). As the SC is a more peripheral structure in the sound localization pathway which contains neurons exhibiting spatial tuning, it would be interesting to see if latency tuning is already realized at this level. I observed that not only peak-response rates but also response latencies varied with sound source direction in SC units. This might be a second important factor determining the representation of spatial information.

Latency and the unit's activity rate shifted in opposite directions with sound source azimuth (Fig. 4-7a). The azimuth values at which the peak response and shortest latency were observed tended to be similar. I found significantly shorter response latencies at preferred sound directions, i.e. at the contralateral side (35 dB SPL: paired t-test: $N = 107$, $t = 4.78$, $p < 0.0001$, contralateral on average 5.3 ms shorter, Fig. 4-7b; 50 dB SPL: paired t-test, $N = 74$, $t = 3.38$, $p = 0.0012$; contralateral on average 4.1 ms shorter). Latencies, in addition, are shorter at 50 dB SPL than at 35 dB SPL stimulus intensity (non-preferred: paired t-test: $N = 68$, $t = 3.11$, $p = 0.0028$, on average 5.1 ms shorter; preferred: Wilcoxon MPSR-test: $N = 68$, $t = 630$, $p < 0.0001$, on average 5.54 ms shorter, Fig. 4-7c). These two factors influencing the latency were also observable for multi units.

4.3.8. Elevation tuning

Elevation response profiles from 41 auditory SC units were determined at or close to the best azimuth direction. Examples of elevation tuning curves are depicted in Fig. 4-8a. Three curves were recorded simultaneously (squares, triangles), one curve was recorded in a different data set (circles). Only 4 units showed narrow elevation tuning like the example shown in Fig. 4-8a (circles). Most SC units showed broad elevation tuning. The majority of cells (71 %) had a halfmaximal width that extended beyond our stimulation range. Nevertheless, tuning curves often showed a single peak at one preferred elevation (Fig. 4-8a).Therefore, I determined best elevation (BE_{PEAK}) as the direction with maximal peak response as was done for the BA_{PEAK}. I

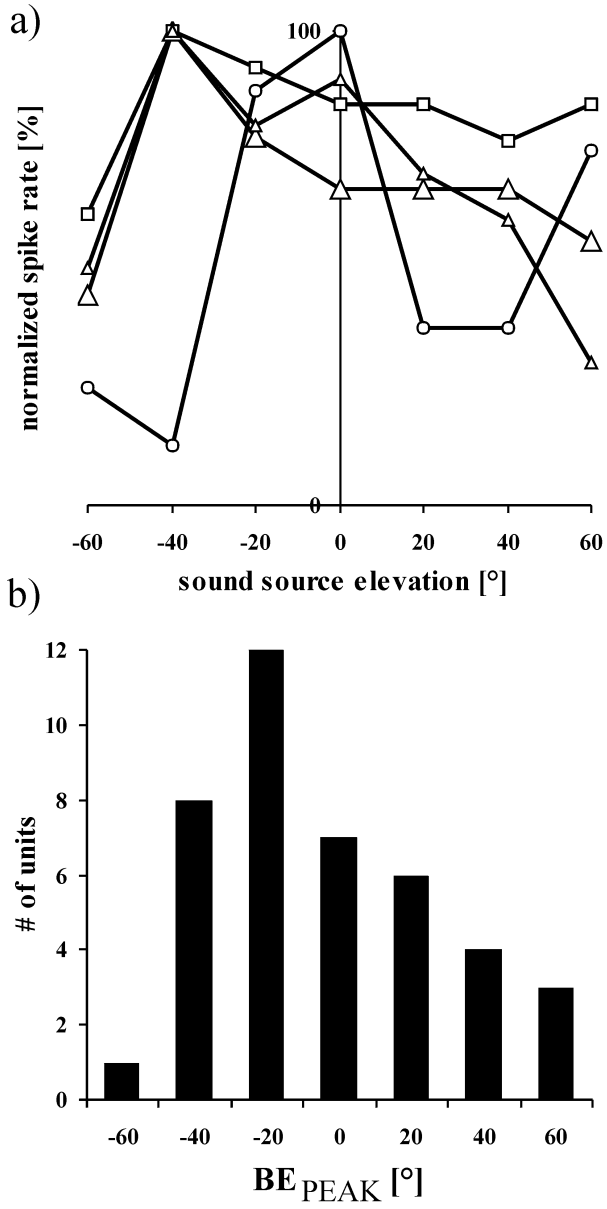

Fig. 4-8) Elevation tuning curves based on peak activity rates. (a) Four examples are given for elevation tuning measured at or near the units' best azimuth response: 3 units with broad tuning (squares, small and large triangles; units were recorded simultaneously, see corresponding azimuth response profiles in Fig. 4) and one unit showing narrow elevation tuning (circles). (b) Distribution of best elevation directions (N = 41). Elevations below the auditory horizon were slightly preferred but in general best elevations covered all tested sound source directions.

found that elevations below the auditory horizon were slightly preferred but in general best elevations covered all tested values (Fig. 4-8b). There was no interdependence between elevational and azimuthal tuning width.

4.3.9. Spatial arrangement

Maps of visual, auditory, and somatosensory space have been described in the SC of several small mammals (e.g.Dräger *&* Hubel, 1976; Palmer *&* King, 1982; King *&* Hutchings, 1987; King *&* Carlile, 1994; Carlile *&* King, 1994). In a given SC, these maps of different modalities were found to be in register, although they might occur in different layers (Meredith *&* Stein, 1983; Palmer *&* King, 1985). For the rat, a map of visual space was already described (Siminoff et al., 1966; Diao et al., 1983). Its orientation fits the general pattern in small mammals: azimuth is represented along the rostrocaudal axis with the frontal space being represented near the rostral pole of the SC, and elevation is represented along the mediolateral axis with upward directions represented at medial SC sites. In the light of these findings I examined preferred azimuth directions of auditory SC neurons for a systematic representation of auditory space. Our analyses were mainly based on the data gathered with 35 dB SPL stimulation intensity.

A possible systematic arrangement of azimuth values (BA_{PEAK} and BA_{MRD}) along the rostrocaudal axis of the SC was first tested in all tuned units recorded, correcting for differences between animals. The ANCOVA was significant ($F_{11,152} = 2.2$, $p = 0.017$, $r^2 = 14\%$) with a significant effect of the rostrocaudal position in the SC ($F_{1,152} = 6.1$, $p = 0.015$) and a significant influence of the animal ($F_{10,152} = 2.2$, $p = 0.022$). The same analysis for BA_{MRD}-values showed no systematic arrangement along the rostrocaudal axis of the SC. The distribution of recording positions along the rostrocaudal axis for all 219 tuned auditory units is shown in Fig. 4-9a. Recording positions are more or less equally distributed along this axis with exception of the most caudal part of the SC (up to 10% from the caudal border).

Investigating the medium population of auditory neurons (located in intermediate layers of the SC) and the ventral population (located in the deep layers of the SC; see Fig. 4-1) separately, revealed a more differentiated pattern. I found that the BA_{PEAK}-values of the medium population did not change systematically with rostrocaudal SC position when tested with an ANOVA ($N = 80$, $b = 0.16$, $t = 0.87$, $p = 0.387$, $r^2 = 0.96\ \%$). In the ventral population, however, BA_{PEAK}-value was a function of rostrocaudal position (Fig. 4-9b; ANOVA: $N = 84$, $b = -0.93$, $t = -4.06$, $p = 0.0001$, $r^2 = 17\ \%$). Although only 17% of the variance in BA_{PEAK}-values could be

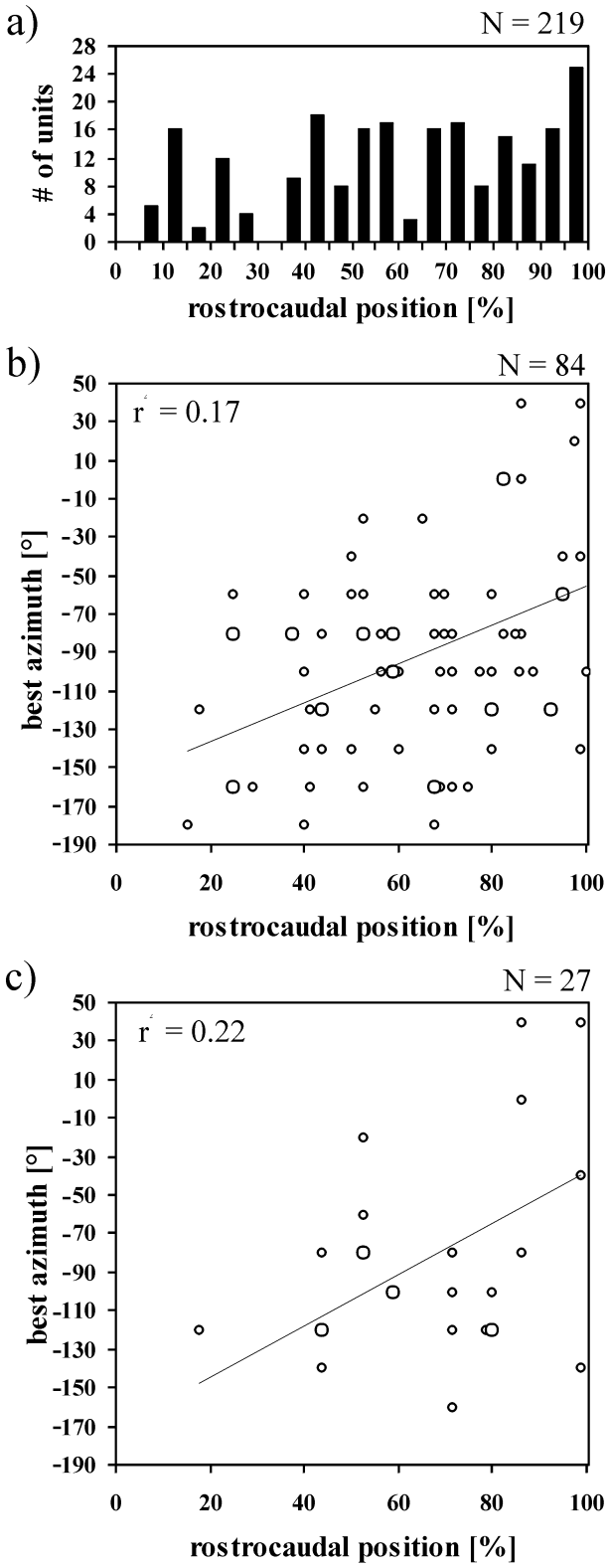

Fig. 4-9) Regression analysis of best azimuth as a function of the recording position along the rostrocaudal axis of the SC. (a) Distribution of recording positions along the rostrocaudal axis for all tuned auditory units (binsize 5%, caudal border at 0%). (b) Lower SC units pooled from 8 animals. (c) Units from one animal (27 recordings from the lower SC). BA_{PEAK} changed systematically with the rostrocaudal position in the SC, but there is a remarkable degree of variability in the representation.

explained by the rostrocaudal position, our data indicates, that a topography of sound source direction is present in the ventral population of auditory units in the rat SC, but highly variable. Surprisingly, the two populations did not show any significant differences in the overall distribution of best azimuth values and halfmaximal width values.

One of the advantages of using multiple electrode was that I could record from many units in a single rat although I could expect that only up to 23% of these units were auditory responsive (and again only a subset of them were tuned to sound source location). Nevertheless I recorded on average from 20 tuned units per animal. In one animal I recorded from a sufficient number of tuned units located in the deep layers (ventral population) to examine their spatial arrangement. A regression analysis on these 27 units revealed again, that BA_{PEAK}-values were a function of the rostrocaudal position in the SC (Fig. 4-9c; N = 27, b = -1.34, t = -2.65, p = 0.0138, r^2 = 22%).

A second advantage of using an electrode array is that usually several spatially tuned units were recorded simultaneously. Examples of PSTHs from simultaneously recorded auditory units are shown in Fig. 4-10. Simultaneously recorded neurons enabled us to determine local variability by comparing units from one electrode. Moreover, I could compare data from units recorded simultaneously from different positions along the rostrocaudal axis of the SC. This provided data with a very rigid test of azimuth representation along this axis. The PSTHs shown in Fig. 4-10 provide examples (i) for local variability between units (units b and c are recorded from the same electrode) and (ii) for differences between units recorded at different positions along the rostrocaudal axis (electrodes 1 (unit a), electrode 2 (units b, c), and electrode 3 (unit d) were arranged with 660 µm spacing between adjacent electrodes).

I tested pairs of units for differences in best azimuth due to their position along the rostrocaudal axis of the SC. Out of each dataset with parallel recordings from a row of electrodes along the rostrocaudal axis I took only the pairs with the maximal possible distance between recorded units. I identified 73 such pairs of simultaneous recorded units in 65 recording sites (6 pairs at 25 dB SPL; 42 pairs at 35 dB SPL and 25 pairs at 50 dB SPL).

Again, I focused on pairs gathered at 35 dB SPL stimulation intensity. A paired t-test revealed that there is a significant difference in BA_{PEAK}-values between the more anterior recorded cell of a pair compared to the more posterior recorded unit (N = 42, t = -2.17, p =

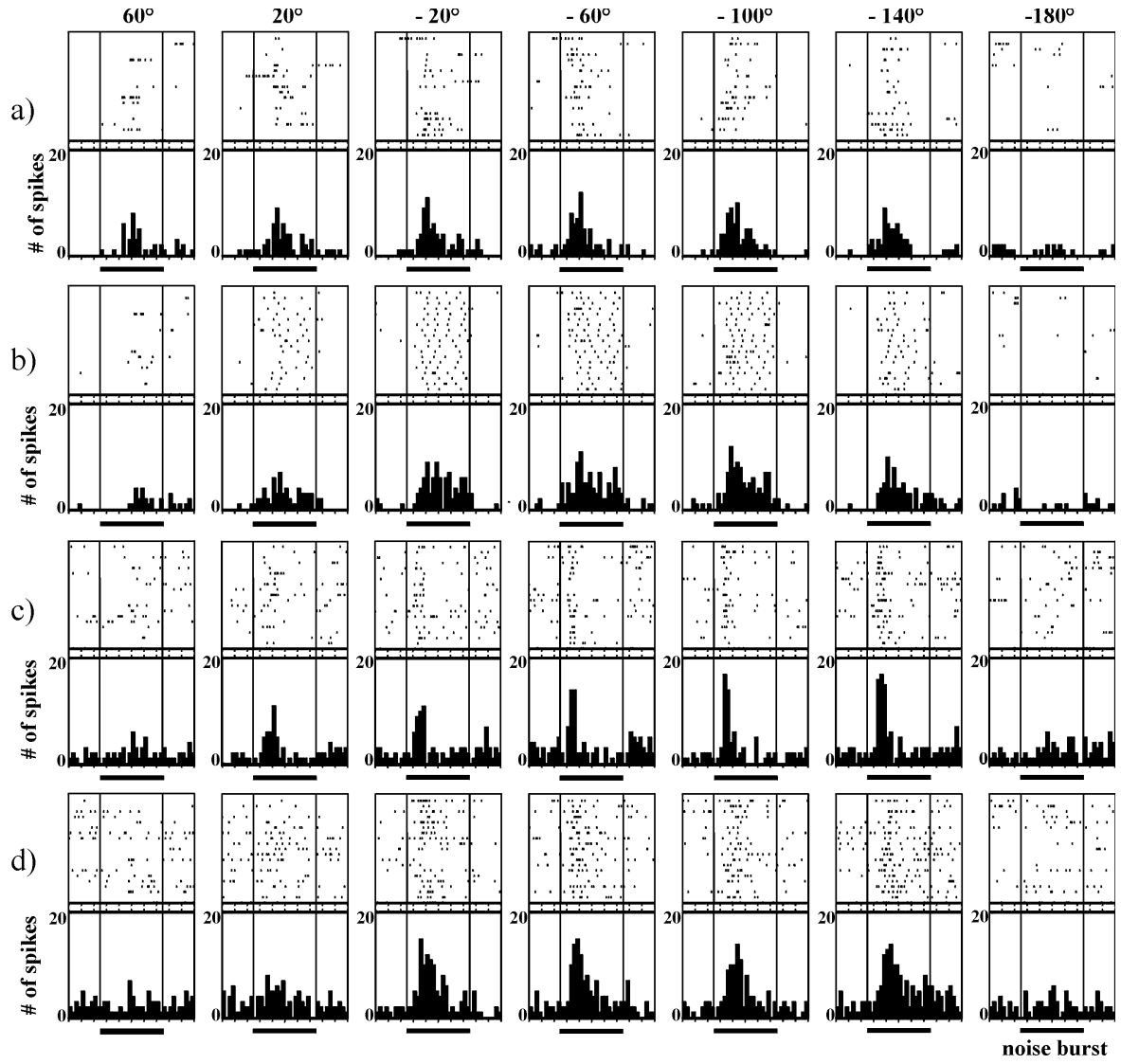

Fig. 4-10) PSTHs and dot displays of 4 simultaneously recorded SC units (20 stimulus repetitions, duration = 200 ms, binsize = 5 ms, noise burst duration = 100 ms). We recorded single units (a, b, d) and multi units (c), separated by waveform characteristics. Electrodes 1 (unit a), electrode 2 (units b, c), and electrode 3 (unit d) were arranged with 660 µm spacing between adjacent electrodes. All units showed a maximal response in the range from -60° to -140° within the contralateral hemifield. Azimuthal tuning curves of units (a) to (d) are shown in Fig. 11, column b.

0.036). The mean best azimuth is about 25.7° more frontal for the more anterior recorded cell. When I separated the sample into cells belonging to the medium or ventral population (intermediate vs. deep layers), I found a more significant difference in BA_{PEAK}-values between the more anterior recorded cell of a pair and the more posterior recorded cell for the ventral

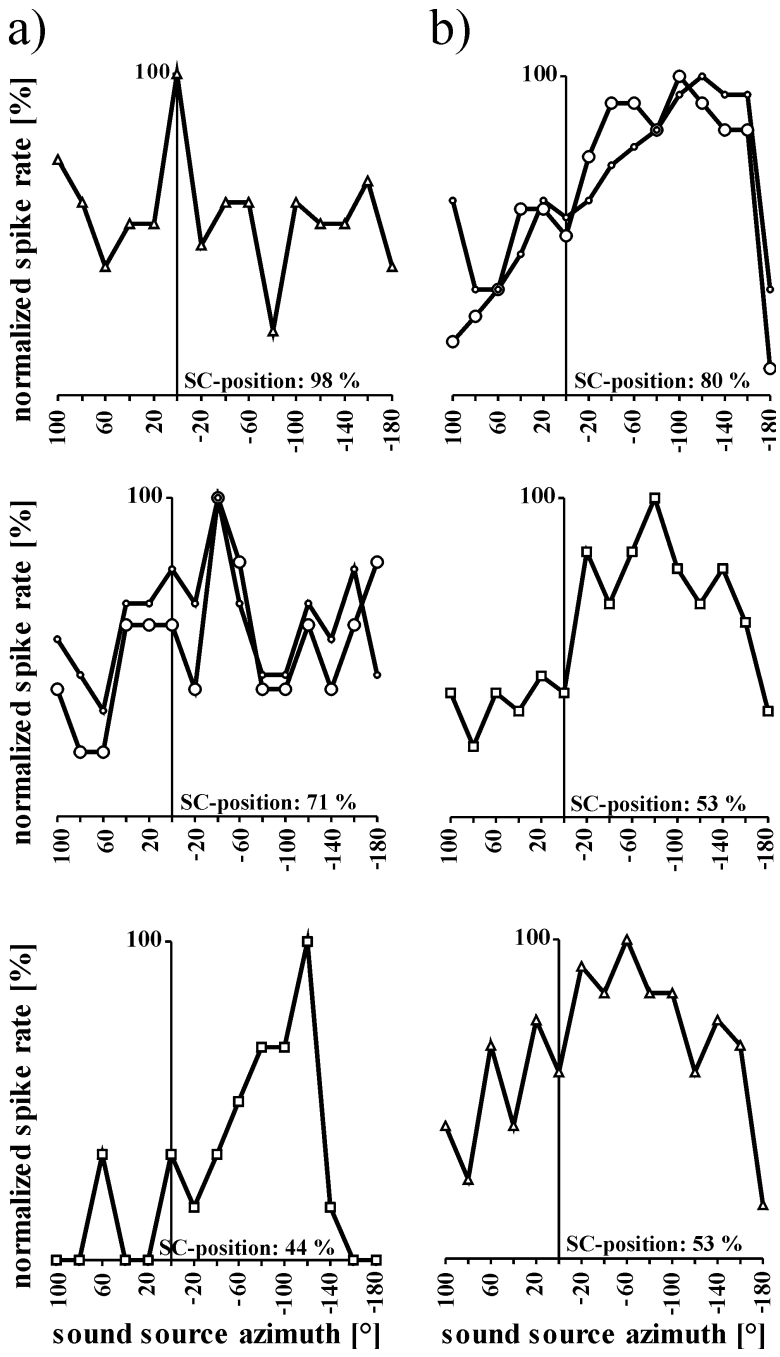

Fig. 4-11) Tuning curves of simultaneously recorded units (arranged in columns). The peak response rate in PSTHs was normalized and plotted as a function of sound source direction (generated from 20 stimulus repetitions at every sound source direction). Same symbols with different sizes indicate recordings from one electrode. Different symbols indicate recordings from different electrodes. Recording positions are described as percent distance from the caudal pole along the whole rostrocaudal extend of the SC (rostral pole = 100 %) and depicted above the maximal response of each tuning curve. (a) Units showing a steep peak in their tuning to a single sound source direction. (b) Broadly tuned units responding to all sound source directions in the contralateral hemifield. Best azimuth directions of simultaneously recorded neurons exhibit a similar amount of variability as was found in the regression analyses.

population (paired t-test: N = 21, t = 2.73, p = 0.0128) and no significant difference for pairs for the medium population (paired t-test: N = 21, t = 3.81, p = 0.802). The BA_{PEAK} of anterior units in the medium population was on average 47.6° more frontal than the BA_{PEAK} of the more posterior located unit (mean electrode distance = 959 µm). Nevertheless, there was again a

remarkable degree of variability of spatial organization observed in the simultaneously recorded pairs. Examples of tuning curves reflecting variability in spatial arrangement of BA_{PEAK}-values are shown in Fig. 4-11. In Fig. 4-11, column a an arrangement is depicted, where more frontal best azimuth values were located at more rostal positions in the SC, as would be expected from the overlying map of visual space (Siminoff et al., 1966; Diao et al., 1983). Two-thirds of the pairs of units belonging to the ventral population were arranged that way. One-third of the pairs of units, however, was arranged as shown in Fig. 4-11, column b: the more temporal best azimuth value was recorded at the more rostral position in the SC. In the medium population, only one half of the pairs showed a best azimuth arrangement with the more frontal value recorded at more rostral positions in the SC.

To get an estimate for the degree of local variability in the rat SC, I compared the spatial tuning of units recorded simultaneously from the same electrode. The median of the maximal difference between BA_{PEAK}-values recorded from one electrode was 40° (ranging from 0° to 140°, N = 19). In 90% of the units recorded from a single electrode, BA_{PEAK}-difference was less than 100°, which matches exactly the variability in BA_{PEAK}-values I could observe at a given SC position in the variance analysis of lower SC neurons (Fig. 4-9b).

4.4. Discussion

The aim of this study was to examine whether auditory responses of units in the rat SC represent sound source location in a systematic manner. I found that the majority of auditory units exhibited a preference for particular regions of space. A single peak in the azimuth response profile was found in 73% of the units. Tuning width at halfmaximal response activity was usually broad and strongly intensity dependent. The preferred sound directions covered a wide range of azimuths and elevations within the contralateral hemifield. The spatial tuning as determined from rate activity was paralleled by similar tuning in latency shifts. At lower intensity levels but clearly suprathreshold, there was a tendency for the best azimuth to vary topographically, but only in the ventral population of SC auditory units (located in the deep layers). Anterior positions were represented in more rostral parts of the SC and more peripheral

positions represented in more caudal parts of the nucleus. These findings are consistent with the general pattern found in small mammals.

The data gathered in this study suggest that the rat SC might contain two different populations of auditory neurons. These populations were separated by a layer of tissue at a depth of about 1500 µm (presumably the intermediate white layer), from which auditory neurons could seldom be recorded. Tuned auditory units dorsal to the transition zone (i.e. medium population) are located in the intermediate white and intermediate gray layer, and units ventral to this transition zone (i.e. ventral population) are located in the deep layers of the SC. Since the lesions were not made through all electrodes, a more detailed reconstruction could not be performed.

The two populations of auditory neurons showed several differences in their characteristics. Auditory SC neurons in the medium population exhibited bimodal responses to auditory and visual stimuli to a higher degree but showed no spatial arrangement. However, mainly non-visual, but topographically organized auditory neurons were in the ventral population of auditory SC neurons located ventral to the intermediate white layer. The fact that I found multimodal but non-topographically arranged neurons in the medium population was somewhat strange and contradicted the hypothesis that for correct multimodal integration spatially aligned representations of the different senses would be necessary (Stein & Meredith, 1993). Nevertheless, auditory neurons in both populations exhibited comparable characteristics in best azimuth tuning and tuning width, suggesting the same binaural processing mechanisms and the same encoding of sound source azimuth in both populations. As far as I know, a similar classification of SC subpopulations has not been observed in other mammals so far.

I found a significant spatial organization of auditory neurons in the rat SC, showing a gradient of best azimuth directions ranging from frontal directions in the rostal SC to the representation of lateral/backward directions in the caudal SC (Fig. 4-9b). This finding was verified by the examination of pairs of simultaneously recorded neurons. The representation of contralateral auditory space in each SC, however, is highly scattered. At a given rostrocaudal position, best azimuth values could differ up to about 140°. In ferrets variability of best azimuth values at a SC position was in general smaller, but could also reach differences of about 60° (King & Hutchings, 1987). A major input from auditory structures to the SC comes via the nucleus of the brachium of the inferior colliculus (BIC, King et al., 1998). Again, a systematic

arrangement of best azimuth values was described for the ferret BIC (Schnupp & King, 1997). There, the local variability of best azimuth values reaches about 100° at a given BIC site, much higher than in the ferret SC. The representation in the rat SC seems to be more comparable to the ferret BIC. Representation of acoustic space in the cat SC, on the other hand, seems to be much more precise (Middlebrooks & Knudsen, 1984)

Simultaneous recording of several auditory neurons along the rostrocaudal axis of the SC provided the basis for a methodical unbiased test of the topographic representation of auditory space. First, spatial tuning obtained from responses of simultaneously recorded neurons reflects the same stimulation conditions and internal status of the animal. Second, I could directly relate the distance between recording electrodes to the differences in best azimuth tuning. I found, as mentioned above, a similar topography in pairs of simultaneous recordings as in the variance analysis of the whole data set. Additionally, a similar amount of variability was observed in the tuning of these pairs: In 67% of the pairs, arrangement of BA_{PEAK}-values was in accordance with the overlying visual map (nasal best azimuth at rostral position), but 33 % showed an inverse arrangement (the more temporal best azimuth was recorded at a more rostral position) at a mean electrode distance of 960 µm. Simultaneous recordings further revealed that there is a mean shift in best azimuth of about 50°/mm of SC distance along rostrocaudal axis (whole length is about 3.5 mm), which would allow for the representation of the whole hemisphere of contralateral space in each SC. Comparable data can be calculated from the linear fit in the analysis of variance. There, I found a smaller mean shift of at least 36°/mm SC distance.

Spatially tuned auditory cells should provide the neural substrate for the ability to localize sound sources. The SC is one of the structures where responses of spatially tuned neurons are directly linked to motor output generation, i.e. to orienting responses towards a novel stimulus (Westby et al. 1990). Using the data from this study, I am now able to compare neural and behavioral data in the rat. Minimal audible angles have been tested in the rat for midline sound localization, i.e. the ability to discriminate between speaker located on the left and right site of the frontal meridian, or for lateral sound localization, i.e. the ability to discriminate between speakers located in the same hemisphere. When tested with speaker positions of at least 20° separation and symmetrical arrangement around 0° azimuth rats showed a high performance in discriminating sound source azimuth (Ito et al., 1996; Kavanagh & Kelly, 1986). Performance

dropped dramatically when both speakers were arranged within the same hemisphere (Potash & Kelly, 1980; Kavanagh & Kelly, 1986). Rats do not seem to be able to localize sound sources within one hemifield even though they can do so between hemifields.

Comparing these behavioral findings to neuronal characteristics in the SC does not provide a clear picture. High localization abilities are thought to correspond to higher densities of cells with best azimuth values at certain sound directions, analogous to the visual fovea. I found an accumulation of best azimuth directions to lateral parts of the contralateral hemifield (-60° to -140°). In behavioral tests the rat showed poor localization abilities in this directional range. Although I recorded 35% of the units in the most rostral 20% of the SC (Fig. 4-9a), best azimuth values around 0° were observed rarely (Fig. 4-6a, 4-6c, Fig. 4-9b, 4-9c), directly contradicting the observed good sound localization performance at frontal directions. Best azimuth tuning, therefore, seems to be not the only parameter determining sound localization abilities. In the rat, auditory responsive neurons were represented along the whole rostrocaudal extend of the SC. This representation is similar to that described for ferrets (King & Hutchings, 1987) and cats (Middlebrooks & Knudsen, 1984). In the guinea pig and the hamster, however, the representation of auditory neurons was found to spare rostral parts of the SC (guinea pig: King & Palmer, 1983; hamster: Chalupa & Rhoades, 1977; Rhoades et al., 1983).

As another characteristic of azimuthal tuning, tuning width could be an important parameter determining sound localization abilities. In the rat, spatial tuning was generally broad, in most cases similar to the so called 'hemifield' neurons in cats (Middlebrooks & Knudsen, 1984), covering a major part of the contralateral side. Activity in the ipsilateral hemisphere was typically reduced. This broad tuning compared to former studies is partially due to the fact that I based our analysis on peak activity of onset responses. Nevertheless, tuning is still broader in the rat than found before in the cat, ferret, or guinea pig SC (ferret: King & Hutchings, 1987; guinea pig: Palmer & King, 1982). Increased tuning width at more lateral directions (represented more caudal in the SC) was found in the cat (Middlebrooks & Knudsen 1984) and in the ferret (King & Hutchings, 1987). Although not significant, in the rat there was a tendency for auditory units to exhibit sharper tuning at more rostral SC positions.

In summary, auditory spatial information at supra-threshold levels, at which most natural stimuli occur, is represented in the rat SC by rather broadly tuned neurons. For azimuth

representation, they are arranged in a systematic manner along the rostrocaudal axis of the SC with frontal directions being represented at the rostral pole. The representation is obviously scattered, but I confirm that the rat SC contains a coarse topographic map of auditory space, as observable in our recordings. Since behavioral sound localization performance in the rat does not directly match the spatial characteristics found in single SC neurons, it might presumably be derived by some degree of neuronal integration at this stage of auditory processing. One possible mechanism could be a population code of spatial direction in the SC, as shown before in the ascending auditory pathway (Fitzpatrick et al., 1997). Such a code was in detail investigated in the auditory cortex (Middlebrooks et al., 1994; Middlebrooks et al. 1998). Encoding in population activity in the SC was so far only described for saccadic eye movements in primates (Lee et al., 1988; Anderson et al., 1998).

5. Influence of spatial attention on auditory midbrain neurons in the barn owl

The role of the barn owl's midbrain in neural mechanisms of sound localization has been studied intensively. Thereby, the midbrain has been defined as an essential structure for reflexive head orienting towards peripheral auditory stimuli. Here I present evidence that the midbrain of barn owls is further modulated by cognitive influences, i.e. by the intention to perform a specific head turn and by spatial attention. I recorded from auditory OT units of one awake barn owl performing a cue-directed selection paradigm. The cue-directed selection paradigm consisted of a visual cue that was followed by two simultaneously presented noise bursts. The visual cue pointed towards the behaviorally relevant stimulus which should be localized by the owl to get a reward. I found that the neural activity differed significantly between OT hemispheres depending on the direction of the head turn in response to auditory stimulation. The development of this direction-dependent bias was closely related to cue presentation, indicating that the cue information was evaluated for planning the head turn. Responses of OT units to auditory stimuli were significantly different depending on the behavioral context, i.e. if the auditory stimulus in the receptive field was relevant or irrelevant, suggesting an influence of attention. I could show that these effects were not caused by simple bimodal interaction of visual cue and auditory stimuli but rather by changes in the internal state of the animal. Many auditory OT units (45%) showed also significantly higher spike rates while the owl performed the head movement, demonstrating that these neurons have an important function in sensorimotor integration.

5.1. Introduction

Orienting towards salient auditory events is an important behavior for survival. In barn owls, sound localization is an essential part of the hunting behavior since they hunt for mice in the dark and rely almost entirely on auditory information. Cognitive influences, such as attention, can improve sound localization capabilities in unclear situations. In chapter 3. of the current thesis, I could show that sound localization behavior in barn owls can be modulated by

spatial attention using a cross-modal spatial cueing paradigm (Johnen et al., 2001): response latencies of head turns towards auditory stimuli were significantly shorter when the animal was attending to the side of the next upcoming stimulus.

The neural basis of sound localization is well understood based on studies from a few model systems as e.g. the barn owl. In barn owls, an essential structure for sound localization is the midbrain optic tectum (OT), where auditory neurons with spatially tuned receptive fields encode for the position of a sound source (Knudsen, 1982). These neurons further elicit, when activated, reflexive orienting of the head towards auditory stimuli (Wagner, 1993; du Lac & Knudsen, 1990). However, an additional pathway for sound localization has been described in the barn owl's forebrain (Knudsen et al., 1993; Cohen & Knudsen, 1999) which also mediates sound localization behavior and which is suggested to perform cognitive processing of auditory stimuli (Cohen & Knudsen; 1995, Knudsen & Knudsen, 1996).

How the processing related to orienting behavior is modulated by attention was so far only investigated in the visual system. There have been three studies that concentrated on the superior colliculus (SC) of monkeys. Two of them found that the activity of visual responsive neurons in the SC was not modulated by cognitive influences (Wurtz, Goldberg & Robinson, 1982; Robinson & Kertzman, 1995). The third study investigated neurons from motor-related sites and found that electrically elicited eye movements were modulated by a shift of spatial attention (Kustov & Robinson, 1996). In contrast to the observations in the monkey superior colliculus (SC), investigations in the parietal cortex of primates could demonstrate a variety of cognitive influences. It was found that neural activity in the parietal cortex of monkeys was modulated by an influence of spatial attention which enhance the neural response to behavioral relevant stimuli at attended positions (Robinson et al., 1995; Colby & Goldberg, 1999) and an influence of goal-related intention that underlies the programming and execution of a specific movement (Andersen, 1995, Snyder et al., 1997, 2000).

The neural basis of attentional modulation in the auditory system of barn owls has not been investigated so far. I therefore started out to record activity from auditory OT units of awake barn owls performing a cue-directed selection paradigm. The cue-directed selection paradigm consisted of a visual cue that was followed by two simultaneously presented noise bursts from two independent sound sources on each side. The cue indicated the behaviorally

relevant side in the actual trial. The owl should localize the source of the noise burst on the cued side and ignore the noise burst on the other side. I investigated the activity of auditory OT units during stimulation with behavioral relevant or irrelevant stimuli and during orienting towards acoustic stimuli. I expected to find attentional modulation of activity in the OT as activity there lead to auditory evoked orienting.

5.2. Methods

Two separate experiments were carried out: first, a behavioral experiment to measure the threshold for level discrimination and, second, an experiment that combined behavioral and neurophysiological measurements to investigate auditory spatial attention (see below). In the first experiment two barn owls (*Tyto alba*) participated and in the second only one of them. Owls were obtained from the institute's breeding colony and previously participated in a crossmodal-spatial cueing paradigm (Johnen et al, 2001). All procedures were approved by the Regierungs-präsidium Köln and followed the European Communities Council Directive on care and use of laboratory animals (86/609/EEC).

The behavioral setup was mounted in an anechoic chamber (IAC, base: 2 x 2 m, height: 2.6 m) which was darkened during the measurements. The sound field was calibrated using a microphone (1/4‘‘, type 4135, Brüel & Kjaer, Naerum, Denmark) with preamplifier (type 2660, Brüel & Kjaer) and power-supply (type 2804, Brüel & Kjaer). RMS sound pressure levels (dB SPL) are expressed relative to 20 µPa. The frequency response of the system was flat (+/- 6 dB) between 1 and 20 kHz (loudspeaker cutoff frequency).

5.2.1. Behavior: Threshold measurements

As a preparatory study for the cue-directed selection paradigm, I determined the sensitivity of two owls to level differences between uncorrelated noise bursts presented from spatially separated loudspeakers (TW 70, 8 Ohm, Visaton, Germany) that were fixed at a position of 40° in azimuth on both sides. Noise bursts with a duration of 100 ms were generated by two independent waveform generators (WG1; all instruments from Tucker Davis

Technologies, Gainesville, FL, U.S.A) and regulated by two attenuators (PA4) to a reference level of 40 dB SPL. Level differences between bursts ranged from 0 dB (both bursts at a level of 40 dB SPL) to 4 dB (one source attenuated by 4 dB) in steps of 0.5 dB. The sequence of which speaker side should be the louder in the next trial was derived from sequences proposed by Gellerman (1933). As a control a level difference of –1 dB for both sides were randomly intermixed in 10% of the trials, i.e. the sound source opposite to the sequence-derived side was 1 dB louder. Thereby, I could test if owls used other cues than the level differences to choose a response side.

5.2.2. Behavior: Cue-directed selection paradigm

Testing in the cue-directed selection paradigm involved combined behavioral and neurophysiological measurements. Only one owl participated in this experiment. Here, the methods used for the behavioral tests are explained. The following chapters refer to the methods that were used for neurophysiological measurements with this paradigm.

The same behavioral setup was used. A visual cueing stimulus was added to indicate the relevant side in the next trial. A custom-built display for the cueing stimulus was placed in front of the owl, about 45 cm from the owl's head. The display contained three horizontally arranged LEDs, in total covering 5° of visual angle. The central LED was only extinguished during reward delivery and served as a reference for orientation towards the display. Cue presentation was initiated automatically, when the owl gazed towards frontal directions. As a cueing stimulus, one lateral LED was switched on for 500 ms, indicating the relevant stimulus hemisphere. The two noise bursts (100 ms) followed as target stimuli after a variable cue-target delay. The cue-target delay was defined as the time between the offset of the cue LED and the onset of the target sound and was ranging from 200 to 500 ms in steps of 100 ms. A schematic drawing of the behavioral setup and the timing of stimulus presentation is given in Fig. 5-1.

In the cue-directed selection paradigm, cue presentation was obligatory and provided valid directional information for the behavioral response. The simultaneously presented noise bursts had always 0 dB difference, so that the behavioral response was not guided by loudness differences. The static loudspeaker was positioned ipsilateral to the implanted OT hemisphere,

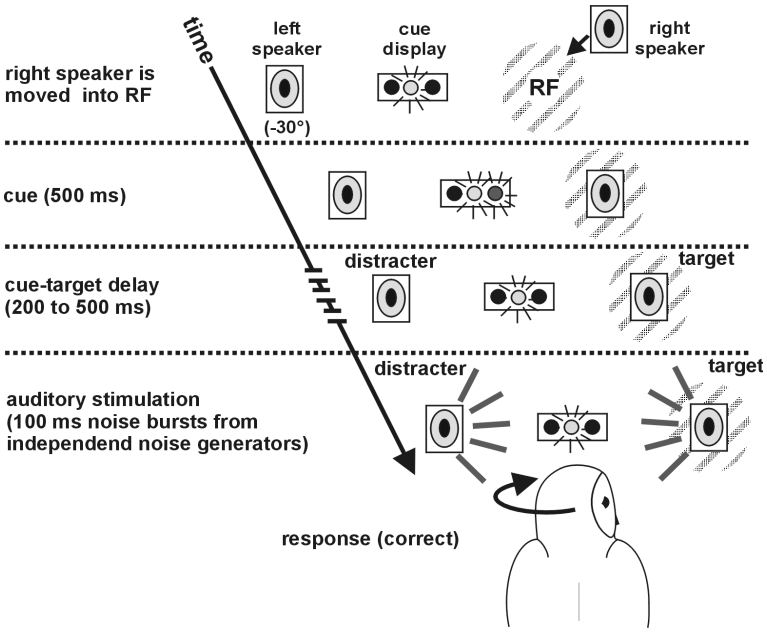

Fig. 5-1) Schematic illustration of the experimental setup and the stimulus sequence in the cue-directed selection paradigm. At the beginning of a trial, when the owl gazed to frontal directions, an indirect visual cue pointed towards the side of the following target. After a randomized delay, two auditory stimuli came up simultaneously: a target and a distracter. For a correct response, owls had to make a head saccade towards the target. Head movements were monitored using a customized magnetic tracking system.

and the hoop-mounted speaker which could be moved into the receptive field (RF) of auditory units was positioned on the contralateral side. By convention, negative azimuthal angles refer to ipsilateral positions with respect to the implanted OT hemisphere and positive to contralateral positions, respectively. In elevation, negative angles refer to positions below the auditory horizon and positive angles to positions above the horizon. The position of the static speaker was at –40° azimuth and 0° elevation. The position of the hoop-mounted speaker was set to the center of RF's, ranging from 25° to 45° azimuth and 20° to –40° elevation.

5.2.3. Behavioral response

In both behavioral paradigms, the required behavioral response was a head saccade towards the source of the relevant target stimulus, i.e. the louder stimulus or the stimulus in the hemisphere indicated by the cue. Head movements were obtained with a magnetic tracking system (3Space Fastrak, 120 Hz sampling frequency, Polhemus Inc., Colchester, VT, U.S.A) attached to the reference post on the owl's forehead during the experiment. Using the tracking technique, the direction of gaze could be reliably determined from the orientation of the head,

because eye movements are virtually absent in owls (Steinbach and Mooney 1972). All correct trials with an apparent head saccade, as monitored by video cameras, were rewarded with small portions of chick meat delivered from a custom-built feeder.

5.2.4. Neurophysiology: Implants

Varnish-coated tungsten electrodes (9-11 Meg; F.H.C, Bowdoinham, ME, USA) were mounted in custom-built microdrives. Microdrives consisted of two canulae and a miniature screw (M1.6x16). The canula diameters were 0.8 mm and 0.5 mm. After they were shortened to appropriate length (9 mm and 13 mm, respectively), the smaller canula was inserted into the larger, thereby forming a movable cannula that served to hold the electrode. The tip of the miniature screw was beveled to remove the thread for about 3 mm. The beveled part of the screw and one side of the larger canula were attached to a socket consisting of dental acrylic, so that the screw could turn without producing drive. The threaded part of the screw and one side of the smaller canula were mounted to a second acrylic block that had an appropriate thread and a female connector. By turning the screw, this block moved along the screw's axis, i.e. away from or towards the socket for about 330 µm during a 360° turn, and the smaller canula slid into the larger. The blunt, uninsulated side of an electrode was cut and solidly attached to the movable block. The bared end was contacted to the female connector using conductive adhesive (EPOXY Produkte GmbH & Co Vertr. KG, Fürth/Odenwald, BW, Germany). Depending on the owl's size, the electrode extended between 12 and 15 mm from the microdrive.

5.2.5. Neurophysiology: Surgery

Owls underwent more than one surgery. Surgeries were repeated only after several weeks or months. In an initial surgery, owls were equipped with a metal reference post that was used to attach the probe of a magnetic tracking system to the head during behavioral measurements and to fix the head in a custom-built stereotaxic frame during surgery to implant the electrodes. After an appropriate recovery period, owls had performed a cross-modal spatial cueing paradigm (Johnen et al., 2001). For the studies reported here, firstly the threshold measurements for the

perception of simultaneous presented noises containing level differences were conducted and secondly the cue-directed selection paradigm. At the end of the threshold measurements, first owl 1 and, with a delay, owl 2 was implanted with electrodes. After surgery to implant the electrodes, owls restarted with the paradigm used for threshold measurements and were then retrained on the cue-directed selection paradigm. Neurophysiological recordings were conducted only in the cue-directed selection paradigm. In this thesis, due to the delay between implanting the owls, threshold measurements are presented for both owls but results from the cue-directed selection paradigm are presented only for owl 1.

Surgery to implant electrodes was similar to that performed in other studies conducting electrophysiological recordings from midbrain neurons in anesthetized owls (Wagner, 1993): Owls were sedated with Diazepam (Valium, 1mg/kg/h) and anesthetized with Ketamin (10-15mg/kg/h). The head was fixed in the stereotaxic frame and a skin incision was made over the dorsal surface of the skull. A part of the dural surface was exposed through craniotomy. Using a custom-built clamp, the microdrive was attached to a remote-controlled stepper motor. The electrode, which was fixed in the microdrive, was positioned at an appropriate position onto the dura and advanced dorsoventrally into the brain in steps of 10 µm or 50 µm steps. Anesthetized owls were stimulated via earphones and the electrodes in the microdrives were used to record neural activity. Activity was tested for auditory responsiveness and for spatial response characteristics as determined by interaural time and level differences (ITD, ILD). Electrophysiological characteristics in combination with stereotaxic coordinates allowed to determine whether the position of the electrode tip was within the OT (Knudsen, 1982). Electrodes were lowered to sites in the tectal map of acoustic space that were useful for behavioral experiments, i.e. to best azimuth angles of more than 25° lateral from the owl's midline. Azimuth is mapped in the barn owl's OT from anteromedial to posterolateral, and elevation is mapped dorsoventrally (Knudsen, 1982). When a useful position was isolated, the socket of the microdrive was glued to the skull with dental acrylic. From that position the microdrive allowed for 1 mm upward and 5 mm downward movement of the electrode. Thus, with respect to the representation of acoustic space in the barn owls midbrain, best azimuthal positions of neurons recorded along the electrode track was reasonably stable, whereas best elevation changed when moving the electrode upward or downward. After the microdrives were

implanted, the craniotomy was closed with dental acrylic and the skin incision was sutured. The wound was treated with antibiotics. Implanted owls behaved completely normal after recovery from the surgery and showed no signs of discomfort. In owl 1, two electrodes were implanted into the left OT hemisphere, and in owl 2, three electrodes into the right OT hemisphere. A new set of electrodes was implanted in owl 1 after 3 months.

5.2.6. Neurophysiological recordings

Neural activity was recorded from the implanted electrodes, when the owls performed the cue-directed selection paradigm. At the beginning of a recording session, new units were isolated by lowering the electrode tips into deeper parts of the OT. Neural activity was passed through a custom-built headstage, then amplified and band-pass filtered (300 - 6000 Hz) with an 8-channel amplifier (Lynx8, Neuralynx, Tucson, AZ, U.S.A), and displayed on an oscilloscope. Single units (SU) and multi units (MU) were separated according to their waveform using customized spike sorting software (Discovery, DataWave Technologies Corporation, Longmont, CO, U.S.A.).

5.2.7. Data analysis: Behavior

Analysis of the owls' behavior was the same in the threshold measurements and in the cue-directed selection paradigm. Behavioral classifications were determined trial by trial from actual stimulus conditions and the horizontal head movement vector performed by the owl. For classification, turning angles and turning directions were analyzed: Turning angles were calculated from the maximum of the horizontal head movement trajectory. Their direction could be to the right or to the left from the frontal gaze direction at the beginning of the trial. Additionally, response latency was calculated as the time elapsing between the onset of the auditory stimulus and the point when the head saccade exceeded a threshold of 5° minimum turning angle. Due to this threshold criterion, latency measurements were generally prolonged by about 8 ms with respect to the precise start of the head saccade.

Head turns were assigned to three response types by a computer algorithm using simple classification attributes: (i) correct responses, i.e. head turns towards the relevant sound source with a minimum angle of 5°, (ii) incorrect responses, i.e. head turns away from the relevant sound source with a minimum angle of 5°, and (iii) non-responses, when the head-turning angle was < 5°, or the latency of the head turn was > 2000 ms. For correct and incorrect responses, the maximum head-turning angle, i.e. the perceived target direction, had to be stable within 2° for at least 85 ms. Head shakings, which occurred depending on owl and behavioral paradigm in 3% to 13% of the trials, were omitted from analysis.

To quantify the stability of initial fixation, we calculated the magnitude and direction of the largest movement in a 200 ms-sliding window during the time prior to target presentation. Detailed analysis revealed that the owls made only small head movements while waiting for the target stimulus: In 88 % of the trajectories, the movements were smaller than 2° including head saccades and slow drifts. Most of the early movements occurred at onset of the cueing stimulus, when the owls had gazed to frontal directions but not precisely towards the display.

5.2.8. Data analysis: Neural activity

Analysis of neural activity depended on behavioral classifications. Thereby, a direct correlation between behavior and neural activity was made. Spike rates of auditory OT units were analyzed according to behavioral response types (correct, incorrect, and no response) and sides (to the right, to the left). For each unit, matched pairs were compared by a modulation index (MI, equation 1) which calculates normalized differences in the mean spike rates:

$$MI = \frac{\text{mean spike rate [condition A]} - \text{mean spike rate [condition B]}}{\text{mean spike rate [condition A]} + \text{mean spike rate [condition B]}} \quad (1)$$

Particular conditions (A, B) analyzed with MIs are presented in detail in the Results section (see below). I used MIs to analyze several time windows of a trial with a binsize of 20 ms or 100 ms. These included calculations of spontaneous activity, cue-evoked activity, activity during the cue-target delay, response to auditory stimulation, and activity during motor response.

I further used MIs to analyze differences in activity across response types (correct, incorrect, no response). MIs were reversed to relative activity levels in % by using equation 2:

$$\text{relative activity [\%]} = 100 * 2MI / (1 - MI) \quad (2)$$

5.3. Results

5.3.1. Behavior: Threshold measurements

As a result of the preparatory study I calculated the threshold from a logistic fit to the psychometric function ranging from 50% to 100% performance: Owl 1 reached the threshold criterion of 75% performance at a level difference of 1.19 dB and owl 2 at a level difference of 1.68 dB (Fig. 5-2a, b, open symbols). At a level difference of 0 dB, when the level and spectra of the simultaneous noises were virtually identical, both owls operated near chance level. The only parameter which paralleled with an increase in performance was the experimentally introduced level difference (ANCOVA: $F_{17,36} = 5.92$, $p < 0.01$; level difference: $F_{8,36} = 11.91$, $p < 0.01$; owl: $F_{8,36} = 0.11$, $p = 0.998$). The performance at the control stimulus (Fig. 5-2a, b, filled symbols) was below 50%, again indicating that both owls rather responded to the louder stimulus than to other cues. However, since performance is not perfectly symmetrical around 50% there might be also other cues that influence discrimination performance in this paradigm.

5.3.2. Behavior: Cue-directed selection paradigm

The study using the cue-directed selection paradigm was conducted to investigate the influence of a visual cueing stimulus on both general behavior of barn owls and the physiology of neurons in the optic tectum. In this paradigm two noise bursts were presented simultaneously like in the threshold measurements, but without level differences. Thus, owls had no auditory driven cues to choose a specific side. The relevant side was instead indicated by an indirect visual cueing stimulus that was presented from a display in front of the owl (Johnen et al., 2001). Only data from owl 1 are analyzed and presented in this thesis.

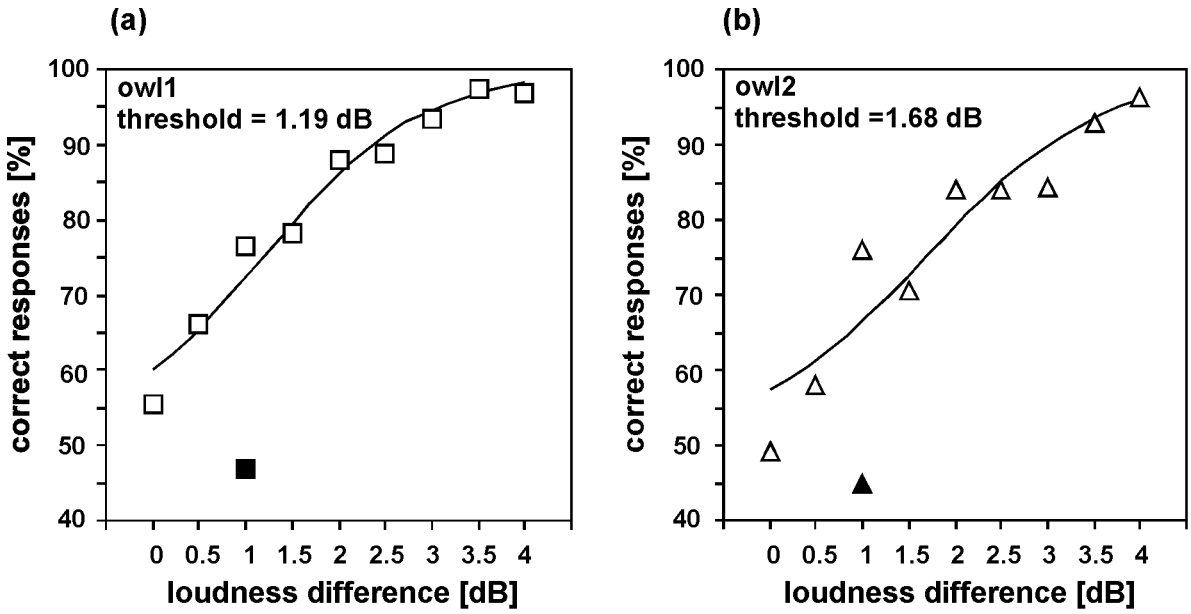

Fig. 5-2) Psychometric functions of two barn owls in the level discrimination paradigm. The task involved two simultaneously presented noise bursts with relative level differences ranging from 0dB to 4dB. Noise bursts were generated from independent generators and were presented from spatially separate speakers, one in each hemisphere. Owls had to orient towards the side of the louder stimulus. (a) Owl 1 reached the threshold criterion (75% performance) at 1.19 dB level difference. (b) Owl 2 reached the threshold at 1.68 dB level difference. At 0 dB level difference, both owls performed at chance level. Open symbols refer to the psychometric function and closed symbols to the control stimulus (1 dB with reversed sides).

I obtained 2350 trials in the cue-directed selection paradigm. Of those, 1057 were correct trials (45 %), 651 incorrect trials (28 %), and 642 no-response trials (27 %). Parallel to the behavioral measurements, neurophysiological recordings from OT neurons were obtained. In all trials with a correct, an incorrect or with no response, neurophysiological recordings were investigated using unit-based or average MIs.

To determine the owl's performance in the cue-directed selection paradigm, the relative occurrence of correct and incorrect responses was compared statistically by testing the probability whether in a cumulative binomial distribution of $n = n_{correct} + n_{incorrect}$ trials the occurrence of correct responses is greater than 50%. This test revealed that owl 1 exhibited a significant performance of 62% to correctly determine the side indicated by the cue (binomial test, $p < 0.01$, $n = 1708$). Thus, owl 1 clearly used the information provided by the cue, but not

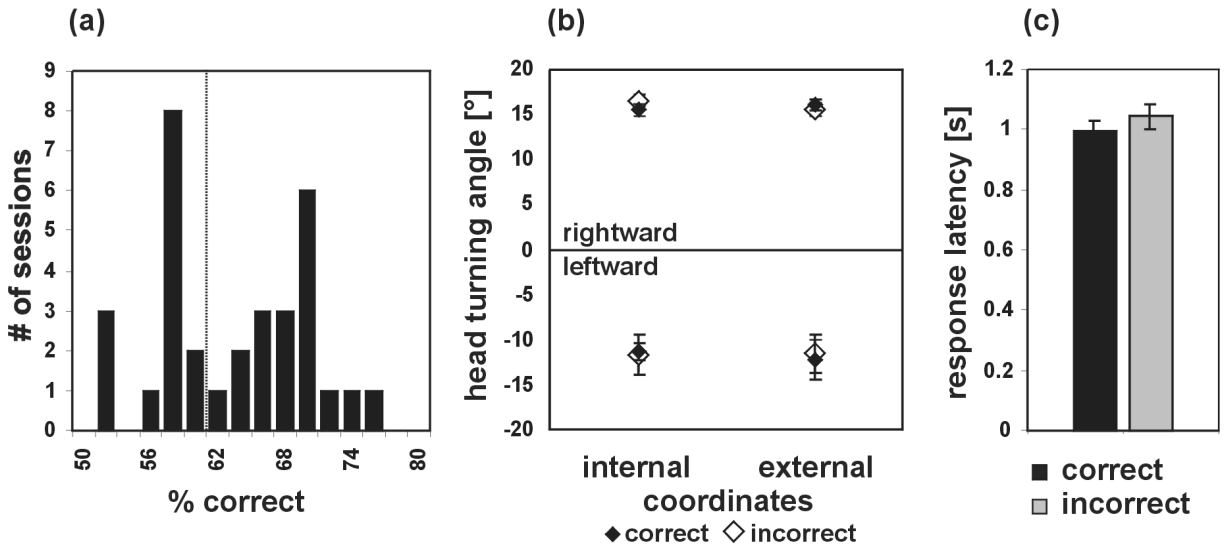

Fig. 5-3) Analysis of the behavior in correct and incorrect trials in owl 1 in the cue-directed selection paradigm. First, head turning responses were classified according to response side (to the right, to the left) and response type (correct, incorrect, no response). Then, behavioral performance and other behavioral parameters were compared between classifications. (a) The animal exhibited significant performance in choosing the target side over all 32 sessions. However, not all of the sessions reached the significance level. As shown in the distribution, performance in a session could vary between 52 % and 76%. Level of significance is indicated by the vertical line. Importantly, performance never fell below 50%, indicating that a reversed interpretation of the cue did not occur.
Other behavioral parameters like head turning angle (b) and response latency (c) did not consistently differ between correct trials and incorrect trials. In (b) head turning angles (means +/- 95% confidence intervals) are depicted for correct trials (closed diamonds), incorrect trials (open diamonds), separated according to response side (positive angles refer to responses to the right and negative angles to responses to the left). Response latencies in (c) are depicted as means +/- 95% confidence intervals.

obligatory. The distribution of the percentage of correct responses in 32 sessions is depicted in Fig. 5-3a.

Other behavioral parameters, that could be tested for differences between correct and incorrect trials, were the response latency, the amplitude of the head turn (in internal coordinates), and the position at the end of the turn (in external coordinates). I found that none of these parameters seemed to be consistently different in incorrect trials compared to correct trials (Fig. 5-3b, c). However, there was a trend that response latencies were shorter in correct than in incorrect trials (Fig. 5-3c, ANOVA, $F_{1,1708} = 2.87$, $p = 0.091$).

5.3.3. Neural activity

In 32 sessions, I recorded from 66 units in one animal, i.e. 1 to 4 units per session. Out of those, 62 units showed a significant excitatory response to auditory stimulation, as determined by paired t-tests between auditory evoked activity and baseline activity (Fig. 5-4, "target"). Since this is the first study recording from OT neurons in awake barn owls, I first looked at general response patterns.

All investigated auditory units were of the bursty type (Knudsen, 1982), usually generating a couple of spikes with short intervals and varying amplitudes when activated. Peristimulus time histograms (PSTHs) of auditory units revealed that auditory responses were vigorous and mainly phasic (e.g. Fig. 5-5a). Spatial tuning characteristics were comparable to those of recordings from anesthetized owls (Knudsen, 1982). In awake owls, best elevation moved from positive angles (above auditory horizon) to negative angles (below auditory horizon) along the electrode track, whereas best azimuth was around 30° to 45° lateral. This pattern was consistent with the organization described for the auditory map in anesthetized owls (Knudsen, 1982). No significant responses of the units to the visual cue could be observed when the activity during cue presentation was compared to baseline activity (paired t-tests, Fig. 5-4, "cue"). This was expected because visual stimulation (cue) and auditory stimulation (target) were spatially separated by at least 25° so that a bimodal activation was very unlikely. Comparisons between activity during the onset of the head turn and baseline activity revealed a significant increase in spike rate in 45% of the units parallel to a motor response (paired t-tests, Fig. 5-4, "response"). This indicated that many OT neurons in our sample had integrative functions in sensorimotor processing.

In the following, quantitative measurements for differential activation will be investigated for the whole population of OT units using MIs. For population-based MIs, all 62 unit-based MIs were averaged in each analysis. The results from the average-MI analyses are presented separately for the three response types (correct, incorrect, no response).

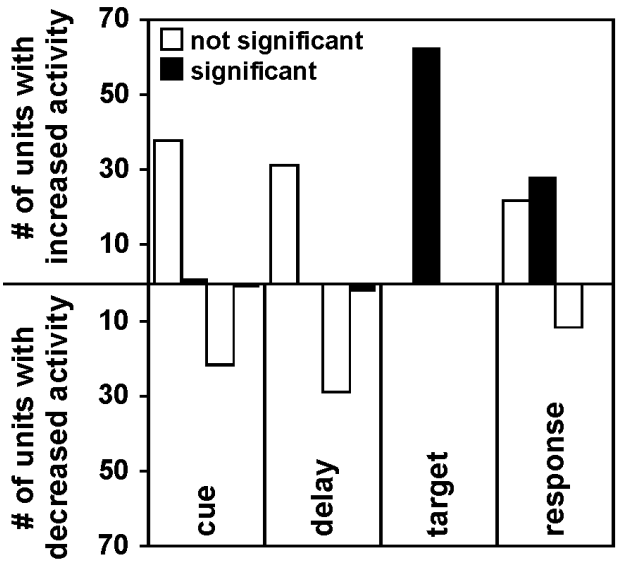

Fig. 5-4) Unit-based analysis of activity of auditory OT units in target trials. Four different time windows (cue presentation, delay, target presentation, and motor response) are compared to baseline activity. Units with higher activity than baseline activity are depicted as positive bars, units with lower activity as negative bars. Significant paired t-tests are shown as black bars and non-significant paired t-tests as open bars. In each time window, bars add up to the 62 units that were investigated. All units showed significant auditory activation. Interestingly, 45% of the units also changed their response rate significantly during motor response, indicating that many of the investigated units had functions related to sensorimotor gating.

I first compared the neural activity in several time windows of a trial to the baseline activity which was measured before cue onset. These time windows were 100 ms and included cue-evoked activity, activity during cue-target delay, response to auditory stimulation, and activity during motor response:

$$MI = \frac{\text{mean spike rate [actual time window]} - \text{mean spike rate [baseline]}}{\text{mean spike rate [actual time window]} + \text{mean spike rate [baseline]}}$$

Thus, positive average MI-values indicate a higher activity in the time window than baseline and negative values a lower activity than baseline.

Units were further analyzed for changes in auditory responsiveness related to behavior by comparing the conditions that the speaker in the RF was behavioral relevant, i.e. served as target, and the condition that it was irrelevant, i.e. served as distracter (see Methods, Fig. 5-1) using the following MI:

$$MI = \frac{\text{mean spike rate [response to target]} - \text{mean spike rate [response to distracter]}}{\text{mean spike rate [response to target]} + \text{mean spike rate [response to distracter]}}$$

The behavioral role of the speakers was defined by the cue. Since target and distracter stimuli were identical noise bursts presented from the same speaker, differences in auditory

responsiveness could only derive from changes in the animal's internal state. Trials in which the speaker in the units RF presented the target will be termed 'target trials', and the other will be termed 'distracter trials'. Positive average-MI values indicate a higher activation in response to the target, negative average-MI values a stronger activity in response to the distracter. Values around zero indicate similar activity in target and distracter trials.

5.3.4. Correct responses

The results of the analysis for correct responses are depicted in Fig. 5-5. An overlay of raw PSTHs of one unit for target trials and distracter trials is shown in Fig. 5-5a. I found that auditory evoked activity was higher when the stimulus in the RF was a target than when it was a distracter. This difference could not be explained by small level differences between the loudspeakers, since target and distracter were identical noise bursts presented from the same speaker. Instead, this difference clearly demonstrated an influence of internal states, presumably dependent on the cue-directed orienting of attention, on auditory midbrain neurons in the barn owl.

The comparison of activity during a trial and baseline activity revealed that neural activity changed systematically during a trial (Fig. 5-5b). Highest activity levels were observed during auditory stimulation. This activation consisted of a significant increase in spike rate in response to target and distracter stimuli (average MIs: paired t-test, $target_{auditory\ stimulation} = 0.71$, $t_{61,62} = 29.23$, $p < 0.01$; $distracter_{auditory\ stimulation} = 0.6$, $t_{61,62} = 12.1$, $p < 0.01$). Importantly, activation levels were higher in response to target (MI = 0.71, relative activity compared to baseline = 490%) than to distracter stimuli (MI = 0.6, relative activity compared to baseline = 300%). A clear and significant activation could further be observed during the motor response, indicating that many of the investigated neurons were sensorimotor neurons. This activation depended on the response side of the owl: in target trials, when the owl turned its head towards the RF side, activity increased above spontaneous levels, and in distracter trials, when the owl oriented towards the opposite side, activity fell below spontaneous levels (average MIs: paired t-test, $target_{motor\ response} = 0.26$, $t_{61,62} = 5.44$, $p < 0.01$; $distracter_{motor\ response} = -0.32$, $t_{61,62} = -3.94$, $p <$

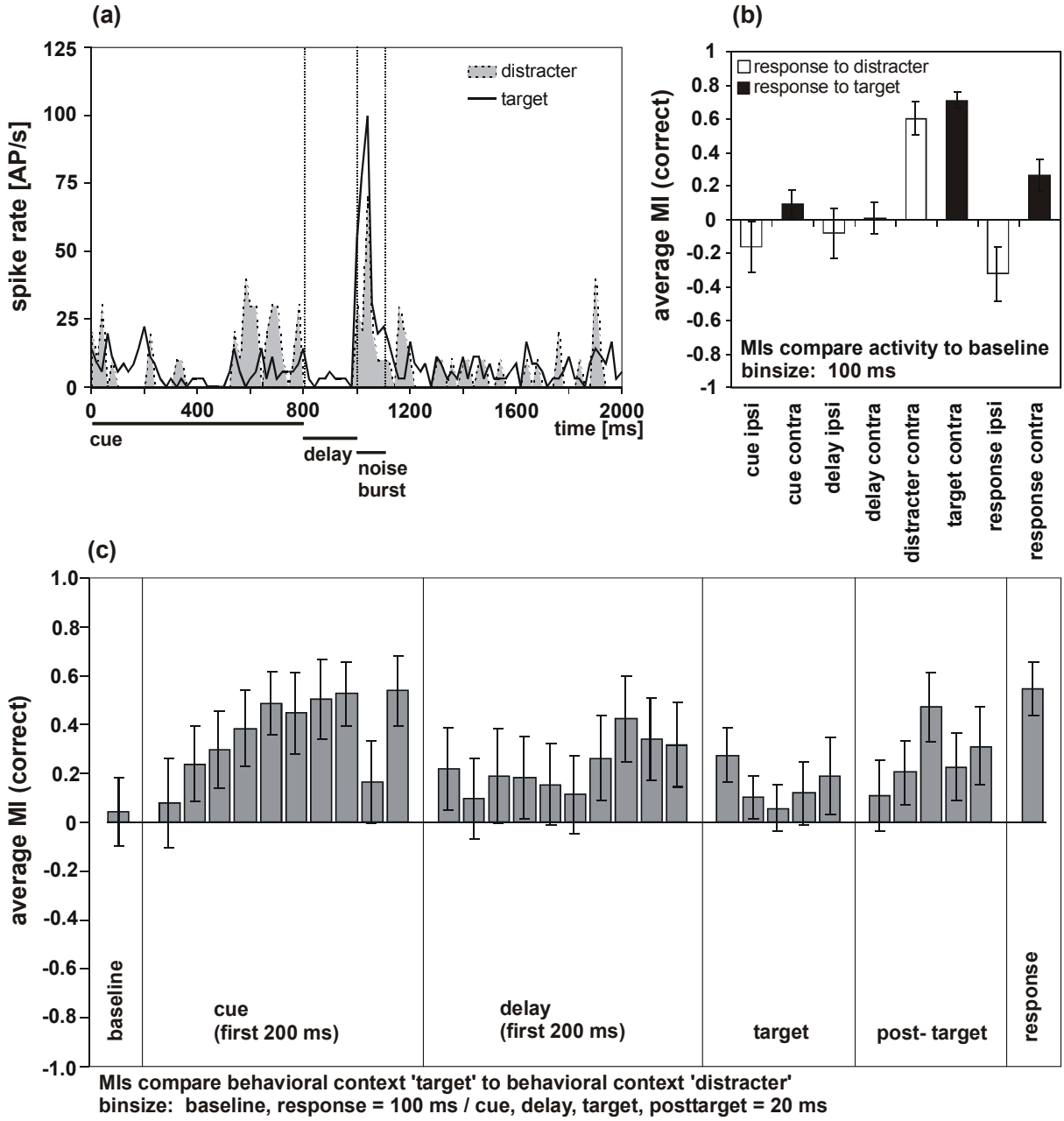

Fig. 5-5) Evaluation of neural activity in correct trials. (a) Change of activity of an OT unit over time separated for responses to the target or to the distracter. Depicted are the peri-stimulus time histograms (PSTHs) for cue presentation, delay period, and auditory stimulation. Trials are aligned to the onset of auditory stimulation. We found that the OT unit responded more vigorously to the target than to the distracter in correct trials. Note that target and distracter stimuli were identical noise bursts presented from the same loudspeaker. The observed difference in spike rate can therefore not be explained by stimulus differences, but rather by a change in the internal state of the animal.

(b) Population-based analysis of the activity of all investigated auditory OT units (n = 62) in target and distracter trials. Depicted are average MIs comparing neural activity in different 100ms-time windows of a trial (cue presentation, cue-target delay, auditory stimulation, and motor response) to baseline activity (mean +/- 95% confidence intervals). Activity was significantly different from baseline levels during auditory stimulation and motor response. Responses to auditory stimulation were more vigorously when the loudspeaker in the RF presented the target. During motor response, head turns towards contralateral directions were paralleled by an increase in activity, and head turns towards the ipsilateral side by a decrease below baseline levels.
(c) Population-based analysis comparing neural activities in target trials and distracter trials using averaged MIs of all investigated OT units (n = 62). Average MIs were depicted as mean +/- 95% confidence intervals for different time windows of a trial (cue presentation, cue-target delay, auditory stimulation, post-target, and motor response). During auditory stimulation, most of the investigated auditory units showed a stronger response to the target than than to the distracter. Importantly, this difference developed during cue presentation, indicating a change in the animal's internal state.

0.01). Using equation 2, I found that this refers to an increase in activity of 70% in target trials and a decrease in activity of 94% in distracter trials compared to baseline.

Activity during cue presentation and cue-target delay changed mainly non-significantly when compared to baseline activity. However, I observed an activity pattern during cue-presentation and delay which paralleled the side-dependent activation during motor response: activity increased above spontaneous levels when the animal would finally turn its head towards the RF side later in the trial (average MIs: paired t-test, $target_{cue\ presentation} = 0.09$, $t_{59,60} = 2.14$, $p < 0.05$; $target_{delay} = 0.01$, $t_{60,61} = 0.2$, $p = 0.84$), and activity fell below baseline when the owl oriented torwards the opposite side, i.e. towards the distracter ($distracter_{cue\ presentation} = -0.16$, $t_{57,58} = -2.05$, $p < 0.05$; $distracter_{delay} = -0.08$, $t_{59,60} = -1.9$, $p = 0.28$). Using equation 2, these changes in relative activity fell in the range of 2% to 38% compared to baseline.

A comparison between the t-tests in Fig. 5-4 and the target-related average MIs in Fig. 5-5b (black bars) demonstrates that MIs are a very reliable measure for differential activation of units with varying baseline levels.

I then went on to analyze the development of activity in the OT over time by quantifying the normalized differences between the conditions that the noise burst in the RF was as target or a distracter using average MIs at a binsize of 20 ms (Fig 5-5c). For cue presentation and cue-target delay, the first 200 ms (10 bins) were analyzed, otherwise the first 100 ms (5 bins). I found

a consistent upward modulation, i.e. a higher activation level in target trials, which was observable throughout all investigated time windows (Fig. 5-5c). This effect, already shown in the raw PSTH in Fig. 5-5a, existed in most of the units investigated. Importantly, this difference in activation developed at the beginning of cue presentation, indicating a cue-related change in the animal's internal state. After the cue, the pattern of activation remained stable until the head turn was performed.

The increase in activity during cue presentation might have been related to small head movements. To test this, I compared small movements between correct and no-response trials: In both response types, small movements occurred to the same amount (movements to the left: t-test, $t_{122,124} = -1.16$, $p = 0.25$; movements to the right: t-test, $t_{122,124} = -0.68$, $p = 0.5$), but neural activity during cue presentation differed clearly (see chapter "*No responses*"). I conclude that in correct trials, when the owl followed the cue information, an attentional effect is reflected consistently in the differential neural activation in both OT hemispheres.

5.3.5. Incorrect responses

The results of the analysis of incorrect responses are depicted in Fig. 5-6. An overlay of raw PSTHs from the same unit as in Fig. 5-5a for target trials and distracter trials is shown in Fig. 5-6a. In incorrect trials, responses to the noise bursts were higher in distracter trials than in target trials. This was the opposite result to what was found in correct trials (Fig. 5-5a) and demonstrated that there was also a change in the animal's internal state which finally resulted in a head turn towards the incorrect side. This internal state, however, ignored the information provided by the cue.

When the activity in different time windows of a trial was compared to baseline activity, highest activation levels were found during auditory stimulation (Fig. 5-6b). As in correct trials (Fig. 5-5b), this activation consisted of a significant increase in spike rate in response to target and distracter stimuli (average MIs, paired t-tests: $distracter_{auditory\ stimulation} = 0.74$, $t_{55,56} = 19.41$, $p < 0.01$; $target_{auditory\ stimulation}$: 0.53, $t_{55,56} = 9.07$, $p < 0.01$). Different from correct trials, auditory

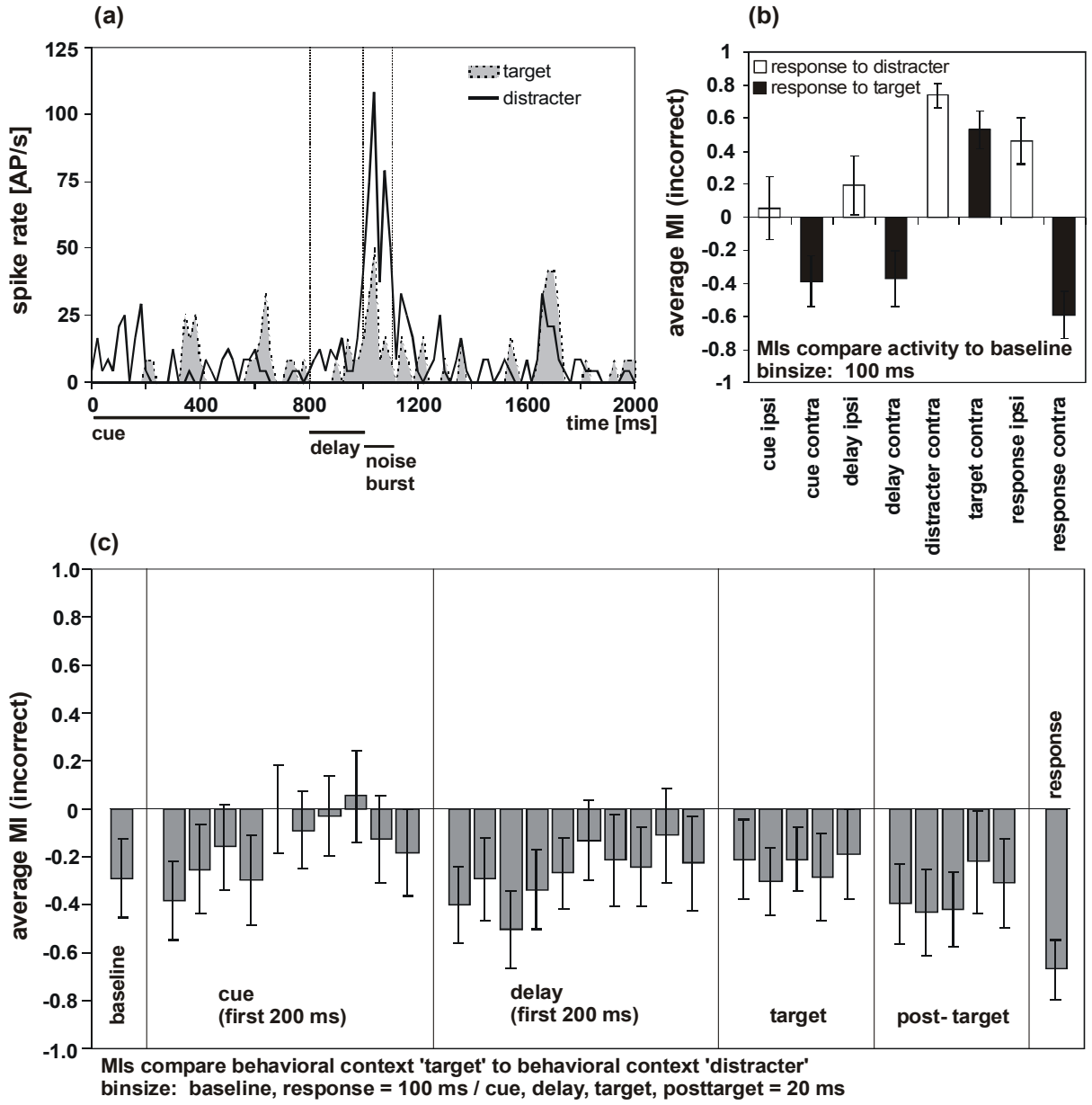

Fig. 5-6) Evaluation of neural activity in incorrect trials. (a) Change of activity of an OT unit over time separated for responses to the target and to the distracter. Depicted are the peri-stimulus time histograms (PSTHs) for cue presentation, delay period, and auditory stimulation for the same units as in Fig. 5-5a. Trials are aligned to the onset of auditory stimulation. In incorrect trials, the unit responded more vigorously to the distracter than to the target. This was opposite to what was found in correct trials. Note that target and distracter stimuli were identical noise bursts presented from the same speaker. The observed difference in spike rate can therefore not be explained by stimulus differences or cue information, but may derive from the animal's intention to perform a head turn towards one side.

(b) Population-based analysis of the activity of all investigated auditory OT units (n = 62) in target and distracter trials. Depicted are average MIs comparing neural activity in different time windows of a trial (cue presentation, cue-target delay, auditory stimulation, and motor response) to baseline activity (mean +/- 95% confidence intervals). Significant changes in activity compared to baseline levels occured during auditory stimulation, motor response, and also in some cases during cue presentation and cue-target delay. Responses to auditory stimulation were more vigorously when the loudspeaker in the RF presented the distracter. During motor response, head turns towards contralateral directions were paralleled by an increase in activity, and head turns towards the ipsilateral side by an decrease below baseline levels. These observations resemble the results of those in correct trials, however, with reversed behavioral relevances for target and distracter (Fig. 5-5b).
(c) Population-based analysis comparing neural activities in target trials and distracter trials using averaged MIs of all investigated OT units (n = 62). Average MIs were depicted as mean +/- 95% confidence intervals for different time windows of a trial (cue presentation, cue-target delay, auditory stimulation, post-target, and motor response). In contrast to correct trials (Fig. 5-5c), most of the investigated auditory units responded more vigorously to the distracter than to the target. This effect was observable and significant already before cue presentation, i.e. at baseline level. This suggests a longer lasting influence that results in differential activation of OT hemispheres. This intention-related activity was partially confounded during cue presentation. However, the original intention was restored during cue-target delay, as visible in the negative MIs, and resulted in an incorrect response.

activity was higher after stimulation with a distracter (MI = 0.74, relative activity compared to baseline = 569%) than with a target (MI = 0.53, relative activity compared to baseline = 226%).

A clear and significant activation could further be observed during motor response, however, in reversed directions compared to correct trials: when the owl turned its head in response to the distracter towards the RF side, activity increased above baseline levels, when it oriented towards the opposite side, activity fell below baseline (average MIs, paired t-tests: $distracter_{motor\ response} = 0.46$, $t_{52,53} = 6.43$, $p < 0.01$; $target_{motor\ response} = -0.59$, $t_{51,52} = -8.16$, $p < 0.01$). Using equation 2, this refers to an increase in relative activity of 170% in distracter trials and a decrease in activity of 287% in target trials compared to baseline. Thus, during a head turn, the direction of the turn seemed to be the most important factor and not the behavioral role of the speaker.

During cue-presentation and the cue-target delay the direction of activation resembled the differential activation as found during motor responses, but average MIs were not always significantly different from baseline. Activity increased above spontaneous levels when the

animal would turn its head towards the RF side later in the trial (average MIs: paired t-test, $distracter_{cue\ presentation} = 0.05$, $t_{48,49} = 0.54$, $p = 0.59$; $distracter_{delay} = 0.19$, $t_{52,53} = 2.11$, $p < 0.05$), and activity fell below baseline when the owl would oriented towards the opposite side ($target_{cue\ presentation} = -0.39$, $t_{52,53} = -4.91$, $p < 0.01$; $target_{delay} = -0.37$, $t_{54,55} = -4.4$, $p < 0.01$). These results are comparable to those in correct trials, but again with reversed directions. Using equation 2, I found in incorrect trials modulations in relative activity during cue presentation and cue-target delay ranging from 10% to +128% compared to baseline.

When the activity in target trials was compared to that in distracter trials, a downward modulation was found, i.e. a lower activation when the stimulus in the unit's RF was a target than when it was a distracter (Fig. 5-6c). This effect, for one example shown in the raw PSTH in Fig. 5-6a, existed in most of the investigated units. Importantly, the bias towards the distracter side did not develop at the beginning of cue presentation as in correct trials (Fig. 5-5c), but was already visible and significant very early in the trial, i.e. during baseline activity (Fig. 5-6c). Related to this early and long-lasting bias, the animal chose a response side and ignored the information provided by the cue. Interestingly, when the cue was presented, the additional side-dependent information partially confounded the intention of the animal to go towards the distracter side. As a result, average MIs changed during cue presentation from negative to positive values, suggesting that the cue influenced the animal's internal state. However, the initial bias was restored during cue-target delay and lead to an incorrect response.

5.3.6. No responses

The results of the analysis of no responses are depicted in Fig. 5-7. PSTHs of no-responses trials, as depicted for the same typical unit as in correct and incorrect trials, showed similar auditory activation levels in target and distracter trials (Fig 5-7a).

When the activity in different time windows of a trial was compared to baseline levels, it was observed that a significant increase in activity compared to baseline levels occurred only during auditory stimulation. Auditory responses in no-response trials were as vigorous as in correct or incorrect responses (Fig. 5-7b, average MIs, paired t-test, $distracter_{auditory\ stimulation} = 0.75$, $t_{61,62} = 30.43$, $p < 0.01$; $target_{auditory\ stimulation} = 0.73$, $t_{61,62} = 26.95$, $p < 0.01$). Importantly,

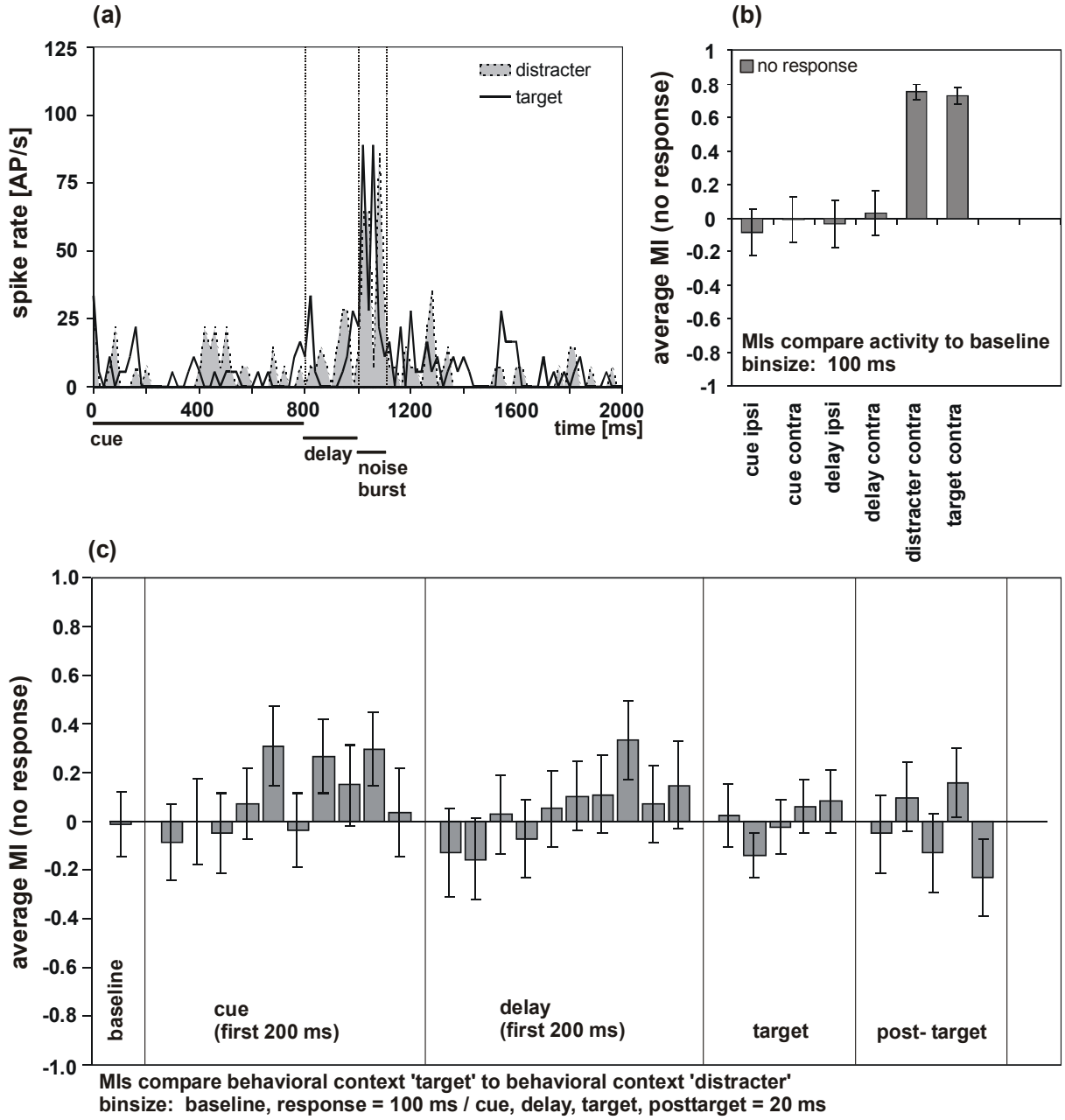

Fig. 5-7) Evaluation of neural activity in no-response trials. (a) Change of activity of the same OT unit as in Fig. 5-5 and 5-6 in target and distracter trials. Depicted are the peri-stimulus time histograms (PSTHs) for cue presentation, delay period, and auditory stimulation. Trials are aligned to the onset of auditory stimulation. In contrast to correct (Fig. 5-5a) and incorrect trials (Fig 5-6a), no obvious differences in neural activation between target and distracter presentation could be observed in no-response trials.

(b) Population-based analysis of the activity of all investigated auditory OT units (n = 62) in target and distracter trials. Depicted are average MIs comparing neural activity for different time windows during a trial (cue presentation, cue-target delay, auditory stimulation, and motor response) to baseline activity (mean +/- 95%

confidence intervals). A significant increase in activity compared to baseline levels only occurred during auditory stimulation. Auditory responses were not significantly different to target compared to distracter stimuli. This observation differed from the effects found in correct trials (Fig. 5-5b) and incorrect trials (Fig. 5-6b).
(c) Population-based analysis comparing neural activities in target trials and distracter trials using averaged MIs of all investigated OT units (n = 62). Average MIs were depicted as mean +/- 95% confidence intervals for different time windows of a trial (cue presentation, cue-target delay, auditory stimulation, post-target, and motor response). In contrast to correct trials (Fig. 5-5c) and incorrect trials (Fig 5-6c), no consistent trend in differential activation of target or distracter could be observed. Thus, neural activity indicated already during cue presentation, that the animal would finally show no response at the end of the trial.

there was no side-dependent increase in activation in response to the target or to the distracter. In fact, auditory activation levels were fairly similar in target trials (MI = 0.75, relative activity compared to baseline = 541%) and distracter trials (MI = 0.73, relative activity compared to baseline = 600%).

As already shown, I found in correct and incorrect trials that during cue-presentation and cue-target delay the direction of activation resembled the side-dependent activation during motor response. In no-response trials, on the other hand, neural activity during cue-presentation and cue-target delay showed no bias either to the side of the target or to the side of the distracter (Fig. 5-7b). This paralleled the observation that the animal would not turn it's head at the end of the trial. Such a non-activation pattern was typical for no-response trials and differed from that in correct and incorrect responses (average MIs: paired t-test, $distracter_{cue\ presentation}$: -0.09, $t_{54,55}$ = -1.2, p = 0.23; $distracter_{delay}$: -0.04, $t_{55,56}$ = -0.51, p = 0.61; $target_{cue\ presentation}$: -0.01, $t_{56,57}$ = -0.13, p = 0.9; $target_{delay}$: 0.03, $t_{56,57}$ = 0.42, p = 0.68). Using equation 2, I found that changes in relative activity fell only in the range of 2% to 20% compared to baseline.

When the activity in target trials was compared to that in distracter trials, no consistent trend in differential activation of target or distracter could be observed (Fig. 5-7c). This pattern, for one unit shown in the raw PSTH in Fig. 5-7a, existed in most of the investigated units. Thus, neural activity indicated already early in the trial, i.e. during cue presentation and cue-target delay, that the animal would show no response at the end of the trial.

5.3.7. Influence of the behavioral response type

In a last step I were interested in the question if auditory evoked responses were comparable between response types or if there were any pattern that suggested a reduced sensitivity of OT units to auditory stimulation in one of the response types. I therefore collapsed the response sides for each of the 3 response types (correct, incorrect, and no response) and performed pairwise comparisons between the neural activity in the three response types using the following MI:

$$MI = \frac{\text{mean spike rate [e.g. response } \textit{correct}\text{]} - \text{mean spike rate [e.g, response } \textit{incorrect}\text{]}}{\text{mean spike rate [e.g. response } \textit{correct}\text{]} + \text{mean spike rate [e.g. response } \textit{incorrect}\text{]}}$$

I found that neural activity in the OT changed only marginally with response type. Pairwise comparisons between correct, incorrect, and no response showed that relative activity declined from correct responses to no responses and then to incorrect responses (Fig. 5-8). This leads to a statistical significant difference both during cue presentation and delay in the activity between correct trials and incorrect trials (average MIs, paired t-tests: cue presentation: 47% higher relative activity in correct responses, paired t-test, $t_{54/55} = 3.17$, $p < 0.01$; cue-target delay: 50% higher relative activity in correct responses, paired t-test, $t_{54/55} = 3.75$, $p < 0.01$). Importantly, this difference was virtually absent during target presentation and motor response (average MIs, paired t-tests: target presentation: 2 % higher relative activity in correct responses, paired t-test, $t_{55/56} = 0.54$, $p = 0.59$; motor response: 13% higher relative activity in incorrect trials, paired t-test, $t_{55/56} = -1.1$, $p = 0.27$). I found also no significant difference between the auditory responses in correct trials and no-response trials (average MIs, paired t-tests: target presentation: 2% higher relative activity in no-response trials, paired t-test, $t_{61/62} = -0.56$, $p = 0.58$) Thus, at least for auditory stimulation, I conclude that the overall excitability of OT neurons did not change between trials containing a correct, incorrect, or no response.

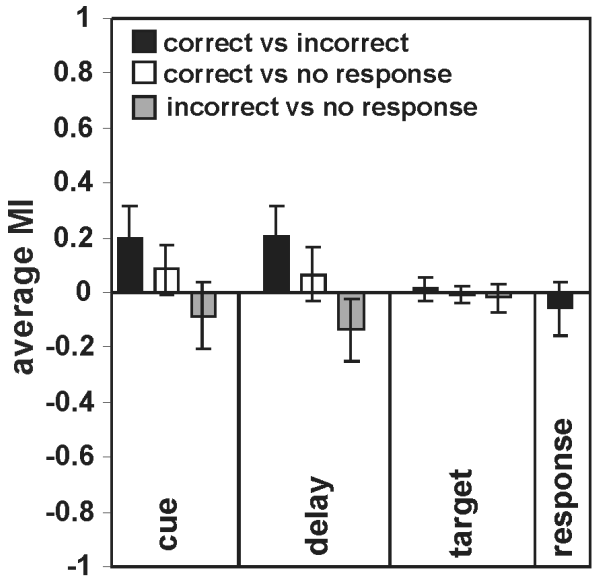

Fig. 5-8) Analysis of neural activity of auditory OT units comparing response types (correct, incorrect, and no response) independent of response sides. Average differences in neural activity between response types were compared using MIs. Depicted are the mean +/- 95% confidence intervals of MI distributions for different time windows (cue presentation, cue-target delay, target presentation, and motor response). As a result, we found by pairwise comparisons that relative activity declined from correct responses to no responses and to incorrect responses. Accordingly, both during cue presentation and delay, activity in correct trials is significantly higher than in incorrect trials. Interestingly, this difference is virtually absent during target presentation and motor response. Thus, for auditory stimulation, we conclude, that neural exitability was not different in trials containing a correct, incorrect, or no response.

5.4. Discussion

This study was conducted to investigate cognitive influences, specifically spatial attention, on sound localization behavior and related neural activity in the OT of barn owls. I recorded from neurons in the OT of one awake animal using permanently implanted electrodes while the owl performed a cue-directed selection paradigm. The cue-directed selection paradigm consisted of a visual cue that was followed by two simultaneously presented noise bursts from two independent sound sources on each side. The cue indicated the behaviorally relevant side in the actual trial. Owls were trained to localize the source of the noise burst on the cued side (target) and ignore the noise burst on the other side (distracter). The owl showed a significant performance in choosing the correct side. When the cue was omitted and the noise bursts had virtually the same level and spectra, this owl and a second owl performed at chance level, i.e. turned to the sound source on each side equally often. This indicates that the cue was in fact used by the owl in choosing the correct target side.

Although the stimulation with two broadband noise bursts that overlap in time and spectrum was a very confusing situation, owls showed neither in the preparatory study nor in the cue-directed selection paradigm any problems in recognizing the simultaneous noise bursts as two events coming from spatially separated sound sources. This observation is consistent with the results of other studies investigating the precedence effect in barn owls. First, when noise bursts with independent waveforms, each with an individual ITD, were presented to spatially tuned neurons in the external nucleus of the inferior colliculus (ICx), an individual set of neurons became excited by each noise (Takahashi & Keller, 1994). Correspondingly, when two correlated noise bursts that generate a single ITD were presented to these neurons, only one population was excited. These results indicated that two independent noise bursts excite individual populations of neurons that could be a correlate for the perception of two separate sound sources. Second, when noise bursts with identical waveforms are presented from two spatially separate speakers, barn owls perceive the existence of a single phantom sound source at an intermediate position (Keller & Takahashi, 1996). In our experiments, I found that owls oriented their heads to a variety of spatial positions during a behavioral session which would not suggest that they perceived a single phantom sound source for most of the trials. Instead, our studies demonstrated that owls were capable of discriminating two simultaneously perceived sounds with and without preceding visual cue.

I were interested in the question, whether cognitive influences modulate the activity of neurons in the OT of barn owls, because it is known that this structure is essentially involved in generating sound localization behavior (Konishi, 1995). In chapter 3. it was shown that this behavior is influenced by spatial attention. In the midbrain of barn owls, information about the location of a sound source is represented in the activity of space-specific neurons which encode sound source direction in their spatial response profile (Knudsen & Konishi, 1978; Knudsen, 1982). Activation of these neurons is sufficient to elicit head orienting as shown by electrical stimulation (du Lac & Knudsen, 1990) or focal lesions (Wagner, 1993). However, a critical question was, whether auditory neurons trigger the orienting behavior by relaying the sensory information to motor-related efferent neurons, or whether they are directly involved in motor control. In our experiment, due to the challenging paradigm for the owl, head movements were sufficiently delayed from auditory stimulation (on average about 1 sec) to allow for a separate

analysis. I found that many auditory neurons (45%) also showed significant movement-related activation that depended on the response side: Orienting towards contralateral directions was paralleled by an increase in spike rate and orienting towards ipsilateral directions by a decrease below spontaneous levels. This indicated that OT units act as sensorimotor neurons and that they are possibly also involved in the control of non-reflexive orienting behavior.

As a main result of the investigation of cognitive influences on neural activity in the barn owl's midbrain I found that OT neurons exhibited a significant difference in auditory activity depending on the behavioral role of the stimulus as target or distracter. This difference could not be devoted to any differences in level or spectrum of the auditory stimuli, because target and distracter were identical noise bursts presented from the same speaker. My results clearly suggest that the neural excitability changed with the behavioral context related to cue information. Importantly, I could show that the change in excitability was triggered by cue onset as determined by analyzing the development of activity in the OT over time (Fig. 5-5c). This again demonstrated that the cue had influenced the decision of the owl to turn its head towards the target side. Thus, this study showed a clear influence of the internal state, presumably a shift in spatial attention, on processing of auditory stimuli in the owl's midbrain.

The change in neural activity in close relation to cue onset strongly indicated the existence of cognitive influences on midbrain neurons in our study. As a main criticism one could ask whether this effect was a sensory response to visual stimulation by the cue itself. This is very unlikely for several reasons: First, visual cue and auditory stimuli were separated in space and time which made a interaction of those stimuli in bimodal neurons very unlikely (Knudsen, 1982). Second, unit-based comparisons between cue-related activity and baseline activity revealed that only 2 out of 62 units showed significant excitatory responses to the cue and 1 unit significant inhibitory responses. On the other hand, all 62 units were clearly auditory (Fig. 5-4). Third, the cue-related activation pattern was reversed in incorrect trials (Fig. 5-6c) compared to correct trials (Fig. 5-5c), although the visual stimulation remained similar. Moreover, such an activation pattern was completely absent during cue presentation in no-response trials. For these reasons, a direct influence of the visual cue on the activity of auditory OT neurons can be ruled out. In fact, this activation pattern may rather be related to changes in the behavioral context between trials.

Only a few studies investigated effects of spatial attention on midbrain structures in other animals so far. Two studies in the monkey SC which concentrated on visually responsive neurons (Wurtz, Goldberg & Robinson, 1982; Robinson & Kertzman, 1995) found that the activity of midbrain neurons was not different between the conditions that the RF was within the focus of attention or outside, showing that these neurons were not modulated by attention. A study that investigated neurons from motor-related SC sites found an influence of attention on electrically elicited eye movements (Kustov & Robinson, 1996). However, this study used electrical stimulation, so that the responsiveness of motor-related midbrain sites to visual or auditory stimuli could not be investigated in detail. Our results extend those of Kustov & Robinson (1996) by adding two important new findings: in the OT of barn owls auditory and movement-related activity is reflected mainly in the same neurons and cognitive influences, as e.g. spatial attention, modulate not only motor-related activity but also sensory information already at the midbrain level.

In contrast to the few investigations of cognitive influences on the midbrain level, a substantial body of work was dedicated to cognitive modulation in the neocortex of primates. It was found that neural activity in the parietal cortex of monkeys and humans was modulated by an influence of spatial attention which enhance the neural response to behavioral relevant stimuli at attended positions (Robinson et al., 1995; for review see Colby & Goldberg, 1999) and an influence of goal-related intention that underlie the programming and execution of a specific movement (Andersen, 1995, Snyder et al., 1997, 2000). These findings can now be compared to our investigations in the barn owl's midbrain. By analyzing the neural activity over time I found that there might be more than one effect influencing the sensorimotor OT neurons: First, the presentation of the cue lead to a change in the internal state, presumably by a shift in attention towards one side. In correct trials this influence was strong and resulted in an increase of activity in the OT hemisphere contralateral and in a decrease of activity ipsilateral to the attended side (Fig. 5-5b, c). In incorrect trials, the effect of the cue also caused an increase in activity in the OT hemisphere contralateral to the RF side (Fig. 5-6b, c). This increase, however, was overlaid by a second effect which may be caused by the owl's own decision to perform a head turn towards one side. This intention for a specific movement originated from internal states and not from experimentally controlled parameters. It caused a bias in the activation of OT hemispheres

which was visible and significant already in the baseline activity, i.e. before cue onset (Fig. 5-6c). For unknown reasons, I could not observe this bias in correct trials. However, in no-response trials no cue-related influence and no internal bias towards one side was found, indicating that the owl did not shift attention anywhere and that it never intended to make a response (Fig. 5-7c). Thus, the cognitive influences described in the parietal cortex of primates can be observed with similar characteristics also in the midbrain of barn owls. However, I consider it very unlikely that these influences had their origin in midbrain neurons, whereas in mammals the parietal cortex seems to be an appropriate neural substrate to generate cognitive modulations in orienting behavior.

The origin of the cognitive influences on the barn owl's OT is not clear so far. There are at least two possible explanations where these influences came from. On one hand, it could be that the visual cue stimulated a distinct population of neurons that enhanced the excitability of the investigated auditory neurons by local interactions. A simple local interaction based on hard-wired neural connections could not account for the observed effects since the awake owl could make movements that cause a high variability in the visual stimulation by the cue. However, a local circuit which is modulated by a form of learning may be the reason for the enhanced excitability of auditory OT neurons. While I suppose that this was not the case, the data don't rule out this possibility. On the other hand, there are important forebrain structures that are also involved in sound localization and that project back to most midbrain nuclei (Knudsen et al., 1993; Cohen et al., 1998; Cohen & Knudsen, 1999). These forebrain structures are one of the likely origins for the observed effects in OT neurons. They receive visual and auditory input via thalamic nuclei and form maps of the visual and auditory space which are independent from the midbrain (Cohen & Knudsen, 1999). They further send projections to motor nuclei in the tegmentum which can elicit head movements directly (Knudsen et al., 1993). Backprojections from the visual Wulst to the OT which have been found in the little owl (*Athene noctua*) could also allow for telencephalic guidance of attention (Casini et al., 1992). Thus, it seems to be clear that the forebrain has a very important function in generating orienting behavior. The neural circuits that have been investigated so far would be suited to explain the observed cognitive influences on structures as e.g. the midbrain via top-down projections.

Unfortunately, none of these explanations – bottom up vs. top-down – is favored by our data. Even the time course of the change in neural excitability during cue onset in correct trials could not indicate whether one of these explanations is unlikely. The time course showed that the observed effects are significant already after 40ms (Fig. 5-5), which suggests a local origin, but fully developed at least after 120 ms which would also allow an origin in the forebrain (Fig. 5-5c). Here, further effort in studying the underlying mechanisms is necessary.

Although the specific mechanisms have to remain unclear I would like to offer an explanation for the behavioral relevance of the cognitive influences which are reflected in the excitability of OT neurons: since the forebrain and the midbrain have independent output pathways to the neck muscles (Knudsen et al. 1993), the sensorimotor computations generated in these structures has to be integrated into *one* behavioral context, i.e. has to be coordinated to prevent conflicting commands. To achieve this, a local computation of visual and auditory inputs in the midbrain which is fed forward to the forebrain may be as effective as a feedback from forebrain structures to the midbrain.

6. General Discussion

6.1. Awake preparations in barn owls and rats

In vertebrates, neural mechanisms of sensory processing were mainly investigated in anesthetized animals, as e.g. spatial tuning characteristics of auditory midbrain neurons in barn owls (Knudsen & Konishi, 1978, Knudsen, 1982). Recently, however, it became more and more important to extend such studies to awake preparations using modern recording methods, because it is known that anesthesia has strong influences on neural characteristics (Schmidt & Konishi, 1998, Gaese, 2001). Moreover, investigations in neural mechanisms of cognitive functions, as e.g. attention or intention, require investigations in awake animals since those functions can be observed only in the awake brain. Therefore, efforts in establishing new electrophysiological methods are necessary that allow for measuring neural activity in awake animals. To achieve this, the use of well-known model systems is of great advantage, as fundamental knowledge in neural functions may be applied to modern paradigms and recording methods.

In the current thesis, I investigated midbrain neurons in awake barn owls and anesthetized rats using extracellular electrophysiology. The recordings in awake owls were performed in the midbrain OT and tried to determine to what extend the midbrain is under cognitive control. The study in rats was conducted in the midbrain SC and investigated whether the rat midbrain is a candidate substrate for sound localization. The study in barn owls could show that the midbrain supports cognitive functions. The rat study demonstrated a map of auditory space in the SC. Additionally, it was shown recently in a study conducted in our group that cognitive influences on auditory SC neurons exist also in the rat (Möller, 2002). Both species, barn owls and rats, are therefore well suited models for investigations in auditory attention using electrophysiology in awake animals. Nevertheless, there was a significant difference between those species: in barn owls, cognitive influences resulted in an increased excitability in the OT hemisphere contralateral to the behaviorally relevant side, whereas in rats,

as tested in a comparable cueing paradigm using direct auditory cues, the neural excitability decreased in the contralateral SC hemisphere. Effects similar to that in owls were repeatedly observed in the visual system of primates (Motter, 1994; Desimone & Duncan, 1995; Treue & Maunsell, 1996; Treue & Trujillo, 1999; Kustov & Robinson, 1996). On the other hand, a reduction in responsiveness due to cognitive influences, as described in rats, could also be found in the visual system of primates (Constantinidis & Steinmetz, 2001b), leaving the particular mechanisms unclear that are involved in generating of cognitive modulations.

6.2. Role of cognitive influences on activity of OT neurons

The midbrain is part of the network that processes sensory stimuli for generating appropriate behavior. The midbrain OT is a multimodal structure that mainly represents information about stimulus position or stimulus movement (Meredith & Stein, 1993). Furthermore, this structure has a premotor function that supports orienting of the head or the eyes towards peripheral stimuli. However, the OT acts not completely autonomously, since other pathways of the network, especially forebrain pathways, can initiate orienting behavior by themselves (Knudsen et al., 1993). This raises the question which substrate will succeed, when midbrain and forebrain are competing in generating a response. For the case that events in the environment are easily to detect and to localize, it can be assumed that the midbrain circuit will generate a fast and precise response and that no involvement of the forebrain is necessary (Konishi, 1983; 1995). In more complex situations it is suggested that forebrain involvement is necessary to maintain behavioral demands and goals (Cohen & Knudsen, 1995; Knudsen & Knudsen, 1996). However, it was completely unknown so far, how the midbrain is influenced by the forebrain in a complex sensorimotor task. In principle, several scenarios are possible: first, the OT is performing computations but its output does not reach motor centers. Secondly, the OT becomes completely inhibited and is therefore excluded from the network. Or thirdly, the OT is supporting forebrain functions by filtering auditory information and by co-activation of premotor units. The experiment presented in chapter 5 was designed to test these possibilities: the cue-directed selection paradigm forces the subject to make a response towards the behaviorally relevant side on the basis of spatial information that was derived from a cueing stimulus.

Auditory stimulation was complex and consisted of two competing sound sources. In such a challenging paradigm, the neural substrates that process spatial information have to use all resources that are available to generate behavior that will be rewarded. Thus, in such a complex task the involvement of the midbrain in processing of auditory spatial information can be tested. Using a cue-directed selection paradigm I observed that activity of auditory OT neurons was modulated depending on the information whether the ipsilateral or the contralateral side will be behaviorally relevant in the ongoing trial. In addition, I found that the activity of many OT neurons changed during the response period depending on the direction of the owl's head turn. These results clearly suggest that the midbrain is supporting cognitive processes by modulating auditory responses and by presetting an intended response. Thus, I would suggest that in case of the barn owl the competing pathways in midbrain and forebrain cooperate to maintain sensorimotor contingency for generating appropriate behavior.

6.3. Outlook

There are two main questions that should be addressed in further studies: are the observed cognitive influences generated in the midbrain, which is possible but very unlikely, or are they generated in the forebrain? Which forebrain structures are involved in supporting cognitive functions as attention or intention?

In the barn owl, forebrain pathways are well investigated with respect to sensory processing (Cohen & Knudsen, 1996, 1999; Cohen et al. 1998) and to anatomical connections (Knudsen et al., 1993). On the other hand, only few studies were conducted to study the forebrain's role in cognitive functions (Knudsen & Knudsen, 1996). It is known, that the auditory midbrain is an important part of the ascending auditory pathway, thus able to feed-forward side-dependent modulations of auditory activity to the forebrain. On the other hand, top-down projections from forebrain structures, as e.g. the neostriatum caudolaterale, the archistriatum (Knudsen et. al, 1993) or the visual Wulst (Casini et al., 1992), to a variety of midbrain nuclei have been described. These projections would allow to influence midbrain nuclei with respect to attention or intention. To test the network structures involved, it would be useful to obtain more data on the functional connections between forebrain and/or midbrain nuclei. This was

extensively done e.g. for the pathways involved in song production in the zebra finch and led to a very detailed picture of the neural mechanisms and structures that modulate song behavior in this species (Brainard & Doupe, 2000).

Recordings in awake, behaving barn owls are a well suited method to investigate the mechanisms that lead to cognitive effects in the described network. However, the use of an appropriate behavioral paradigm is crucial. In our group, two strategies in shifting the animal's attention towards one side were used: first, in the barn owl study, indirect visual cues were presented which pointed towards the behaviorally relevant side before onset of auditory stimulation. The precise position of the upcoming auditory stimuli was not cued and had to be localized by the owl. Visual cueing contained a high percentage of valid stimulation: 80% in the crossmodal-cueing paradigm and 100% in the cue-directed selection paradigm. Thus, owls had a benefit from using the cue information. Secondly, direct auditory cues were used in the rat study that was performed in parallel in our group. Here, auditory cues and targets were presented from the same speaker in the valid condition, thereby directly indicating the position of the relevant stimulus. The percentage of valid cueing was also 80%, again allowing for having a benefit from attending to the cued side. Although there were differences between the precise implementation of the paradigms (crossmodal vs. unimodal cueing, indirect vs. direct cues) the induced behavioral effects were very similar (Gaese, 1999; Johnen et al., 2001) and also similar to those found in humans (Spence & Driver, 1996, 1997).

Nevertheless, an important difference between the described cueing paradigms becomes obvious when they are used in combination with electrophysiological recordings in behaving animals: Crossmodal cueing involves more than one modality, as e.g. vision plus audition in the study in barn owls. Unimodal cueing, on the other hand, stresses one modality twice in a trial, since cue and target are from the same sensory quality. This was the case for auditory stimulation in the rat study. Thus, each paradigm has its own advantages and drawbacks for the investigation of neural mechanisms that lead to cognitive modulation of midbrain neurons. The use of unimodal cueing requires to control for time constants of neural excitability in the modality investigated. This is necessary to avoid artifacts caused by the neuron's stimulation history, as e.g. adaptation. But a great advantage of unimodal cueing is that cue and target are represented in the same modality, so that processing of both stimuli can be monitored in the

neural substrate. Thereby, the involved structures and their particular contributions to the processing of cue and target can be studied easily. Using cross-modal cueing, on the other hand, minimizes problems with stimulation artifacts, since cue and target can be separated in space and time. This ensures that only the target is presented in the receptive field of the neuron. The drawback of crossmodal cueing, however, is that the neural substrate integrating visual and auditory information can not be discovered easily. In the barn owl, forebrain structures which could be candidates for audio-visual integration and involved in generating cognitive effects have not been investigated so far. For further studies, thus, a more fundamental strategy in discovering appropriate neural substrates is necessary, e.g. by combining different types of cueing paradigms (unimodal, crossmodal) or by combining experiments under anesthesia with experiments in awake animals to get a more detailed picture about multimodal integration and cognition in the barn owls forebrain.

7. Summary

1. Attention improves processing of sensory stimuli. It may be directed to object features or to spatial positions. Orienting of attention towards spatial positions has been termed 'spatial attention'. In the visual system, an influence of attention on sensory processing was repeatedly demonstrated on the behavioral and neural level. Auditory attention, on the other hand, has been relatively neglected so far. In the current thesis, two animal models were chosen to investigate auditory spatial attention: first, the barn owl as a sound localization model and a highly-developed auditory specialist and, secondly, the rat as an auditory generalist.

2. An influence of spatial attention on sound localization has solely been demonstrated in humans so far. Since barn owls are important animal models in sound localization, I asked whether auditory attention can influence sound localization abilities in these birds. Using a cross-modal cueing paradigm that was adapted from a human study, I could show that barn owls can initiate a head turn towards a sound source significantly earlier (37.4ms / 16% difference in mean response latency) when they are informed about the likely position of the next upcoming auditory stimulus. This benefit in reaction time was explained by the owl's ability to shift attention towards expected positions which resulted in a speeded detection of the stimulus and an earlier response.

3. In a cueing paradigm the subjects are informed that the behaviorally relevant stimulus will be presented soon. In the mammalian species tested so far, this warning elicits a reduction in response latency that is related to the timing of the trial and not to the expected position of the next relevant stimulus. Such a reduction in latency is not influenced by spatial attention but by undirected forms of attention, as e.g. arousal or alertness. In the barn owl, I found clear effects of spatial attention, but, interestingly, no time-related attentional

influences. Why the evaluation of the relative timing of events was not an evolutionary constraint for the barn owl remains open.

4. The construction of internal space is considerably different between the auditory and the visual system which makes research on the mechanisms of auditory spatial attention very important. The representation of auditory space in the brain of barn owls is well investigated. Two representations have been described which are directly involved in sound localization: one map-like in the midbrain and one patchy in the forebrain. Neurons in each representation may elicit, when activated, sound localization behavior. Surprisingly, less was known about internal representations of auditory space in the rat. Therefore, I investigated auditory neurons in a candidate structure in the rat's midbrain, the colliculus superior (SC) and asked whether auditory space is represented there. I found that most auditory neurons (73%) were broadly, but significantly tuned to the spatial location of a sound source. Preferred locations shifted from frontal to lateral in parallel to the neuron's position along the rostrocaudal axis of the SC. Both characteristics, spatially tuned neurons and their systematical arrangement, demonstrate the existence of a representation of auditory space in the rat SC, however, with substantial differences to that in the midbrain of barn owls.

5. The neural basis of spatial attention in the auditory system is unknown so far. This is partially due to the lack of appropriate methods to record from awake animals. In the current thesis I adapted a method that was used in awake rats to record from the midbrain optic tectum (OT) of awake barn owls. During recording sessions owls performed a cue-directed selection paradigm, i.e. a modified cueing paradigm. By combining behavioral and neural measurements, I could test whether attention-related effects in behavior are reflected in the representation of auditory space in the barn owl's midbrain. I found two cognitive influences on auditory midbrain neurons that were seemingly independent: first, the intention to perform a specific movement, which induced a difference between the baseline activities of both OT hemispheres, and second, spatial attention, which enhanced or reduced neural responses to auditory stimulation depending on the behavioral relevance of the stimuli. Both

effects are suited to explain the speeded detection and the shorter latency found in the behavioral study.

6. Many auditory neurons (45%) in the barn owl's OT change their activity also during head movements: head turns towards contralateral directions are paralleled by an increase in spikerate and head turns towards ipsilateral directions by a decrease in spikerate below spontaneous levels. The role of midbrain neurons in premotor activation was so far indirectly shown by focal lesions or electrical stimulation.

7. These data suggest that the midbrain of barn owls and rats is an important structure for sound localization. It combines sensory and motor functions, thus acting as sensorimotor interface. The existence of cognitive influences on the midbrain level indicates that midbrain neurons support not only reflexive but also non-reflexive functions, as e.g. goal-directed orienting towards auditory stimuli.

8. Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BA	best azimuth
BE	best elevation
BIC	brachium of the inferior colliculus
HMW	tuning width at halfmaximal response
ICx	external nucleus of the inferior colliculus
ILD	interaural level difference
ITD	interaural time difference
LED	light emitting diode
MI	modulation index
MRD	mean response direction
MU	multi unit
OT	optic tectum
PSTH	peristimulus time histogram
RF	receptive field
SC	superior colliculus
SPL	sound pressure level
SU	single unit

9. References

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