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

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Abstract

Historically, the study of multisensory processing has examined the function of the definitive neuron type, the bimodal neuron. These neurons are excited by inputs from more than one sensory modality and, when multisensory stimuli are present, they can integrate their responses in a predictable manner. However, recent studies have revealed that multisensory processing in the cortex is not restricted to bimodal neurons. The present investigation sought to examine the potential for multisensory processing in non-bimodal (‘unimodal’) neurons in the retinotopically-organized posterolateral lateral suprasylvian (PLLS) area of the cat. Standard extracellular recordings were used to measure responses of all neurons encountered to both separate- and combined-modality stimulation. While bimodal neurons behaved as predicted, the surprising result was that 16% of ‘unimodal’ visual neurons encountered were significantly facilitated by auditory stimuli. Because these ‘unimodal’ visual neurons did not respond to an auditory stimulus presented alone, but had their visual responses modulated by concurrent auditory stimulation, they represent a new form of multisensory neuron: the subthreshold multisensory neuron. These data also demonstrate that bimodal neurons can no longer be regarded as the exclusive basis for multisensory processing.
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, 1965), 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 three 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 non-bimodal (‘unimodal’) neurons remain largely unexamined. A few studies have observed the suppressive (Meredith 2002; Dehner et al. 2004; Meredith et al. 2006) or facilitatory (Newman and Hartline 1981; Bizley et al. 2006; Sugihara et al. 2006) effects of an apparently ineffective stimulus on responses of ostensibly ‘unimodal’ neurons to cues from another modality. 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.

**Materials and Methods**

All procedures were performed in compliance with the Guide for Care and Use of Laboratory Animals (NIH 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 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, as a support during the recording experiment. Routine postoperative care was provided and approximately 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, recording 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/h Ketamine; 0.5 mg/kg/h Acepromazine) and, to prevent spontaneous movements, a muscle relaxant (Pancuronium bromide 0.3 mg/kg initial dose; 0.2 mg/kg/h 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 37ºC.

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); visual (moving light or dark bars). These qualitative sensory tests were systematically performed for each neuron encountered along the electrode track from the lip to the fundus of the PLLS cortex. The
associated visual receptive fields were manually mapped on a translucent hemisphere (92 cm diameter), 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 diameter), whose movement direction, velocity and amplitude across the visual receptive field was 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 (and standard deviation, 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, two-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] x 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 µm or 250 µm intervals) was categorized as auditory (56/360), visual (212/360), bimodal (47/360) or unresponsive (45/360); the majority of which (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 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. 2A, 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). While 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, of the 233 visual neurons quantitatively tested using the same standardized single- and combined-modality paradigm as used above for bimodal neurons, 16% (37/233) showed a significantly (paired t-test, P<0.05) increased response when the stimuli were combined despite being unresponsive to auditory stimulation alone. This subthreshold facilitation of the visual response is depicted in Fig. 2D. When the responses were graphed as an x-y scatter-plot, all values plotted above the line of unity (Fig. 2F). Furthermore, as depicted in Fig. 2G, the average for the population of subthreshold-facilitated responses was significantly greater for the combined visual-auditory stimuli than the visual stimulus alone (AV=11.4±1.4 vs. V=8.7±1.2 mean spikes/trial, P<0.001, paired t-test). Thus, these ostensibly ‘unimodal’ visual neurons demonstrated significant cross-modal facilitatory effects in the presence of combined-modality stimulation, with 73% demonstrating response increments in the range of 21-60% (Fig. 2H). As shown in Fig. 3,
the visual receptive fields of the majority (62%) of neurons showing subthreshold cross-modal facilitation centered in or near central visual space (<40° eccentricity).

The possibility of the auditory stimuli generating non-specific alerting, rather than sensory effects in the PLLS was examined in an additional 34 visual neurons. Of these neurons, 10 showed a significant response facilitation (paired t-test, P<0.05) when the visual stimulus was combined with a contralateral auditory cue. However, when the auditory cue was repositioned within the ipsilateral auditory field, only 3 of the same neurons had their visual responses facilitated by the additional cue. Thus, most of the affected PLLS neurons appeared to be differentially influenced by contralateral versus ipsilateral auditory stimuli in a manner that correlates better with a monaural auditory receptive field (found in other auditory cortical areas, Middlebrooks et al. 1980) than a uniform level of activation consistent with alerting effects.
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 multisensation, and a great deal is known about its function as well as relevance to specific multisensory behaviors (for review, see Stein and Meredith 1993; Calvert et al. 2004). In contrast, there is a profound lack of knowledge about how the rest of the brain, represented by non-bimodal (i.e., presumed ‘unimodal’) neurons, is affected by multisensory stimulation. Only a few studies have reported multisensory effects on ‘unimodal’ neurons (Newman and Hartline 1981; Meredith 2002; Dehner et al. 2004; Meredith et al. 2006; Bizley et al. 2006; 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 non-specific 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 since 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, while 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 a novel type of neuron, the subthreshold multisensory neuron, which in contrast to traditional bimodal (and trimodal) neurons, has response activity induced by an effective sensory modality modulated by concurrent inputs from another that are ineffective when presented alone.

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 while the subthreshold multisensory neurons were located more deeply in the bank and fundus of the sulcus and revealed more centrally placed receptive fields. This dichotomy may reflect different strategies given to the different portions of the visual field by the PLLS, whereby events in the periphery can be dealt with in an either (visual) or (auditory) basis or by dramatic changes in activity levels as a result of multisensory integration, while those more centrally located have their 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. In this study, 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 non-bimodal neurons to assess the potential for subthreshold multisensory effects.
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References


Figure Legends

**Figure 1.** PLLS location of recording sites and penetrations. The schematic of lateral view of cat cortex shows the anterior-posterior levels from which the row of coronal sections containing 12 recording penetrations within the PLLS were taken (top panel). Below, the PLLS is magnified to show the distribution of auditory (light grey), bimodal (black), visual (dark grey) and unresponsive (dashes) recording sites within the PLLS.

**Figure 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=standard deviation; dashed line=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. The populations of bimodal neurons (n=49, black) and subthreshold multisensory neurons (n=37, grey) showed a significantly greater response (mean spikes/trial ± SEM, P<0.001, paired t-test) for the combined visual-auditory stimuli (AV) than the visual stimulus alone (V). H. The majority of bimodal neurons (black bar) and all of the subthreshold multisensory neurons (grey bar) 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).

Figure 3. PLLS bimodal and subthreshold multisensory neurons had different visual receptive field distributions. Top: Visual field (concentric circles) shows receptive field locations of bimodal neurons (dark grey) and subthreshold multisensory neurons (light grey) which themselves were segregated to different regions of the PLLS. Bottom: Bimodal neurons (dark grey) had visual receptive field centers that were almost exclusively located in the visual periphery (93% at >40º eccentricity), whereas subthreshold multisensory neurons (light grey) had more centrally-located visual receptive fields (62% at <40º eccentricity).
Figure 2.
Figure 3.