Top-Down Control of Multimodal Sensitivity in the Barn Owl Optic Tectum

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We studied the effects of electrically microstimulating a gaze-control area in the owl's forebrain, the arcopallial gaze fields (AGFs), on the responsiveness of neurons in the optic tectum (OT) to visual and auditory stimuli. Microstimulation of the AGF enhanced the visual and auditory responsiveness and stimulus discriminability of OT neurons representing the same location in space as that represented at the microstimulation site in the AGF. At such OT sites, AGF microstimulation also sharpened auditory receptive fields and shifted them toward the location represented at the AGF stimulation site. At the same time, AGF microstimulation suppressed the responsiveness of OT neurons that represented visual or auditory stimuli at other locations in space. The top-down influences of this forebrain gaze-control area on sensory responsiveness in the owl OT are strikingly similar to the space-specific regulation of visual responsiveness in the monkey visual cortex produced by voluntary attention as well as by microstimulation of the frontal eye fields. This experimental approach provides a means for discovering mechanisms that underlie the top-down regulation of sensory responses.

Key words: optic tectum; superior colliculus; arcopallium; gaze control; attention; barn owl

Introduction

Spatial attention, gaze control, and the regulation of sensory responsiveness are tightly linked (Moore et al., 2003). For example, when humans make a saccadic eye movement to a new location, behavioral sensitivity to stimuli at that location increases in the tens of milliseconds before the eyes move to that location (Shepherd et al., 1986; Hoffman and Subramaniam, 1995), suggesting that the intention to move the eyes shifts spatial attention to the endpoint of the impending eye saccade. Moreover, when a monkey makes an eye saccade toward a stimulus that is located within the receptive field of a visual neuron in the cortical area V4, the responsiveness of the V4 neuron increases in the period immediately preceding the eye movement (Fischer and Boch, 1981a,b; Moore et al., 1998) in the same way that it does when the monkey attends to the stimulus without moving its eyes (Desimone and Duncan, 1995; Maunsell, 1995).

Recently, an experimental approach for studying the top-down regulation of sensory responsiveness has been developed that takes advantage of this tight linkage between gaze control and spatial attention (Moore and Fallah, 2001, 2004). Applying large amplitude electrical microstimulation pulses to a gaze control area in the monkey's forebrain, the frontal eye fields (FEFs), causes the animal to make saccadic eye movements that are highly consistent in both direction and magnitude, defined as the movement field of the FEF site (Robinson and Fuchs, 1969; Bruce et al., 1985). When the FEF site is stimulated with currents well

below those required to induce an eye movement, monkeys exhibit an increase in behavioral sensitivity to stimuli located within the movement field of the stimulation site, as though microstimulation shifts the monkey's spatial attention to the location of the movement field (Moore and Fallah, 2001, 2004). Moreover, weak microstimulation of the FEF also causes a brief increase in the responsiveness of visual neurons in area V4 that have receptive fields that contain the movement field of the FEF site (Moore and Armstrong, 2003; Armstrong et al., 2006). These microstimulation-induced increases in neuronal responsiveness in V4 mimic the increases in V4 responsiveness that are observed in monkeys cued to attend to stimuli at a particular location (Moran and Desimone, 1985; McAdams and Maunsell, 1999).

Recently, we demonstrated that the linkage between gaze control circuitry and top-down control of neural responsiveness applies to the auditory modality as well (Winkowski and Knudsen, 2006). We applied weak electrical microstimulation to the barn owl forebrain gaze control area, the arcopallial gaze field (AGF), while measuring the responses of space-tuned auditory neurons in the optic tectum (OT). We found that microstimulation of the AGF increased the responsiveness and sharpened the spatial tuning of auditory neurons that encoded the location represented by the AGF stimulation site, analogous to the effects of FEF stimulation on visual responsiveness in the V4 of monkeys.

This study builds on the findings of the first report. Here, we measure the effect of AGF microstimulation on visual as well as auditory responsiveness in the OT and explore the dependence of these top-down effects on the relative alignment of the spatial tunings in the forebrain and midbrain.

Materials and Methods

Animals and surgical preparation. A total of 18 barn owls were used in this study. Surgical and experimental procedures were approved by the Stan-

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ford University Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health and Society for Neuroscience guidelines.

Owls were prepared for multiple electrophysiological experiments. Before an experiment, an owl was anesthetized with halothane (1.5%) and a mixture of nitrous oxide and oxygen (45:55), and a headpiece was mounted to the skull. Plastic cylinders that permitted access to the brain were implanted over the optic tectum and AGF bilaterally. Chloramphenicol antibiotic (0.5%) was applied to the exposed brain surface, and the recording chambers were sealed. All wounds were cleaned with betadine and infused with a local anesthetic. After recovering from surgery, the owl was returned to its flight room.

On the day of an experiment, the owl was anesthetized with halothane (1.5%) and a mixture of nitrous oxide and oxygen (45:55) and was placed in a restraining tube in a prone position within a sound-attenuating booth. The head was secured to a stereotaxic device, and the visual axes were aligned relative to a calibrated tangent screen (the eyes of the owl are nearly stationary in the head). Owls were maintained on the mixture of nitrous oxide and oxygen throughout the experiment.

Auditory stimulation. Auditory stimuli were generated using customized Matlab (MathWorks, Natick, MA) software (courtesy of J. Bergan, Harvard University, Cambridge, MA) interfaced with Tucker Davis Technologies (Alachua, FL) hardware (RP2). Auditory stimuli were delivered through matched earphones (ED-1914; Knowles Electronics, Itasca, IL) coupled to damping assemblies (BF-1743) inserted into the ear canals ~ 5 mm from the eardrum. The amplitude and phase spectra of the two earphones were equalized to within ± 2 dB and ± 2 μ s, respectively. Auditory tuning was measured by presenting dichotic noise bursts (100 or 250 ms duration, 2–10 kHz, 0 ms rise/fall times, 10–20 dB above unit threshold; interstimulus interval = 1.2 s). Tuning to interaural timing differences (ITDs) and interaural level differences (ILDs) was assessed by presenting 10–20 series of noise bursts with ITD (or ILD) varied in a random, interleaved manner while ILD (or ITD) was held constant at the best value for the site.

Visual stimulation. A computer-generated visual stimulus was projected onto a calibrated tangent screen (Brainard, 1997). The owl was positioned so that the visual axes were in the horizontal plane aligned with 0° elevation and 0° azimuth of the screen. All locations are given in double pole coordinates of azimuth relative to the midsagittal plane and elevation relative to the visual plane.

Visual tuning was measured by presenting a negative contrast (black) dot, subtending 2° of visual angle at a distance of 1 m from the owl, on a gray background. Tuning for visual azimuth (or elevation) was assessed by presenting 10–20 series of stationary dot flashes (duration 250 ms; interstimulus interval 1.2 s) with visual azimuth (or elevation) varied in a random, interleaved manner while visual elevation (or azimuth) was held constant at the best value for the site.

Neurophysiology. Epoxy-coated tungsten microelectrodes were used to record extracellularly from single and multiple units (sites) in the AGF and OT and to deliver electrical microstimulation to the AGF. An electrode was positioned in the AGF using targeting information provided by a previous study (Cohen and Knudsen, 1995). The electrode was first advanced, with a mechanical microdrive, to the site in the superficial layers of the OT at which units are tuned to 0° azimuth and 0° elevation. The auditory region of the AGF was located ~ 2 mm rostral, 0.5 mm lateral, and 3 mm dorsal to this site. After the stimulation electrode was positioned in the AGF (details below), a recording electrode was advanced into the OT. We studied the effects of AGF microstimulation on the responses of single or multiple units in the deep layers (layers 11–13) of the OT. In most experiments, we studied the effect of a single AGF site on responses at more than one OT recording site by advancing the recording electrode to new position in the OT. Categorization of OT sites as “aligned” or “nonaligned” was performed *post hoc* based on changes in OT responsiveness caused by electrical microstimulation (see Fig. 4). Spike times were saved on a computer using TDT hardware (RA-16) controlled by customized Matlab (MathWorks) software.

AGF microstimulation. The AGF was identified based on stereotaxic coordinates and functional properties. In the AGF, space was organized in a clustered representation in which neighboring neurons were tuned

Figure 1. The AGF and properties of AGF stimulation sites. **A**, Nissl stain of a transverse section through the owl forebrain at the level of the AGF. The image shows the location of an electrolytic lesion (arrow), confirming the electrode position that yielded the data shown in Figures 2 and 3. White arrowheads indicate border of arcopallium. Scale bar, 1 mm. Dorsal (d) and medial (m) are indicated at the bottom left. **B**, Scatter plot comparing AGF activity during visual stimulation and during auditory stimulation. Ordinate, Average firing rate during visual stimulation; abscissa, average firing rate during auditory stimulation. Each symbol represents one AGF site at which responsiveness to both visual and auditory stimuli was quantitatively tested. The diagonal line represents the line on which the points would fall if firing rates were equal for auditory and visual stimuli. **C**, Variability of tuning for best ITD and best ILD within AGF clusters. Ordinate and abscissa, Variability (SD) of best ITD and best ILD, respectively, within each AGF cluster. Each symbol represents one AGF cluster that was used for an electrical stimulation experiment. Red symbols, AGF clusters that caused a significant modulation of OT responsiveness; black symbols, AGF clusters at which no effect on OT responsiveness was observed. **D**, Effect of AGF microstimulation on auditory responses at aligned sites depended on the strength of electrical stimulation. Each color/symbol combination represents one AGF–OT pair at which the effect of increased AGF microstimulation strength was tested. Ordinate, Magnitude of increase in responsiveness (percentage change in firing rate to auditory stimulation at best ITD and best ILD) at each OT site with AGF microstimulation; abscissa, amplitude of the current pulses used to activate the AGF cluster.

to a similar location (i.e., similar values of ITD and ILD), but neighboring groups of neurons were tuned to unpredictably different locations (Cohen and Knudsen, 1995). In our initial experiments, we recorded from several sites, spaced 50–100 μ m apart, as the electrode was advanced dorsoventrally through the AGF. At each site, we measured unit tuning to ITD and ILD. We centered the electrode between the first and last site in the series and assessed the effect of weak electrical microstimulation of that AGF site on sensory responses in the OT. In a subset of experiments, an electrolytic lesion was made (cathodal current, 5 μ A, 10 s) at the stimulation site after the experiment was complete to confirm the stimulating electrode's position in the AGF (Fig. 1A).

Electrical stimulation was delivered to the AGF site through a tungsten microelectrode (0.5–1.0 M Ω at 1 kHz). Stimulation waveforms were generated with a Grass stimulator (S88) and two Grass stimulus isolation units (PSIU-6). The waveforms consisted of 25 ms trains of biphasic current pulses, 200 Hz, and 200 μ s phase duration. The electrical stimulation trains were timed so that they just preceded unit discharges elicited by sensory stimulation: for auditory trials, electrical stimulation ended when the sound began; for visual trials, electrical stimulation ended 5–10 ms before visual responses at the site began.

Current amplitude was quantified as the voltage drop across a 1 k Ω resistor in series with the return path of the circuit. For each AGF stimulation site, the current threshold to evoke an eye saccade was determined by incrementally increasing the stimulation current until a small-amplitude saccadic eye movement (a deflection in the position of a

retinal landmark, the pecten oculus, viewed ophthalmoscopically) was observed. Once the threshold for eliciting an eye saccade from an AGF site was determined, the amplitude of the current pulses was set to a low level (usually 5 μ A) and incrementally increased until either an effect on OT auditory responses was observed or the current amplitude reached 40 μ A; the lowest current level that evoked an eye saccade was 55 μ A.

Data analysis. Net responses at each OT site were quantified by subtracting the average firing rate that occurred during the 100 ms interval before the onset of electrical microstimulation across all trials (baseline activity) from the average firing rate occurring during a 100 ms window beginning 5 ms after the offset of the electrical stimulus. This window was chosen to avoid contamination of the data by the stimulation artifact. Identical poststimulus periods were sampled for trials during which electrical microstimulation was and was not applied to the AGF site. Net firing rate across all trials for a single condition was averaged. Paired *t* tests were used to compare the firing rates for trials with and without microstimulation. Auditory best ITD and best ILD and visual receptive field center (VRF_{ctr}) were quantified as the weighted average of responses that were greater than half of the maximum response (half-max). Tuning widths were defined as the continuous range of ITDs, ILDs, visual azimuths, and visual elevations that elicited responses greater than half-max.

To assess the time course of response modulation in the OT by AGF microstimulation, we divided the time period after offset of electrical microstimulation into 20 ms windows and assessed whether the difference between the responses with and without electrical microstimulation was different from zero.

To determine whether AGF microstimulation sharpened OT spatial tuning at individual sites, we measured the width of the tuning curve with and without AGF microstimulation for a series of trials (>10 repetitions) and compared the distributions of tuning width with and without AGF microstimulation with a paired *t* test. To determine whether AGF microstimulation sharpened OT spatial tuning across the population, we computed the difference in average tuning widths with and without AGF microstimulation for each OT site and assessed whether the differences in tuning widths (Δ widths) were different from zero.

To assess whether AGF microstimulation shifted the spatial tuning toward the values represented by the microstimulation site, we used the robust fitting linear regression function (robustfit) in Matlab to fit a line to the data. This function uses an iteratively reweighted least-squares algorithm and is less sensitive to outliers than ordinary linear regression.

We used receiver operating characteristic (ROC) analysis to determine whether AGF microstimulation had an effect on the discriminability of sensory stimuli within OT receptive fields. We compared the distribution of responses to a stimulus that was near the center of the receptive field (best value; see above) with responses to a stimulus that produced approximately half of the maximum response (flank value). Best values and flank values were determined independently for trials with and without electrical microstimulation. We determined the probability that the firing rate distribution produced by the best value stimulus exceeded a criterion ("correct detection") versus the probability that the firing rate distribution produced by the flank value stimulus exceeded that same criterion ("false positive"). The criterion was incremented from 0 to the maximum firing rate in steps of 1. Each point on the ROC curve corresponds to an incremental step of the criterion; the entire ROC curve represents all criteria. The ROC curve quantifies the extent to which neural responses discriminate between the two stimuli. The area under the ROC curve indicates the performance of an ideal observer in identifying the stimulus based only on the responses (Green and Swets, 1966; Britten et al., 1992). We computed the difference between the areas under the ROC curve with and without AGF microstimulation for both the left and right flanks of the tuning curves and assessed whether the differences between ROC areas with and without AGF microstimulation (Δ ROC area) were different from zero.

Data from all sites were included for the population analyses. Population tuning curves were constructed for each test by normalizing the mean firing rate for each OT site to the maximum mean firing rate for that site and for that test, measured without AGF stimulation. All tuning curves were aligned according to their best value (plotted as ITD = 0 μ s

or ILD = 0 dB; visual azimuth or elevation = 0°) and averaged. Note that, for this analysis, the best ITDs and best ILDs of the AGF stimulation sites and the predicted best visual azimuths and predicted best visual elevations for the AGF were to either side of the corresponding best values represented at the OT recording site.

Histology. Approximately 2 d after an electrolytic lesion was made in the AGF, the owl was deeply anesthetized with 5% isoflurane mixed with nitrous oxide and oxygen (45:55). The thoracic cavity was opened and Nembutal (0.5 cc, 50 mg/ml) and heparin (0.3 cc) were injected into the left ventricle of the heart. The owl was perfused transcardially with 300–500 ml of 0.1 M phosphate buffer (PB) containing lidocaine (3 ml/L), followed by 500 ml of formalin in PB. The brain was removed and sunk in 30% sucrose in fixative for 2 d before sectioning. The brain was sliced in 40 μ m frozen sections. Sections from electrolytically lesioned tissue were mounted onto slides and stained with cresyl violet.

Results

The effect of AGF microstimulation on auditory responsiveness was characterized at 125 recording sites in the OT and 53 stimulation sites in the AGF in 18 owls. The effect of AGF microstimulation on visual responsiveness was characterized at 34 recording sites in the OT and 20 stimulation sites in the AGF in six owls. In the presentation of the results, we describe first the properties of AGF stimulation sites, the selection of microstimulation parameters, and the importance of spatial matching between the AGF stimulation and OT recording sites for producing response enhancements. Second, we describe the changes in auditory and visual response properties in the OT that resulted from AGF microstimulation.

AGF stimulation sites

The auditory AGF

Before initiating microstimulation experiments, the sensitivity of AGF units to auditory and visual stimuli was tested at each microstimulation site. All stimulation sites (*n* = 53) were responsive to sound and were tuned sharply for both ITD and ILD, indicating restricted tuning for auditory azimuth and elevation, respectively. The average width at half-max for ITD tuning was 60 ± 30 μ s (SD), and the average width at half-max for ILD tuning was 15 ± 5 dB. Most stimulation sites exhibited best ITDs and best ILDs that corresponded to frontal space (mean best ITD = 23 ± 20 μ s contralateral ear leading and mean best ILD = 1 ± 3 dB greater in the right ear), reflecting the magnified representation of frontal space that exists in this structure (Cohen and Knudsen, 1995).

Neural responses to visual stimuli were tested qualitatively at all sites with moving bars and dots. Such tests revealed little or no visual responses at most sites. In addition, visual responsiveness was tested quantitatively at a subset of 12 sites. Only one site (1/12; 8%) exhibited visually driven activity in response to a stationary dot. For all 12 of these AGF sites, unit activity during visual stimulation was compared with unit activity during auditory stimulation. For these measurements, the auditory stimulus was a noise burst at the best ITD and best ILD for the site, and the visual stimulus was a stationary flashed dot positioned at the location in space corresponding to the best ITD and best ILD for that site [based on acoustic, head-related transfer function data (Olsen et al., 1989)]. To compare the effectiveness of visual stimuli relative to auditory stimuli in driving neural responses in the AGF, we plotted the maximum firing rate during visual stimulation as a function of the maximum firing rate during auditory stimulation (Fig. 1*B*). In every case, the maximum firing rate during auditory stimulation was higher than the maximum firing rate during visual stimulation.

These results indicate that the portion of the AGF that was stimulated in these experiments represented, primarily, auditory stimuli. Therefore, we refer to this region as the auditory (AGFa).

Functional clusters

Electrode penetrations were made through the AGFa, and auditory tuning was measured at regular (50 or 100 μm) intervals along these penetrations. Only penetrations that encountered auditory responses continuously for at least 300 μm were used for microstimulation. When this criterion was met, the electrode was repositioned at the dorsoventral center of the AGFa patch of tissue ($n = 53$ AGFa stimulation sites). As described in previous reports (Cohen and Knudsen, 1995), neighboring units in the AGFa were tuned to similar values of ITD and ILD, but neighboring groups of units were tuned to different, unpredictable values of these auditory spatial cues. Therefore, for a given electrode penetration through the AGFa, values of best ITD and best ILD were either highly consistent throughout the 300 μm patch, indicating that the electrode remained within a spatial cluster, or they varied. We found that the likelihood that AGFa microstimulation would modulate the auditory responsiveness of neurons in the OT depended on the consistency of best ITDs and best ILDs within the patch of tissue in the AGFa (Fig. 1C). When the SD of best ITDs within a patch was $<7 \mu\text{s}$ and the SD of best ILDs was $<2 \text{ dB}$ (24/53), microstimulation of the AGFa always (24/24) modulated sensory responses in the OT. When the SD of either best ITDs or best ILDs exceeded these values (29/53), the probability that microstimulation would modulate sensory responses in the OT decreased (14/29 of the AGFa stimulation sites caused increases in responsiveness, whereas 15/29 AGFa stimulation sites had no effect).

The data demonstrate that centering the stimulating electrode within a patch of tissue that represented a single location in space dramatically increased the likelihood that AGFa microstimulation would affect neural responsiveness in the OT. For this reason, we searched for such putative clusters (300 μm dorsoventral extent, exhibiting little variation in best ITD and best ILD), and we centered our stimulating electrode within them.

Stimulation current strength

The effect of AGFa microstimulation on the responsiveness of OT neurons depended critically on the strength of the electrical stimulation applied to the AGFa site. The effect of microstimulation current strength on OT responsiveness was tested parametrically for six AGFa–OT pairs (Fig. 1D). The effect of AGFa microstimulation on OT responsiveness increased steeply for current amplitudes $>5 \mu\text{A}$, peaked at current amplitudes ranging from 10 to 30 μA , and then decreased rapidly with further increases in current amplitude. At current amplitudes $>60 \mu\text{A}$, an effect of AGFa stimulation on OT responsiveness was not apparent at any site.

For all AGFa microstimulation sites, we applied the lowest current strength at which a significant modulation of auditory responsiveness was observed when tested at increments of 5 or 10 μA . The average current amplitude used was 13 μA (range: 4–40 μA). According to estimates of current spread in the mammalian cortex, 40 μA should have directly activated neuronal somata within a radius of $\sim 110 \mu\text{m}$ of the electrode tip (Stoney et al., 1968).

Effects of AGFa microstimulation on sensory responses at a single site in the OT

Neurons in the barn owl's AGFa and OT are tuned for the location of auditory stimuli (Knudsen, 1982; Olsen et al., 1989; Co-

hen and Knudsen, 1995). When tested with dichotic stimuli, this auditory spatial tuning is expressed as tuning to ITD (the primary cue for azimuth) and ILD (the primary cue for elevation). For AGFa–OT pairs with similar ITD and ILD tuning, microstimulation in the AGFa caused a dramatic increase in the responsiveness of OT neurons to auditory stimuli centered within their receptive fields. The results shown in Figure 2 were obtained by stimulating an AGFa site that was tuned to $-13 \mu\text{s}$ ITD (left ear leading) and -1.3 dB ILD (left ear greater) while recording from an OT site that was tuned to $-10 \mu\text{s}$ ITD and -2.7 dB ILD. The top rasters on the left in Figure 2, A and D, show the typically sharp tuning of this OT site to ITD and ILD, respectively. Randomly interleaved with purely auditory trials were two other sets of trials involving a brief train of low-level electrical microstimulation delivered to the AGFa site (stimulation current = 12 μA ; current to evoke eye movements = 105 μA). During one set of trials, a 25 ms train of electrical stimulation was applied to the auditory AGFa site without any sensory stimulation (Fig. 2A,D, middle rasters). During this set of trials, electrical microstimulation in the AGFa had no effect on unit discharge rate at the OT site (baseline firing rate = 4 spikes/s; firing rate after stimulation = 3 spikes/s, $p > 0.5$, paired t test). During the other set of trials, electrical microstimulation of the AGFa site preceded the presentation of an auditory stimulus (Fig. 2A,D, bottom rasters). During this set of trials, electrical microstimulation of the AGFa site increased the responsiveness of the OT site to sounds with ITDs and ILDs at or near the best values for this OT site by an average of 34% across all trials (ITD: 28% increase in responsiveness, $p < 0.01$, paired t test; ILD: 40% increase, $p < 0.03$, paired t test), while having no effect on the responsiveness of this site to sounds with ITDs or ILDs away from these best values (ITD: $p > 0.5$ paired t test; ILD: $p > 0.3$ paired t test).

For the same AGFa–OT pair, AGFa microstimulation (12 μA) also caused a space-specific increase in the responsiveness of the OT site to visual stimulation (Fig. 3). The OT site exhibited robust sustained visual responses, at a latency of 50 ms, to stimuli presented at or near the site's VRF_{center}. The VRF_{center} was located at left 5° , -13° (below the horizon), and the width at half-max for the visual tuning curves was 5° in azimuth and 8° in elevation. For the microstimulation trials, the timing of the electrical stimulation was adjusted to end just before the onset of visual responses. In this case, the microstimulation train ended at 45 ms after stimulus onset (Fig. 3A,D, bottom rasters). Microstimulation of the AGFa site increased the responsiveness of the OT site to visual stimuli presented within a restricted portion of the OT site's VRF ($p < 0.03$, paired t test) and had no effect on OT responses to stimuli presented at other locations ($p > 0.5$, paired t test). The average magnitude of the increase in OT responsiveness to visual stimuli presented near the VRF_{center} across all trials was 52% for this site (azimuth: 43% increase, $p < 0.03$, paired t test; elevation: 60% increase, $p < 0.02$ paired t test).

Effect of AGFa–OT spatial alignment on OT responsiveness

The influence of AGFa microstimulation on sensory responsiveness in the OT depended on the mutual alignment of the spatial tunings for the AGFa–OT pair. To explore this spatial dependence, we computed the absolute difference between the ITD and ILD tunings for each AGFa–OT pair, yielding a ΔITD and a ΔILD value for each pair. We then plotted the effect of AGFa microstimulation measured at each OT recording site as a function of the ΔITD and ΔILD values for the AGFa–OT pair (Fig. 4A). In this plot, a color was assigned to each AGFa–OT pair to represent the average magnitude of the microstimulation-induced change

Figure 2. Effect of electrical microstimulation of a site in the AGFa on auditory responsiveness at an OT site with similar spatial tuning. **A**, Raster display of OT unit activity with and without AGFa microstimulation. Top, Auditory stimulation was presented without AGFa microstimulation; middle, no auditory stimulation was presented, and microstimulation was applied to the AGFa site; bottom, auditory stimulation was presented, and microstimulation was applied to the AGFa site. Ordinate of top and bottom raster plots, Stimulus ITD; abscissa, time relative to stimulus onset; shaded area shows the duration of the auditory stimulus; white box, the occurrence of electrical stimulation in AGFa. **B**, Mean neuronal firing rate plotted as a function of stimulus ITD for trials with (red) and without (black) AGFa microstimulation. Red arrow, Best ITD of the AGFa stimulation site. Error bars represent SEM. Microstimulation of the AGFa caused a 28% increase in the responses to sounds with ITD values near the best ITD. For this AGFa–OT pair, microstimulation had no effect on either the sharpness of the ITD tuning curve (width of ITD tuning_{nostim} = 32 μ s, width of ITD tuning_{stim} = 32 μ s; $p > 0.5$, paired t test) or the best value of the ITD tuning curve (best ITD_{nostim} = -10 μ s, best ITD_{stim} = -12 μ s; $p > 0.12$, paired t test). **C**, Peristimulus time histograms (PSTHs) of the effect of AGFa microstimulation on auditory responses in the OT. PSTHs with AGFa microstimulation (red) and without AGFa microstimulation (black) are shown. The stimulation artifact has been removed. **D–F**, Conventions are the same as in **A–C**. **D**, Effect of AGFa microstimulation on an ILD tuning curve. Ordinate of top and bottom raster plots, Stimulus ILD. **E**, Mean firing rate plotted as a function of stimulus ILD. Microstimulation of the AGFa site (arrow, best ILD) increased OT responsiveness to the values of ILD near the best value (40% increase) and caused the best ILD to shift toward the value represented by the AGFa stimulation site (best ILD_{nostim} = -2.7 dB; best ILD_{stim} = -1.4 dB; $p < 0.02$, paired t test). For this AGFa–OT pair, AGFa microstimulation had no significant effect on the width of the ILD tuning curve (width of ILD tuning_{nostim} = 8.7 dB, width of ILD tuning_{stim} = 8.4 dB, $p > 0.5$, paired t test). **F**, Peristimulus time histograms of the effect of AGFa microstimulation on auditory responses in the OT. PSTHs with AGFa microstimulation (red) and without AGFa microstimulation (black) are shown. The stimulation artifact has been removed. The magnitude of the increase in responsiveness caused by AGFa microstimulation was statistically the same regardless of whether ITD (28% increase) or ILD (40% increase) was tested ($p > 0.35$, Mann–Whitney U test).

in auditory responsiveness at each OT site. When the auditory spatial tunings for an AGFa–OT pair were similar (Δ ITD ≤ 22 μ s and Δ ILD ≤ 10 dB), AGFa microstimulation tended to increase the responsiveness of OT neurons to auditory stimuli (Fig. 4A).

Conversely, when the auditory spatial tunings for an AGFa–OT pair were different (Δ ITD > 22 μ s and Δ ILD > 10 dB), microstimulation of the AGFa tended to suppress or have no effect on OT responsiveness to auditory stimuli.

To estimate the boundary at which there was an equal likelihood of AGFa microstimulation causing either an increase or a decrease in OT responsiveness, we assembled the data into two groups (“increase” or “decrease”). We then used logistic regression to calculate the probability of finding an increase in auditory responsiveness as a function of Δ ITD and Δ ILD. As shown in Figure 4B, when the difference in the spatial tunings for an AGFa–OT pair was ≤ 22 μ s ITD and ≤ 10 dB ILD, the probability of observing an increase in OT responsiveness was high (> 0.5). Therefore, we refer to such pairs as “aligned” pairs. In contrast, when the difference between the spatial tunings for an AGFa–OT pair was greater than these values, the probability that AGFa microstimulation would cause an increase in OT responsiveness was low. Therefore, we refer to such AGFa–OT pairs as “non-aligned” pairs.

Modulation of auditory responses

Aligned AGFa–OT pairs

The effects of AGFa microstimulation on the auditory responsiveness of OT sites with aligned and nonaligned spatial tuning (see above) were analyzed separately. We measured the effects of AGFa microstimulation ($n = 53$ AGFa sites) on 67 aligned OT sites. For all AGFa–OT pairs ($n = 67/67$), we assessed the effect of AGFa microstimulation on the OT responses to a range of ITD values. At a subset of aligned AGFa–OT pairs ($n = 20/67$), we assessed the effect of AGFa microstimulation on ILD tuning. Although these sample sizes are different, the magnitude of the effect of AGFa microstimulation on neural responses in the context of presenting a range of ITD values or ILD values was statistically the same ($p > 0.1$, Mann–Whitney U test) and therefore, when appropriate, we present the results together. Across the population of aligned AGFa–OT pairs ($n = 67$ AGFa–OT pairs using 53 AGFa stimulation sites), activation of the AGFa had no significant effect on OT responses when microstimulation was applied in the absence of an auditory stimulus (mean firing rate_{prestim} = 8 spikes/s; mean firing rate_{poststim} = 11 spikes/s; $p = 0.09$, paired t test). When AGFa microstimulation was combined with an auditory stimulus, microstimulation of the AGFa increased the responsiveness of OT neurons to auditory stimuli

in auditory responsiveness at each OT site. When the auditory spatial tunings for an AGFa–OT pair were similar (Δ ITD ≤ 22 μ s and Δ ILD ≤ 10 dB), AGFa microstimulation tended to increase the responsiveness of OT neurons to auditory stimuli (Fig. 4A).

with ITDs and ILDs at or near the best values for the pair by an average of $27 \pm 16\%$ ($p < 10^{-7}$, paired t test) (Fig. 5), but had no effect on OT responsiveness to sounds with ITD or ILD values outside the half-max values for the AGFa site ($p > 0.39$, paired t test).

The enhancement of OT responsiveness depended on the timing of AGFa microstimulation. The effect of microstimulation timing was tested systematically for 10 different AGFa sites and, for each, a corresponding aligned OT site (Fig. 6A). When AGFa stimulation ended >75 ms before sound onset, no reliable response enhancement was observed ($p > 0.1$, t test). As the interval between the termination of the microstimulation and the onset of the sound decreased, response enhancement increased (Fig. 6A, asterisks) ($p < 0.02$, t test), and reached its maximum for intervals ≤ 50 ms. When microstimulation occurred after auditory responses had commenced, enhancement remained high (Fig. 6A, asterisks) ($p < 0.04$, t test).

Once response enhancement was instantiated, it typically persisted for ~ 120 ms. To determine the time window in which AGFa influences OT neural activity under these conditions, peristimulus time histograms for each condition (i.e., with and without AGFa microstimulation) were constructed and normalized to the maximum firing rate without AGFa microstimulation for each OT site. For this analysis, we used the 67 aligned AGFa–OT pairs at which AGFa microstimulation modulated auditory responsiveness and the microstimulation ended immediately before sound onset. The average time course of response enhancement was determined by computing the difference between the peristimulus time histograms with and without AGFa microstimulation for each OT site ($n = 67$) and then averaging the differences across all sites. The population average time course of the response enhancement to best ITD and best ILD sounds when microstimulation ended at the onset of the auditory stimulus is shown in Figure 6B. Response enhancement was observed soon after the offset of AGFa microstimulation and persisted for ~ 120 ms beyond the end of AGFa microstimulation.

AGFa microstimulation sharpened auditory spatial tuning at aligned OT sites. The change in tuning width for each site was determined by computing the difference between the width of the tuning curve at half-max with and without AGFa microstimulation ($\Delta\text{width} = \text{tuning width}_{\text{stim}} - \text{tuning width}_{\text{nostim}}$). The shift in the population distribution of Δwidths toward negative values (Fig. 7A) indicates a sharpening of tuning curves with AGFa microstimulation. The mean Δwidth was $-7.5 \mu\text{s}$, a decrease of 17% ($p < 0.0001$, t test). For individ-

Figure 3. Effect of AGFa microstimulation on visual responsiveness at an OT site with similar spatial tuning. Conventions are the same as in Figure 2. **A**, Raster display of OT unit activity under various conditions. Top, Visual stimulus was presented with no AGFa microstimulation; middle, no visual stimulus was presented, and microstimulation was applied to the AGFa site; bottom, visual stimulus was presented, and microstimulation was applied to the AGFa site. Ordinate of top and bottom raster plots, Horizontal position of visual stimulus (azimuth); abscissa shows time relative to stimulus onset; shaded area shows duration of the visual stimulus; white box indicates the occurrence of electrical stimulation in the AGFa. **B**, Mean neuronal firing rate plotted as a function of the stimulus azimuth for trials with (red) and without (black) AGFa microstimulation. Red arrow, Predicted best azimuth of the AGFa stimulation site based on auditory tuning. Error bars represent SEM. Microstimulation of the AGFa caused OT responses near the VRF_{ctr} to increase by 43% ($p < 0.03$, paired t test). For this AGFa–OT pair, microstimulation had no effect on either the sharpness of the visual tuning curve (width of azimuth tuning curve_{nostim} = 9° , width of azimuth tuning curve_{stim} = 7° , $p > 0.2$, paired t test) or the horizontal position of the VRF_{ctr} (best value of the azimuth tuning curve_{nostim} = 5° to the left, best value of the azimuth tuning curve_{stim} = 4° to the left; $p > 0.1$, paired t test). **C**, Peristimulus time histograms (PSTHs) of the effect of AGFa microstimulation on visual responses in the OT. PSTHs with AGFa microstimulation (red) and without AGFa microstimulation (black) are shown. The stimulation artifact has been removed. **D–F**, Conventions are the same as in **A–C**. **D**, Effect of AGF microstimulation on an elevation tuning curve. Top, Ordinate of raster plot, Stimulus elevation. **E**, Mean firing rate plotted as a function of vertical position of stimulus (elevation). Microstimulation of the AGFa (arrow, predicted best elevation) caused OT responses near the VRF_{ctr} to increase by 60% ($p < 0.02$, paired t test). For this AGFa–OT pair, AGFa microstimulation had no effect on the sharpness of the VRF (width of elevation tuning curve_{nostim} = 8° , width of elevation tuning curve_{stim} = 11° , $p > 0.5$, paired t test) or on the vertical position of the VRF_{ctr} (best elevation_{nostim} = 13° below the horizon, best elevation_{stim} = 12° below the horizon, $p > 0.5$, paired t test). **F**, Peristimulus time histograms of the effect of AGFa microstimulation on visual responses in the OT. PSTHs with AGFa microstimulation (red) and without AGFa microstimulation (black) are shown. The stimulation artifact has been removed. The magnitude of the increase in responsiveness caused by AGFa microstimulation was statistically the same regardless of whether azimuth (43% increase) or elevation (60% increase) was tested ($p > 0.25$, Mann–Whitney U test).

ual sites, the sharpening effect of AGFa microstimulation was significant at 21/67 (31%) of the aligned pairs ($p < 0.05$, paired t test) (Fig. 7A, black bars). Analogously, at the subset of sites ($n = 20/67$ aligned AGFa–OT pairs) at which we tested the effects of AGFa microstimulation on the sharpness of ILD tuning (Fig. 7B), the distribution of Δwidths was shifted toward negative values

Figure 4. The effect of AGFa microstimulation on auditory responses in the OT depends on the relative alignment of the binaural tunings of the AGFa and OT sites. **A**, The effect of AGFa microstimulation plotted as a function of the absolute difference between the best ITDs (abscissa, Δ ITD, AGFa – OT) and best ILDs (ordinate, Δ ILD, AGFa – OT) for all AGFa–OT pairs. Each symbol represents an AGF–OT pair. The color of each symbol reflects the magnitude and direction of the effect of AGFa microstimulation on OT auditory responses to best ITD and best ILD. **B**, Probability of AGFa microstimulation causing an increase in OT auditory responses. Axes are the same as in **A**. The dashed white line indicates the boundary at which there was an equal probability of AGFa microstimulation causing an increase or a decrease in OT responsiveness. The line intersects the abscissa at Δ ITD = 22 μ s and the ordinate at Δ ILD = 10 dB.

($p < 0.05$, t -test). At 5/20 (25%) of the sites (Fig. 7B, black bars), ILD tuning curves were significantly sharpened with AGFa microstimulation ($p < 0.05$, paired t test).

In addition, AGFa stimulation shifted auditory spatial tuning at aligned OT sites toward the values represented at the AGFa site. For this analysis, we used all aligned AGFa–OT pairs and calculated the difference between the best ITD and best ILD values for the OT site with and without AGFa stimulation and plotted these values as a function of AGFa–OT alignment for best ITD and best ILD, respectively (Fig. 8). The direction of the best ITD shift in the OT caused by AGFa stimulation was positively correlated with the direction of the AGFa–OT misalignment in best ITD (Fig. 8A) ($n = 67$ aligned AGFa–OT pairs, slope = 0.16 ± 0.04 SEM, $p < 0.001$, t test). Similarly, the direction of the best ILD shift in the OT caused by AGFa stimulation was positively correlated with the direction of the AGFa–OT misalignment in best ILD (Fig. 8B) ($n = 20$ pairs of aligned AGFa–OT pairs, slope = 0.17 ± 0.05 SEM, $p < 0.002$, t test). The shifting effect of AGFa stimulation cannot be seen in the population tuning curves (Fig.

Figure 5. Summary of the effects of AGFa microstimulation on auditory responses at aligned sites in the OT. **A**, Population ITD tuning curves measured with (filled circles, thick line) and without (open circles, thin line) AGFa microstimulation. Microstimulation of the AGFa caused a 27% increase in responses near the OT best value ($p < 10^{-7}$, paired t test). Ordinate shows normalized response; abscissa shows stimulus ITD normalized to the best ITD (plotted as ITD = 0 μ s). In most cases, error bars are smaller than the symbol. **B**, Population ILD tuning curves. Conventions are the same as in **A**. Abscissa shows stimulus ILD normalized to best ILD (plotted as ILD = 0 dB). In most cases, error bars are smaller than the symbol. The average increases in responses were statistically the same regardless of whether ITD (27%) or ILD (20%) was tested ($p > 0.1$, Mann–Whitney U test).

5) because, for this calculation, the AGFa tuning was to either side of the tuning peak at the paired OT site. Hence, the effect was averaged out.

AGFa microstimulation also improved the discriminability of auditory stimuli at aligned OT sites as revealed by ROC analysis. For experiment shown in Figure 9A, the OT site was tuned to 27 μ s right ear leading (best ITD) and to 0 dB ILD. ROC analysis was used to compare the responses of this site to a stimulus with an ITD closest to its best ITD (Fig. 9A, open diamond) with its responses to a stimulus that elicited responses that were closest to the half-max response, both on the left flank (Fig. 9A, open square) and on the right flank (Fig. 9A, open triangle) of the tuning curve. The area under the ROC curve for the left flank (Fig. 9A, open diamond and open square) without AGFa microstimulation was 0.77 (Fig. 9B, thin line) and with AGFa microstimulation was 0.93 (Fig. 9B, thick line). The difference between

Figure 6. Effects of microstimulation timing and the time course of the effect of AGFa microstimulation on auditory responsiveness. **A**, Comparison of the effects of AGFa microstimulation at different times relative to sound onset. Each symbol represents the mean change in auditory responsiveness as a function of the offset of AGFa microstimulation relative to the onset of the sound stimulus averaged across 10 AGF–OT pairs. AGFa microstimulation-driven changes in OT auditory responsiveness reached significance (asterisks, $p < 0.02$, t test) when the AGFa microstimulation pulses ceased 50 ms before sound onset and remained high even when microstimulation was applied after OT auditory response onset ($p < 0.04$, t test). Abscissa, Time relative to (re.) sound onset. **B**, Time course of AGFa microstimulation-driven effects on OT auditory responsiveness. The average difference in normalized OT responses with and without AGFa microstimulation plotted as a function of time (abscissa). Shading indicates SEM. Microstimulation occurred during the 25 ms before sound onset.

these areas (Δ ROC) was 0.16. A similar increase in ROC area occurred for stimuli on the right flank of the tuning curve (Δ ROC = 0.08). For this AGF–OT pair, the average Δ ROC was 0.12. Across the population, the increase in stimulus discriminability was similar for both the left and right flanks of both ITD and ILD tuning curves ($p > 0.3$, Mann–Whitney U test). Across all measures and all AGFa–OT pairs ($n = 67$ ITD; $n = 20$ ILD), the median Δ ROC was 0.03 (Fig. 9C) ($p < 10^{-5}$, Wilcoxon signed rank test for zero median).

Nonaligned AGFa–OT pairs

When the AGFa–OT pairs represented different locations in auditory space, microstimulation of the AGFa site tended to suppress auditory responsiveness at the OT site (Fig. 4). Averaged across the population of nonaligned sites ($n = 58$ nonaligned

Figure 7. Effect of AGFa microstimulation on the sharpness of auditory tuning at aligned sites in the OT. **A**, Changes in ITD tuning widths at half-max (width of tuning_{stim} – width of tuning_{nostim}) caused by AGFa microstimulation. The distribution is shifted toward negative values indicating that ITD tuning in the OT was sharper with AGFa microstimulation. The mean of the distribution = -7.5μ s, a 17% decrease in ITD tuning width with AGFa microstimulation. Statistically significant changes in tuning width are indicated by black bars. **B**, Changes in ILD tuning widths caused by AGFa microstimulation. The distribution is shifted toward negative values, indicating that ILD tuning in the OT was sharper with AGFa microstimulation. The mean of the distribution = -0.5 dB, a 5% decrease in ILD tuning width with AGFa microstimulation. Statistically significant changes in tuning width are indicated by black bars.

AGFa–OT pairs), microstimulation of the AGFa suppressed the responsiveness of OT neurons at nonaligned sites by an average of 21% (Fig. 10) (ITD: 18% decrease, $p < 10^{-6}$, paired t test; ILD: 24% decrease, $p < 10^{-5}$, paired t test). AGFa stimulation had no significant effect on the widths of ITD tuning (ITD width_{nostim} = $42 \pm 11 \mu$ s; ITD width_{stim} = $46 \pm 11 \mu$ s; $p > 0.06$, paired t test; $n = 58$ nonaligned pairs), or on the widths of ILD tuning in the OT (ILD width_{nostim} = 12 ± 4 dB; ILD width_{stim} = 11 ± 5 dB, $p > 0.3$; paired t test; $n = 25$ nonaligned pairs) or on the values of ITD or ILD to which OT sites were tuned.

Modulation of visual responses

Aligned AGFa–OT pairs

Microstimulation of the AGFa also modulated the responsiveness of OT sites to visual stimuli when the auditory spatial tunings of the stimulation and recording sites were aligned (Fig. 4).

Figure 8. Effect of AGFa microstimulation on best values of auditory tuning curves at aligned sites in the OT. **A**, Shift in best ITD in the OT ($OT_{stim} - OT_{nostim}$) caused by AGFa microstimulation was positively correlated with the difference in the best ITDs of the AGFa–OT pair (slope using robust linear regression = 0.16 ± 0.04 SEM, $p < 0.001$). Triangles, Data that have appeared previously (Winkowski and Knudsen, 2006); circles, data that have been collected since that time. **B**, Shift in best ILD tuning in the OT caused by AGFa microstimulation was positively correlated with the difference in best ILDs for the AGFa–OT pair (slope using robust linear regression = 0.17 ± 0.05 SEM, $p < 0.002$).

We measured the effects of AGFa microstimulation on visual responsiveness at 16 of the 67 aligned AGFa–OT pairs. For these experiments, the 25 ms train of electrical stimulation delivered to the AGFa was timed to precede visual responses in the OT by 5–10 ms; visual latencies in the OT ranged from 40 to 90 ms. Across all aligned AGFa–OT pairs ($n = 16$), AGFa microstimulation caused an average increase of 31% in the responsiveness of OT units to visual stimuli presented at or near the VRF_{cntr} (Fig. 11) ($p < 0.001$, Mann–Whitney U test). The same microstimulation had no effect on OT discharge rates when the visual stimulus was outside the visual receptive field (-0.5% ; $p > 0.5$, Mann–Whitney U test) or when there was no visual stimulus presented (mean baseline firing rate = 7 spikes/s; mean baseline firing rate after AGFa microstimulation 6 spikes/s, $p > 0.2$, Man-

Figure 9. Effect of AGFa microstimulation on stimulus discriminability at aligned AGF–OT pairs. **A**, ITD tuning at a single aligned OT site with (filled symbols, thick line) and without (open symbols, thin line) AGFa microstimulation. Arrowhead indicates the best ITD ($27 \mu s$ right ear leading). Diamonds indicate ITD values that were closest to the best ITD. Squares and triangles represent the stimulus values that produced responses near the half-max response on the left and right flanks of the tuning curves, respectively. **B**, ROC curves for the responses with (thick line) and without (thin line) AGFa microstimulation on the left flank of the tuning curves (comparison of diamonds with squares). The ΔROC area was 0.16. **C**, Population distribution of ΔROC areas for left and right flanks of ITD and ILD tuning curves. The distribution is shifted toward positive values indicating an increase in area under the ROC curve with AGFa microstimulation. The median of the distribution is 0.031 ($p < 10^{-5}$, Wilcoxon signed rank test for zero median). **D**, Population distribution of ΔROC areas for left and right flanks of visual azimuth and elevation tuning curves. The distribution is shifted toward positive values; the mean of the distribution is 0.056 ($p < 0.0001$, t test).

n–Whitney U test). The magnitude of the effect of AGFa microstimulation on neural responses in the context of presenting a range of visual azimuth values or visual elevation values was statistically the same ($p > 0.5$, Mann–Whitney U test), and therefore, when appropriate, we present the results together.

In addition to increasing responsiveness, AGFa microstimulation also improved the discriminability of visual stimuli (Fig. 9D). Across all 16 aligned AGFa–OT pairs, the mean ΔROC_{stim} increased by 0.055 ($p < 0.001$, t test).

AGFa microstimulation sharpened visual spatial tuning at a subset of sites. The half-max tuning width for visual azimuth decreased significantly with AGFa microstimulation at 6/16 (38%) aligned OT sites (Fig. 12A, black bars) ($p < 0.05$, paired t test); this sharpening effect did not reach significance across the population ($p = 0.07$, Wilcoxon signed rank test for zero median; $n = 16$). The half-max tuning width for visual elevation decreased significantly at 2/16 (13%) aligned OT sites (Fig. 12B, black bars) ($p < 0.05$, paired t test); this sharpening effect was not significant across the population ($p = 0.4$, Wilcoxon signed rank test for zero median; $n = 16$). AGFa microstimulation caused little or no shift in VRF_{cntr} , and the nominal shifts that did occur did not correlate with AGFa–OT alignment ($n = 16$, $p = 0.11$).

Nonaligned AGFa–OT pairs

For nonaligned AGFa–OT pairs ($n = 18$), AGFa microstimulation suppressed the responsiveness of OT units to visual stimuli presented at or near the VRF_{cntr} . The average magnitude of the suppression across all nonaligned pairs was 30% (Fig. 13). AGFa microstimulation had no consistent effect on the sharpness of

Figure 10. Summary of the effects of AGFa microstimulation on auditory responses at non-aligned sites in the OT. **A**, Population ITD tuning curves measured with (filled circles, thick line) and without (open circles, thin line) AGFa microstimulation. Microstimulation of the AGFa caused responses near the best value to decrease by 18% ($p < 10^{-6}$, paired t test). Ordinate shows normalized response; abscissa shows stimulus ITD normalized to the best ITD (plotted as ITD = 0 μ s). **B**, Population ILD tuning curves. Conventions are the same as for **A**. Microstimulation of the AGFa caused responses near the best value to decrease by 24% ($p < 10^{-5}$, paired t test). Abscissa shows stimulus ILD normalized to best ILD (plotted as ILD = 0 dB). Error bars represent SEM.

visual spatial tuning or on the location of VRF_{ctr} (data not shown).

Similar effects on auditory and visual responsiveness

The direction and magnitude of the effect of AGFa microstimulation on neural responsiveness in the OT was independent of sensory modality. We measured the effects of AGFa microstimulation on both auditory and visual responsiveness at 23 OT sites. At every site, when AGFa microstimulation caused an increase in auditory responsiveness, it also caused an increase in visual responsiveness (Fig. 14). Conversely, when AGFa microstimulation caused a decrease in auditory responsiveness, it also caused a decrease in visual responsiveness.

Discussion

Electrical microstimulation of the AGFa, a gaze-control area in the owl's forebrain, selectively increases the responsiveness and

Figure 11. Summary of the effects of AGFa microstimulation on visual responses at aligned sites in the OT. **A**, Population visual azimuth tuning curves measured with (filled circles, thick line) and without (open circles, thin line) AGFa microstimulation. Microstimulation of the AGFa caused a 36% increase in responses near the best value ($p < 0.001$, paired t test). Ordinate shows normalized response; abscissa shows horizontal position of the visual stimulus (azimuth) normalized to the best azimuth (plotted as azimuth = 0°). **B**, Population visual elevation tuning curves. Conventions are the same as for the population visual azimuth tuning curves. Microstimulation of the AGFa caused a 25% increase in responses near the best value. The average increases in responses were statistically the same regardless of whether visual azimuth (36%) or visual elevation (25%) was tested ($p > 0.5$, Mann–Whitney U test). Error bars represent SEM.

stimulus discriminability of OT neurons that represent the same location in space as that represented by the AGFa microstimulation site. At the same time, microstimulation of the AGFa selectively decreases the responsiveness of OT neurons that represent stimuli at other locations in space. These space-specific effects act similarly on the representations of both auditory and visual stimuli. These effects are strikingly similar to the space-specific modulations of neural responsiveness in the extrastriate visual cortex that have been reported in monkeys trained to direct attention to stimuli at specific locations in space. The following discussion summarizes these effects and evaluates their correspondence with neurophysiological and behavioral observations made in other species. The proposal is made that these top-down influences represent processes that operate in the context of voluntary spatial attention.

Figure 12. Effect of AGFa microstimulation on the sharpness of visuospatial tuning at aligned sites in the OT. **A**, Changes in visual azimuth tuning widths at half-max (width of tuning_{stim} – width of tuning_{nostim}) caused by AGFa microstimulation. Tuning for visual azimuth in the OT was sharper at 6/16 (38%) of the sites (black bars). This effect did not reach significance for the population; median of the distribution = -1.47° ($p = 0.07$, Wilcoxon signed rank test for zero median). **B**, Changes in visual elevation tuning widths caused by AGFa microstimulation. The median of the distribution = -0.78° ($p = 0.4$, Wilcoxon signed rank test for zero median).

Forebrain gaze fields

The region of the forebrain that was electrically activated in this study was the auditory portion of the AGF. Previous work in barn owls has shown that the AGFa encodes auditory space in a clustered representation, that AGF microstimulation causes saccadic movements of the eyes and head, and that pharmacological inactivation of the AGF eliminates the ability of owls to make delayed responses to contralateral auditory stimuli based on working memory (Cohen and Knudsen, 1995; Knudsen et al., 1995; Knudsen and Knudsen, 1996). These findings indicate that the AGFa represents locations in space to which the owl intends to direct its gaze.

Figure 13. Summary of the effects of AGFa microstimulation on visual responses at non-aligned sites in the OT. **A**, Population visual azimuth tuning curves measured with (filled circles, thick line) and without (open circles, thin line) AGFa microstimulation. Microstimulation of the AGFa caused a 30% reduction in responses near the best value ($p < 10^{-6}$, paired t test). Ordinate shows normalized response; abscissa shows horizontal position of the visual stimulus (azimuth) normalized to the best azimuth (plotted as azimuth = 0°). **B**, Population visual elevation tuning curves. Conventions are the same as for the population visual azimuth tuning curves. Microstimulation of the AGFa caused a 29% reduction in responses near the best value ($p < 10^{-4}$, paired t test). The average reductions in responses were statistically the same regardless of whether visual azimuth (30%) or visual elevation (29%) was tested ($p > 0.5$, paired t test). Error bars represent SEM.

The data presented in this study demonstrate, further, that the AGFa also sends top-down, space-specific signals to the OT that modulate its responsiveness to sensory stimuli. The current strengths that evoked this top-down signal were well below those required to elicit eye or head saccades, suggesting that gaze control and top-down sensory modulation are separable. A similar functional separation of gaze control signals from top-down sensory modulation signals has been proposed for the monkey FEF (Moore et al., 2003).

Both the positioning of the stimulating electrode in the AGFa and the strength of the stimulating current had to be precisely controlled to elicit a modulating effect on sensory responsiveness in the OT. These properties can be accounted for by the clustered representation of space in the AGFa. In the AGFa, neighboring clusters of neurons represent locations that can be widely sepa-

Figure 14. Comparison of the effect of AGFa microstimulation on visual and auditory responses in the OT. Percentage change in OT visual responses to stimuli at the VRF_{cntr} (ordinate) plotted as a function of the percentage change in OT auditory responses to stimuli at the best ITD and best ILD (abscissa) for 23 AGFa–OT pairs.

rated in space (Cohen and Knudsen, 1995, 1999). We hypothesize that the spread of electrical stimulation to more than one functional cluster results in contradictory, negatively interacting spatial signals from the AGFa. For this reason, the stimulating electrode must be centered in a cluster and the current amplitude must be sufficiently weak to activate primarily a single cluster. A similar explanation has been proposed to account for a decrease in the effect on visual motion perception that results from increasing microstimulation current strengths in area MT of monkeys (Murasugi et al., 1993).

Top-down enhancement of multimodal responses in the OT

The effect of AGFa microstimulation on OT responsiveness was largely independent of the sensory modality tested. Responses in the OT to both auditory and visual stimuli were enhanced when the OT and the AGFa sites represented similar locations and were suppressed when the OT and AGFa site represented different locations. When sensory responses were enhanced, the response increase scaled with the strength of responses to the stimulus without AGFa stimulation. Therefore, the maximum response increases occurred at the OT site's preferred location. This response enhancement affected OT neurons tuned within $\sim 9^\circ$ [$<22 \mu\text{s}$ ITD and $<10 \text{ dB}$ ILD (Olsen et al., 1989)] of the location represented at the AGFa site. Thus, AGFa microstimulation enhances OT responses to stimuli located within approximately an 18° ($\pm 9^\circ$) region of space, centered on the location encoded by the AGFa site.

In addition to enhancing responses, AGFa microstimulation also increased the discriminability of auditory and visual stimuli located within receptive fields for aligned AGFa–OT pairs. This improvement was observed for responses on both the left and right flanks of the tuning curves. This effect is similar to recently reported results of FEF microstimulation on visual stimulus discriminability of neurons in the extrastriate visual cortex of behaving monkeys (Armstrong and Moore, 2007) and is consistent with the well characterized effects of spatial attention on spatial discrimination.

AGFa activation exerted two additional influences, at least on

auditory responses, at aligned sites in the OT. First, AGFa microstimulation sharpened auditory spatial tuning (average = 17%). By itself, this effect decreases the number of neurons representing a particular region of space. However, AGFa microstimulation also shifted auditory spatial tuning toward the location represented at the AGFa stimulation site, an effect that dynamically increases the number of OT sites that process auditory information from the location encoded by the AGFa site. Together, sharper and shifted spatial tuning curves improve the spatial resolution of the representation of the location encoded by the top-down signal.

We explored the temporal effects of AGFa microstimulation on OT auditory responses in two ways (Fig. 6): (1) by systematically varying the temporal relationship between the offset of AGFa microstimulation and the onset of a sound stimulus (initiation of the effect) and (2) by measuring the duration of the response enhancement once initiated (persistence of the effect). The longest temporal disparity between the offset of AGFa microstimulation and the onset of sound that caused a reliable increase in OT auditory responses was 50 ms (Fig. 6A). This interval is similar to the 75 ms interval for the effects of electrical microstimulation of the FEF on target detection in awake behaving monkeys (Moore and Fallah, 2004). In our study, once an enhancement effect with AGFa microstimulation was initiated, the effect lasted ~ 120 ms (Fig. 6B). This value is similar to the duration of response enhancement of neural responses in cortical area V4 after FEF microstimulation (Moore and Armstrong, 2003) (see discussion of Moore and Fallah, 2004).

Neurons in the OT are selective for stimulus location, but they are not selective for most other stimulus features, such as auditory frequency or visual shape. Instead, OT neurons tend to respond strongly to stimuli that occur rarely and suddenly. Thus, activity across the OT represents the salience of stimuli in a map of space, and this salience signal is enhanced by a top-down signal from the AGFa. This mapped information from the OT could, in turn, enhance the representation of spatially selected stimuli in regions of the brain that analyze specific features within feature representations of space. In addition, this salience map could contribute information directly to circuits that resolve the competition among sensory representations for access to working memory (Knudsen, 2007).

References

- Armstrong KM, Moore T (2007) Rapid enhancement of visual cortical response discriminability by microstimulation of the frontal eye field. *Proc Natl Acad Sci USA* 104:9499–9504.
- Armstrong KM, Fitzgerald JK, Moore T (2006) Changes in visual receptive fields with microstimulation of frontal cortex. *Neuron* 50:791–798.
- Brainard DH (1997) The psychophysics toolbox. *Spat Vis* 10:433–436.
- Britten KH, Shadlen MN, Newsome WT, Movshon JA (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. *J Neurosci* 12:4745–4765.
- Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB (1985) Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. *J Neurophysiol* 54:714–734.
- Cohen YE, Knudsen EI (1995) Binaural tuning of auditory units in the forebrain archistriatal gaze fields of the barn owl: local organization but no space map. *J Neurosci* 15:5152–5168.
- Cohen YE, Knudsen EI (1999) Maps versus clusters: different representations of auditory space in the midbrain and forebrain. *Trends Neurosci* 22:128–135.
- Desimone R, Duncan J (1995) Neural mechanisms of selective visual attention. *Annu Rev Neurosci* 18:193–222.
- Fischer B, Boch R (1981a) Selection of visual targets activates prelunate cortical cells in trained rhesus monkey. *Exp Brain Res* 41:431–433.
- Fischer B, Boch R (1981b) Enhanced activation of neurons in prelunate

- cortex before visually guided saccades of trained rhesus monkeys. *Exp Brain Res* 44:129–137.
- Green DM, Swets JA (1966) Signal detection theory and psychophysics. New York: Wiley.
- Hoffman JE, Subramaniam B (1995) The role of visual attention in saccadic eye movements. *Percept Psychophys* 57:787–795.
- Knudsen EI (1982) Auditory and visual maps of space in the optic tectum of the owl. *J Neurosci* 2:1177–1194.
- Knudsen EI (2007) Fundamental components of attention. *Annu Rev Neurosci* 30:57–78.
- Knudsen EI, Knudsen PF (1996) Disruption of auditory spatial working memory by inactivation of the forebrain archistriatum in barn owls. *Nature* 383:428–431.
- Knudsen EI, Cohen YE, Masino T (1995) Characterization of a forebrain gaze field in the archistriatum of the barn owl: microstimulation and anatomical connections. *J Neurosci* 15:5139–5151.
- Maunsell JH (1995) The brain's visual world: representation of visual targets in cerebral cortex. *Science* 270:764–769.
- McAdams CJ, Maunsell JH (1999) Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J Neurosci* 19:431–441.
- Moore T, Armstrong KM (2003) Selective gating of visual signals by microstimulation of frontal cortex. *Nature* 421:370–373.
- Moore T, Fallah M (2001) Control of eye movements and spatial attention. *Proc Natl Acad Sci USA* 98:1273–1276.
- Moore T, Fallah M (2004) Microstimulation of the frontal eye field and its effects on covert spatial attention. *J Neurophysiol* 91:152–162.
- Moore T, Tolia AS, Schiller PH (1998) Visual representations during saccadic eye movements. *Proc Natl Acad Sci USA* 95:8981–8984.
- Moore T, Armstrong KM, Fallah M (2003) Visuomotor origins of covert spatial attention. *Neuron* 40:671–683.
- Moran J, Desimone R (1985) Selective attention gates visual processing in the extrastriate cortex. *Science* 229:782–784.
- Murasugi CM, Salzman CD, Newsome WT (1993) Microstimulation in visual area MT: effects of varying pulse amplitude and frequency. *J Neurosci* 13:1719–1729.
- Olsen JF, Knudsen EI, Esterly SD (1989) Neural maps of interaural time and intensity differences in the optic tectum of the barn owl. *J Neurosci* 9:2591–2605.
- Robinson DA, Fuchs AF (1969) Eye movements evoked by stimulation of frontal eye fields. *J Neurophysiol* 32:637–648.
- Shepherd M, Findlay JM, Hockey RJ (1986) The relationship between eye movements and spatial attention. *Q J Exp Psychol A* 38:475–491.
- Stoney SD, Thompson WD, Asanuma H (1968) Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *J Neurophysiol* 31:670–681.
- Winkowski DE, Knudsen EI (2006) Top-down gain control of the auditory space map by gaze control circuitry in the barn owl. *Nature* 439:336–339.