Multisensory Processing in “Unimodal” Neurons: Cross-Modal Subthreshold Auditory Effects in Cat Extrastriate Visual Cortex

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INTRODUCTION

At the single-neuron level, multisensory processing is evident when the response to one sensory modality, measured as the number of spikes elicited, is influenced by the presence of a stimulus from another (Meredith and Stein 1983). Historically (e.g., see Horn and Hill 1966), multisensory neurons have been identified as those that respond with excitation to separate stimuli from more than one sensory modality. For example, such neurons respond with excitation to visual and to auditory stimulation. This response pattern is considered bimodal (responses to 3 different sensory modalities are termed trimodal). Not surprisingly, a considerable effort has been expended toward understanding how bimodal/trimodal neurons integrate responses to combined-modality stimuli (for review, see Stein and Meredith 1993), the results of which have provided a great deal of insight into the neuronal basis of multisensory processing. However, neurons that do not respond to more than one modality (i.e., appear to be unimodal) have been, by definition, excluded from multisensory studies. Consequently, the potential for subthreshold multisensory effects on vast numbers of nonbimodal (unimodal) neurons remains largely unexamined. A few studies have observed the suppressive (Dehner et al. 2004; Meredith 2002; Meredith et al. 2006) or facilitatory (Bizley et al. 2006; Newman and Hartline 1981; Sugihara et al. 2006) effects of an apparently ineffective stimulus on responses of ostensibly unimodal neurons. These observations suggest that the classical, bimodal neuron is not the only type of neuron to receive and process multisensory information. Moreover, it seems that subthreshold cross-modal effects have the potential to impact multitudes of areas of the brain currently regarded as unimodal.

To test the notion that cross-modal subthreshold inputs might affect processing in a well-documented unimodal brain region, the present investigation used single- and combined-modality stimulation to evaluate possible multisensory effects in unimodal neurons in the posterolateral lateral suprasylvian (PLLS) visual area of the cat. Located in the lateral bank of the middle portion of the suprasylvian sulcus, the PLLS is a retinotopically organized extrastriate visual area (Palmer et al. 1978) that contributes to motion processing (Rauschecker et al. 1987). Although widely regarded as a visual area, some bimodal neurons have been found within the PLLS (Yaka et al. 2002), and this region is bordered laterally by the auditory dorsal zone (Stecker et al. 2005). Therefore we hypothesized that auditory stimuli presented in combination with visual stimuli would reveal cross-modal subthreshold effects on seemingly unimodal visual neurons in the PLLS.

METHODS

All procedures were performed in compliance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health, publication 86–23), the National Research Council’s Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research (2003), and the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Surgical procedures

Cats (n = 5) were anesthetized with pentobarbital (40 mg/kg), and their heads were placed in a stereotaxic frame. Sterile techniques were used to perform a craniotomy that exposed the PLLS cortex. A stainless-steel recording well was secured to the animal’s head using dental acrylic to act as a support during the recording experiment. Routine postoperative care was provided, and ~7 days elapsed before the recording experiment.

PLLS recording

Recording experiments were conducted similar to those described previously (Dehner et al. 2004; Meredith et al. 2006). Briefly, record-
ing was initiated by anesthetizing the animal (35 mg/kg ketamine; 2 mg/kg acepromazine) and securing the implanted well to a supporting bar. The saphenous vein was cannulated for continuous administration of fluids, supplemental anesthetics (8 mg·kg⁻¹·h⁻¹ ketamine; 0.5 mg·kg⁻¹·h⁻¹ acepromazine) and, to prevent spontaneous movements, a muscle relaxant (pancuronium bromide, 0.3 mg/kg initial dose; 0.2 mg·kg⁻¹·h⁻¹ supplement). The animals were intubated through the mouth and maintained on a ventilator with expired carbon dioxide maintained at ~4.5%. Heart rate was monitored and a heating pad was used to maintain temperature at 37°C.

For recording, the implant well was opened and a glass insulated tungsten electrode (tip exposure: ~20 μm, impedance: <1.0 MΩ) was inserted into the PLLS cortex and advanced using a hydraulic microdrive. Neurons were sought at 125-μm intervals (or 250 μm for some penetrations) to ensure that different neurons were recorded at each site and to avoid bias toward any particular type of response. Neurons were isolated by their spontaneous or sensory-evoked activity and were first identified qualitatively by their responses to manually presented stimuli: auditory (clicks, claps, whistles, and hisses) and visual (moving light or dark bars). These qualitative sensory tests were systematically performed for each neuron encountered along the electrode track from the tip to the fundus of the PLLS cortex. The associated visual receptive fields were manually mapped on a translucent hemisphere (92 cm diam), and the center of the receptive field was noted as a measure of eccentricity.

After the initial sensory assessment, neurons at each site were examined quantitatively to evaluate their possible cross-modal effects, and essentially the same sensory tests were delivered to each neuron encountered along an entire recording penetration. These tests consisted of computer-triggered visual and auditory stimuli, presented alone and in combination (V, A, AV). Each stimulus presentation was separated by 7 s, and each condition was presented 25 times; combined stimuli were presented with onsets where visual preceded auditory by 40 ms to compensate for the differences in their response latencies. Separate and combined modes of stimulation were interleaved to compensate for possible shifts in baseline activity. Visual cues were light bars projected onto the translucent hemisphere (92 cm diam) of which the movement direction, velocity, and amplitude across the visual receptive field were computer-controlled. Free-field auditory cues were electronically generated white-noise bursts (50-ms duration, 55 dB SPL) from a hoop-mounted speaker 44 cm from the head delivered in spatial register with the visual receptive field. Neuronal responses were digitized (rate: >25 kHz), and individual waveforms were templated using Spike2 (Cambridge Electronic Design) and routed to a computer for storage and later analysis.

**Data analysis and histology**

Once the waveform for each recorded neuron was identified and templated, a peristimulus time histogram was constructed for each neuron for each of the test conditions (V, A, and AV) using Spike2 software. From the histogram, the response duration for the AV condition was determined and the mean spikes per trial (±SD) was calculated for that period for all three stimulus conditions. For each neuron, the mean spikes/trial from the 25 trials of the combined stimuli (AV) and the most effective single stimulus (V or A) were compared (paired, 2-tailed t-test). Responses that showed a significant difference (P < 0.05) were defined as response interactions (Meredith and Stein 1986) and classified accordingly (e.g., enhancement in bimodal neurons = combined-modality response significantly greater than the best single-modality response; facilitation in unimodal neurons = combined-modality response greater than the only single-modality response). The level of enhancement/facilitation was calculated = [(AV response – V response)/V response] × 100% (modified from Meredith and Stein 1986). The depth of each neuron within a penetration was noted and correlated with its sensory activity. Several recording penetrations were performed in a single animal, and each recording penetration was electrolytically marked at its terminus. At the conclusion of the recording experiment the animal was overdosed, perfused and the brain fixed (formalin). The brain was blocked stereotaxically and postfixed in 30% sucrose/formalin. Frozen sections (50 μm) were cut in the coronal plane through the recording sites, processed using standard histological procedures and counterstained with cresyl violet. A projecting microscope was used to trace sections and to reconstruct the recording penetrations and locations of the examined neurons from the lesion sites.

**Results**

**Qualitative sensory tests**

From the five cats examined a total of 12 recording penetrations traversed the PLLS cortex, which collectively contained 360 recording sites. Based on manual stimulation of identified single units, each recording site (at 125- or 250-μm intervals) was categorized as auditory (56/360), visual (212/360), bimodal (47/360), or unresponsive (45/360); the majority of sites (72%, 259/360) were excited by visual stimulation (either visual or bimodal). As depicted in Fig. 1, there was a tendency for auditory sites to be segregated near the lip of the sulcus, visual sites were deep within the banks and fundus, and bimodal sites were wedged between those two.

**Quantitative sensory tests**

A total of 520 neurons were presented quantitative visual, auditory, and combined sensory tests. From these tests, 41 neurons (8%) were identified as auditory, 233 (45%) visual, 49 bimodal (9%), and 197 unresponsive (38%). The vast majority of visual neurons (84%; n = 196/233) were not significantly affected by the presence of an auditory stimulus. On the other hand, bimodal neurons (n = 49) responded as would be predicted. Like the responses shown in Fig. 2, A, bimodal neurons were reliably activated by visual stimuli as well as auditory stimuli presented alone. In addition, combined-modality stimulation typically evoked a response increase over either of the single-modality responses. When the responses of each bimodal neuron were graphed as an x-y scatter plot of visual response (the best single-modality response) versus the combined response, the majority of bimodal neurons (86%; 42/49) plotted above the line of unity (Fig. 2C). Furthermore, as shown in Fig. 2G, the response average for bimodal neurons was significantly greater for the combined visual-auditory stimuli than the visual stimulus alone (AV = 8.8 ± 1.3 vs. V = 7.0 ± 1.0 mean spikes/trial, P < 0.001, paired t-test). Whereas many (86%) bimodal neurons showed combined-modality response increases greater than that of the most effective single modality stimulus, only 19 (39%) showed a statistically significant response enhancement. Among bimodal neurons, the level of response increase was relatively modest, with approximately half exhibiting response changes in the range of 21–60% (Fig. 2H). Bimodal neurons also sampled a restricted portion of the visual field, because 93% of them had visual receptive field centers at >40° eccentricity (Fig. 3).

As depicted in Fig. 1, neurons deep to the bimodal region were responsive to visual stimuli but did not respond to any auditory cues presented alone (e.g., had a single, unimodal response distribution). However, using the same standardized single- and combined-modality paradigm as used for bimodal neurons, 16% (37/233) of visual neurons showed a signifi-
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DISCUSSION

Historically, multisensory integration has been studied on the perceptual/behavioral level in humans and at the neuronal level in laboratory animals. In the latter, the bimodal (and trimodal) neuron has become synonymous with multisensory behavior, and a great deal is known about its function as well as relevance to specific multisensory behaviors (for review, see Calvert et al. 2004; Stein and Meredith 1993). In contrast, there is a profound lack of knowledge about how the rest of the brain, represented by nonbimodal (i.e., presumed unimodal) neurons, is affected by multisensory stimulation. Only a few studies have reported multisensory effects on unimodal neurons (Bizley et al. 2006; Dehner et al. 2004; Meredith 2002; Meredith et al. 2006; Newman and Hartline 1981; Sugihara et al. 2006). The present experiments add to those observations by demonstrating that unimodal visual neurons in the cat PLLS exhibit subthreshold multisensory influences. In this case, visually responsive neurons in the PLLS were unresponsive to auditory stimulation alone but showed a significant response increase when visual and auditory stimuli were combined. Because these unimodal neurons were significantly affected by the presence of a stimulus from another modality, they met the definition of being multisensory (Meredith and Stein 1986). In addition, spatial combined-modality tests showed that this cross-modal facilitation was consistent with inputs from monaural auditory receptive fields (as seen in other auditory cortical areas) (Middlebrooks et al. 1980), thereby ruling out nonspecific alerting effects. On the other hand, it might be argued that these subthreshold multisensory neurons found in the PLLS were simply bimodal neurons that were not adequately excited by the stimulation provided. This possibility, however, is unlikely because each neuron was evaluated qualitatively by a wide array of manually presented auditory stimuli that were highly effective for the bimodal neurons but not the subthreshold ones. In addition, there was an organizational basis for the different response modes, whereby the distribution of subthreshold multisensory neurons was largely segregated from the bimodal units. Similarly, the visual receptive field locations for the subthreshold neurons were predominantly centrally positioned, whereas those for bimodal neurons were almost exclusively in the visual periphery. Therefore due to functional and organizational differences, subthreshold multisensory and bimodal responses in PLLS neurons appear to represent separate physiological effects in distinct classes of neurons. Ultimately, these observations confirm the presence of auditory responses in the PLLS.
FIG. 2. Multisensory (bimodal and subthreshold) neurons in the PLLS cortex. For a representative bimodal neuron (A) and subthreshold multisensory neuron (D), responses to visual (light bar moved across the visual receptive field indicated by ramp labeled V), auditory (contralateral white noise 55-dB SPL 50-ms duration denoted by square wave labeled A), and combined stimulation (AV) are shown in the rasters (dot = 1 spike; each row = 1 trial) and histograms (10-ms time bins). As summarized in the bar graphs [B and E; error bars, SD; - - -, spontaneous activity (Sp)], the bimodal and subthreshold multisensory neurons both showed strong responses to the visual stimulus (V) and a significant response increase (*P < 0.05; paired t-test) when the visual stimulus was combined with an auditory stimulus (AV). However, unlike the bimodal neuron, the subthreshold multisensory neuron showed no response to the auditory stimulus alone (A). Consequently, this multisensory effect represents cross-modal subthreshold facilitation. As shown in the scatter plot of the population of bimodal neurons (n = 49, C), the response to the combined auditory-visual stimuli (AV; y axis) was frequently (86%; 42/49) greater than that elicited by the visual (and most effective) stimulus presented alone (V; x axis). The responses of subthreshold multisensory neurons (n = 37) all plotted above the line of unity (F). G: populations of bimodal neurons (n = 49, ■) and subthreshold multisensory neurons (n = 37, □) showed a significantly greater response (mean spikes/trial ± SE, P < 0.001, paired t-test) for the combined visual-auditory stimuli (AV) than the visual stimulus alone (V). H: majority of bimodal neurons (■) and all of the subthreshold multisensory neurons (□) showed facilitatory multisensory effects that spanned a similar range. Neurons plotted right of the line of Unity were facilitated (i.e., AV response exceeded the V response).
Bimodal and subthreshold multisensory neurons were found in only a partial measure of multisensory processing in the brain. This suggests that the exclusive study of bimodal neurons can provide neurons exhibiting subthreshold multisensory effects, demonstrating modestly influenced by concurrent auditory inputs. Events more centrally located can result in visual signals more activity levels resulting from multisensory integration. Those processed on visual or auditory basis or by dramatic changes in visual field by the PLLS. Thus, events in the periphery can be segregated high in the bank of the sulcus, had receptive fields of bimodal neurons (dark gray) had visual receptive field centers that were almost exclusively located in the visual periphery (93% at >40° eccentricity), whereas subthreshold multisensory neurons (light gray) had more centrally located visual receptive fields (62% at <40° eccentricity).

In the PLLS, the two modes of multisensory processing were retinotopically distributed, where bimodal neurons, largely segregated high in the bank of the sulcus, had receptive fields in the visual periphery, and the subthreshold multisensory neurons, located more deeply in the bank of the sulcus, had more centrally placed receptive fields. This dichotomy may reflect different strategies given to the different portions of the visual field by the PLLS. Thus, events in the periphery can be processed on visual or auditory basis or by dramatic changes in activity levels resulting from multisensory integration. Those events more centrally located can result in visual signals more modestly influenced by concurrent auditory inputs.

In summary, the present study shows that some unimodal neurons exhibit subthreshold multisensory effects, demonstrating that the exclusive study of bimodal neurons can provide only a partial measure of multisensory processing in the brain. Bimodal and subthreshold multisensory neurons were found in similar proportions, indicating that approximately half of the multisensory neurons in this region would have gone undetected using conventional methodology. Accordingly, future neuronal studies of multisensory integration should account not only for bimodal activity but also include combined-modality tests of nonbimodal neurons to assess the potential for subthreshold multisensory effects.

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