***Title***: Sound improves neuronal encoding of visual stimuli in mouse primary visual cortex

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## Abstract

In everyday life, we integrate visual and auditory information in routine tasks such as navigation and communication. While it is known that concurrent sound can improve visual perception, the neuronal correlates of this audiovisual integration are not fully understood. Specifically, it remains unknown whether improvement due to sound of detection and discriminability of visual stimuli is reflected in the neuronal firing patterns in the primary visual cortex (V1). Furthermore, presentation of the sound can induce movement in the subject, but little is understood about whether and how sound-induced movement contributes to V1 neuronal activity. Here, we investigated how sound and movement interact to modulate V1 visual responses in awake, head-fixed mice and whether this interaction improves neuronal encoding of the visual stimulus. We presented visual drifting gratings with and without simultaneous auditory white noise to awake mice while recording mouse movement and V1 neuronal activity. Sound modulated the light-evoked activity of 80% of light-responsive neurons, with 95% of neurons exhibiting increased activity when the auditory stimulus was present. Sound consistently induced movement. However, a generalized linear model revealed that sound and movement had distinct and complementary effects of the neuronal visual responses. Furthermore, decoding of the visual stimulus from the neuronal activity was improved with sound, an effect that persisted even when controlling for movement. These results demonstrate that sound and movement modulate visual responses in complementary ways, resulting in improved neuronal representation of the visual stimulus. This study clarifies the role of movement as a potential confound in neuronal audiovisual responses and expands our knowledge of how multimodal processing is mediated at a neuronal level in the awake brain.

## Significance statement

Sound and movement are both known to modulate visual responses in the primary visual cortex, however sound-induced movement has remained unaccounted for as a potential confound in audiovisual studies in awake animals. Here, authors found that sound and movement both modulate visual responses in an important visual brain area, the primary visual cortex, in distinct, yet complementary ways. Furthermore, sound improved encoding of the visual stimulus even when accounting for movement. This study reconciles contrasting theories on the mechanism underlying audiovisual integration and asserts the primary visual cortex as a key brain region participating in tripartite sensory interactions.

## Introduction

Our brains use incoming sensory information to generate a continuous perceptual experience. The neuronal systems underlying sensory perceptions of different modalities interact in a way that often improves perception of the complementary modality (Gingras et al., 2009; Gleiss and Kayser, 2012; Bigelow and Poremba, 2016; Hammond-Kenny et al., 2017; Meijer et al., 2018; Stein et al., 2020). In the audiovisual realm, it is often easiest to understand what someone is saying in a crowded room by additionally relying on visual cues such as lip movement and facial expression (Maddox et al., 2015; Tye-Murray et al., 2016). The McGurk effect and flash-beep illusion are other common perceptual phenomena that demonstrate mutual interactions between the auditory and visual systems (McGurk and MacDonald, 1976; Shams et al. 2002). Despite this current awareness of audiovisual integration at a perceptual level, a detailed understanding of the neuronal codes that mediate this improvement has proved elusive.

Previous studies of neuronal correlates of audiovisual integration found that the primary sensory cortical areas participate in this process (Wang et al., 2008; Ibrahim et al., 2016; Meijer et al.,

2019; Deneux et al., 2019). The primary visual cortex (V1) contains neurons whose light-evoked firing rates are modulated by sound, as well as neurons that are responsive to sound alone (Knöpfel et al., 2019). Orientation and directional tuning of individual neurons are also affected by sound. In anesthetized mice, layer 2/3 neurons in V1 exhibited sharpened tuning in the presence of sound (Ibrahim et al., 2016). But another study in awake mice found no average differences in visual tuning curve bandwidth with and without sound (Meijer et al., 2017). These contrasting findings raise the question of whether the multisensory perceptual improvements described above are reflected in individual V1 neurons in the awake brain. Furthermore, awake animals are subject to brain-wide changes in neuronal activity due to stimulus-aligned, uninstructed movements (Musall et al., 2019), a factor yet unaccounted for in most audiovisual studies.

Sound-induced movement represents a potential confound for audiovisual studies in awake animals because whisking and locomotion modulate neuronal activity in the sensory cortical areas. In V1, movement enhances neuronal visual responses and improves neuronal encoding of the visual scene (Niell and Stryker, 2010; Dardalat and Stryker, 2017). Conversely, in the auditory cortex (AC), locomotion generally suppresses neuronal spontaneous and auditory responses (Nelson et al., 2013; Schneider and Mooney, 2018; Bigelow et al., 2019). Therefore, movement is an important factor in neuronal sensory responses that often correlates with stimulus features.

Thus, audiovisual integration in V1 may not simply represent afferent information from auditory brain regions, as supported by studies demonstrating that V1 neurons are sensitive to the optogenetic stimulation (Ibrahim et al., 2016) and pharmacologic suppression (Deneux et al., 2019) of AC neurons. Indeed, the modulation of V1 activity may instead by a byproduct of uninstructed sound-induced movements which themselves modulate visual responses (Bimbard et al., 2021). However, because previous studies were either performed in anesthetized subjects (Ibrahim et al., 2016), or trials during which the mouse moved were excluded from analysis (Deneux et al., 2019) or pooled together (Iurilli et al., 2012; Meijer et al., 2017), these alternative explanations have not been quantified. We tested to what extent locomotion contributed to audiovisual integration in V1 by performing extracellular recordings of neuronal activity in V1 concurrent with monitoring movement in awake mice presented with audiovisual stimuli. We found that the majority of neurons in V1 were responsive to visual and auditory stimuli. We found that sound and movement exerted distinct yet complementary effects on shaping the visual responses. Importantly, sound improved discriminability of the visual stimuli both in individual neurons and at a population level, an effect that persisted when accounting for movement.

## Results

### Sound enhances the light-evoked firing rate of a subset of V1 neurons

Previous work identified that sound modulates visual responses in V1 (Ibrahim et al., 2016; Meijer et al., 2018; McClure and Polack, 2019), yet how that interaction affects stimulus encoding in individual neurons and as a population remains unclear. Furthermore, whether that interaction can be exclusively attributed to sound or rather to sound-induced motion is controversial (Bimbard et al., 2021). To elucidate the principles underlying audiovisual integration, we presented audiovisual stimuli to awake mice while performing extracellular recordings in V1 (Figure 1A). The visual stimulus consisted of drifting gratings in 12 directions presented at 5 visual contrast levels (Figure 1B). On half of the trials, we paired the visual stimulus with a 70 dB burst of white noise from a speaker positioned next to the screen (Figure 1C), affording 10 trials of each unique audiovisual stimulus condition (Figure 1C). Twelve recording sessions across six mice were spike sorted, and the responses of these sorted neurons were organized by trial type to compare across

audiovisual stimulus conditions. Figure 1D-G demonstrates an example unit tuned for gratings aligned to the 30°-210° axis whose baseline and light-evoked firing rate are increased by the sound.

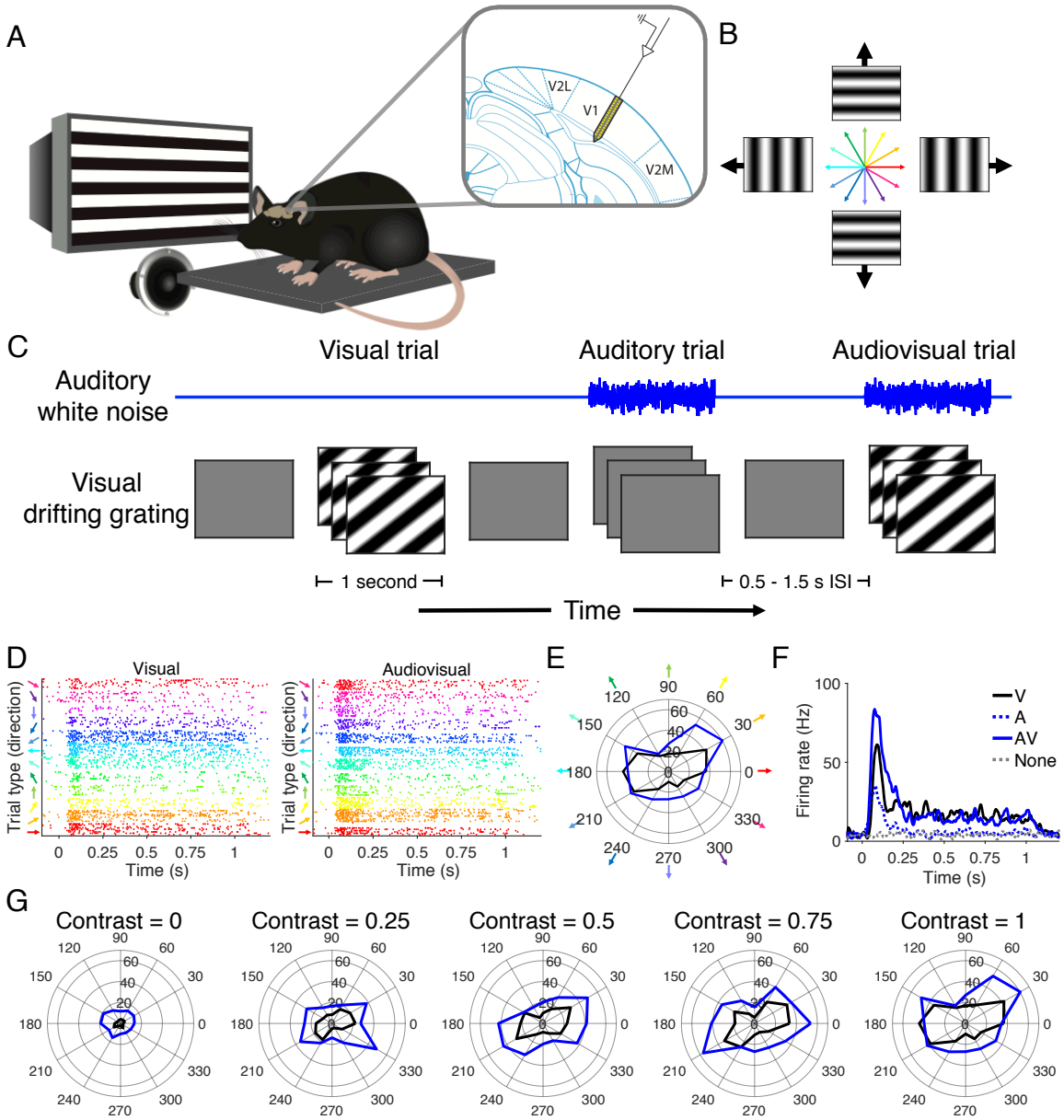


**Figure 1 | Audiovisual stimulus presentation** (A) Diagram (left) demonstrating that mice were head-fixed and presented with audiovisual stimuli from the right spatial field while electrophysiological recordings were performed in V1 (right). (B) Visual stimuli consisted of drifting gratings of 12 directions. (C) Auditory, visual, and audiovisual trials were randomly ordered and spaced with variable inter-stimulus intervals. (D) Raster plots of visual (left) and audiovisual (right) trials of an example neuron exhibiting visual orientation tuning. (E) Polar plot demonstrating the orientation tuning and magnitude of response (Hz) of the same example neuron in *E*. (F) PSTH of the same neuron in *E* demonstrating enhanced firing in response to audiovisual stimuli compared to unimodal stimuli. (G) Example neuron in *E* displays enhanced firing rate with sound across visual contrast levels.

Sound modulated the activity of the majority of V1 neurons. We used a generalized linear model (GLM) to classify neurons as light-responsive and/or sound-responsive based on their firing rate at the onset (0-300 ms) of each trial. Using this classification method, we found that 86.2% (703/816) of units were responsive to increasing visual stimulus contrast levels, and of these visually responsive units, 80.1% (563/703 neurons, 12 recording sessions in 6 mice) were significantly modulated by the presence of sound (Figure 2A). We constructed an average PSTH from the response profiles of sound-modulated light-responsive neurons, which revealed that the largest change in light-evoked firing rate occurs at the onset of the stimulus (Figure 2B). Averaged across neurons, we found a robust increase in the magnitude of the visually evoked response across visual contrast levels (Figure 2C; p(vis)=1.2e-100, p(aud)=1.6e-88, p(interact)=5.7e-4, paired 2-way ANOVA; $p_{c=0}$=2.1e-51, $p_{c=0.25}$=2.6e-62, $p_{c=0.5}$=5.7e-75, $p_{c=0.75}$=1.1e-81, $p_{c=1}$=2.0e-81, post hoc Bonferroni-corrected paired t-test, Table 1). This difference was driven by the majority of neurons (95%) that increased their firing rate in the presence of sound. However, some neurons exhibited lower light-evoked and sound-evoked firing rates relative to baseline.

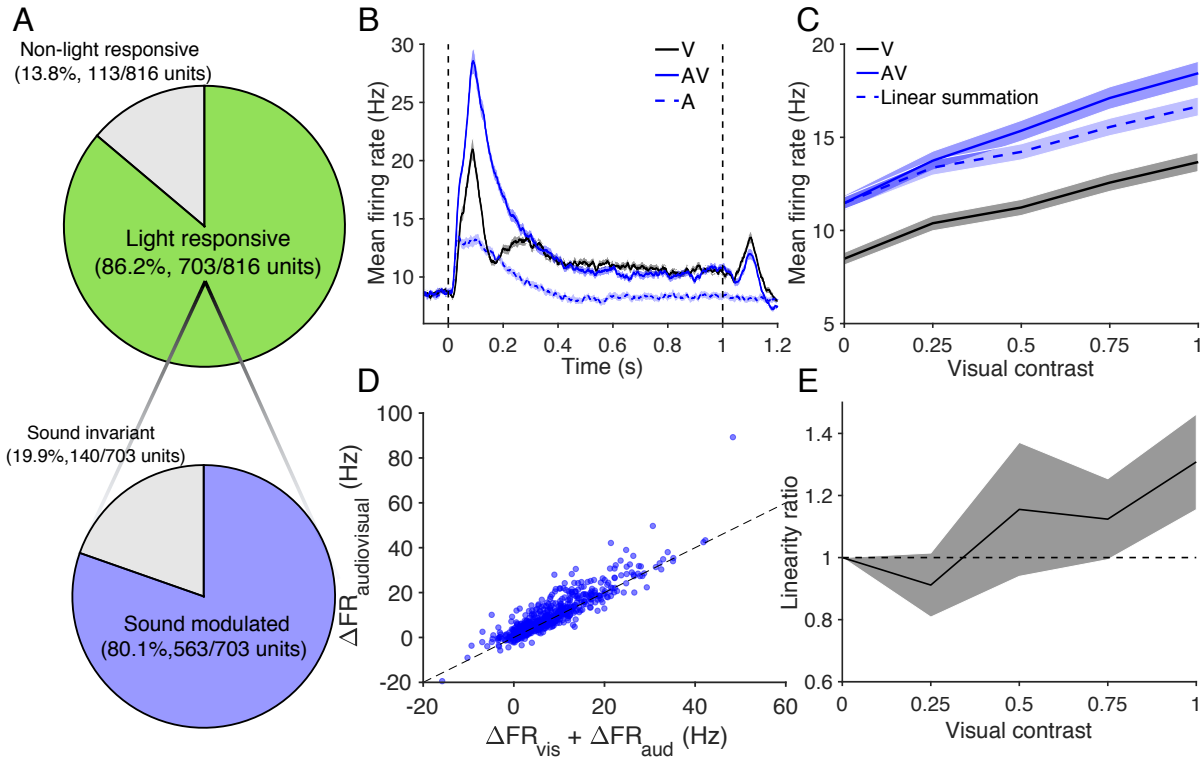


**Figure 2 | Sound enhances visual responses in a supra-linear manner** (A) Sound modulates visually evoked activity in 80.1% of light-responsive neurons in V1. (B) Comparison of visual, auditory, and audiovisual PSTHs averaged across all light-responsive sound-modulated neurons. Visual and audiovisual PSTHs correspond to the highest visual contrast level. (C) The magnitude of audiovisual onset responses (0-300ms) is greater than that of the visual response in light-responsive sound-modulated neurons (n=563, p(vis)=1.2e-100, p(aud)=1.6e-88, p(interact)=5.7e-4, 2-way repeated measures ANOVA; post hoc Bonferroni-corrected paired t-test). The expected linear sum of the unimodal auditory and visual responses is included. (D) At full visual contrast, the observed audiovisual response in the majority of neurons is greater than the linear sum of the unimodal auditory and visual responses. (E) A linearity ratio above 1 demonstrates audiovisual responses in V1 represent supra-linear integration of the unimodal signals (n=563, p=1.6e-12, Kruskal-Wallis test, post hoc Bonferroni-corrected Wilcoxon signed rank test).

This change in firing rate can be described as supra-linear or sub-linear based on whether the audiovisual response is greater or less than, respectively, the sum of the unimodal light-evoked and sound-evoked firing rates. At medium to high visual contrast levels, integration of the audiovisual stimulus was predominantly supra-linear (Figure 2D-E; p=1.6e-12, Kruskal-Wallis test; $p_{c=0.25}$=0.053, $p_{c=0.5}$=0.004, $p_{c=0.75}$=4.6e-8, $p_{c=1}$=2.1e-5, post hoc Bonferroni-corrected Wilcoxon signed rank test, Table 1). In summary, these results show that sound supra-linearly increases the magnitude of the light-evoked response in the majority of V1 neurons.

**Sound reduces the orientation- and direction-selectivity of tuned neurons**

Having observed sound-induced changes in the magnitude of the visual response, we next assessed whether these changes in magnitude affected neuronal tuning. V1 neurons have receptive fields tuned to a specific visual stimulus orientation and, to a lesser extent, stimulus direction (Métin et al, 1988; Rochefort et al., 2011; Fahey et al., 2019). We first tested whether sound altered tuning preferences of V1 neurons. In light-responsive neurons, we calculated the orientation and direction-selective indices (OSI and DSI) as well as pseudo indices based on random permutations of the trials (see Methods), and classified neurons in which the true indices were >95% of the pseudo indices as “orientation-” or “direction-selective.” Using this stringent selection criterion, we found that 13.9% (78/563) of neurons were orientation-selective, whereas 2.1% (12/563) were direction-selective. In these neurons, we observed shifts in the preferred direction from the visual to audiovisual condition (Fig 2 Sup 1A). This shift in visual tuning preference may be due to auditory input, or it may reflect noise in the neuronal responses. To test this, we performed an additional permutation test by repeatedly sampling the visual responses. We found that the resulting distribution of preferred direction shifts resembled the observed distribution under the audiovisual condition (Fig 2 Sup 1B), and the observed mean shift in degrees was within the limits of the sampled distribution (Fig 2 Sup 1C). Therefore, we cannot conclude that the shift in directional tuning preferences is associated with the presence of sound.

In addition to testing a shift in preferred direction, we investigated whether sound altered the neurons’ tuning selectivity. Tuning selectivity captures how strongly an individual neuron responds to stimuli of a certain condition, e.g. grating orientation and drift direction, as compared to others. We found a small reduction in the OSI from the visual to audiovisual conditions (Fig 2 Sup 1D-E; p=0.0018, paired Student’s t-test), which may reflect disproportionate changes in firing rate at the preferred versus orthogonal directions. We also found a reduction in the DSI in the presence of sound (Fig 2 Sup 1F-G; p=0.021, paired Student’s t-test). Combined, these results suggest that sound’s enhancement of the magnitude of light-evoked responses has minimal or potentially diminishing effects on the tuning selectivity of neurons.

**Sound reduces the latency, increases onset duration, and decreases variability of visual responses in neurons**

Behaviorally, certain cross-modal stimuli elicit shorter reaction times than their unimodal counterparts (Diederich and Colonius, 2004; Colonius and Diederich, 2017; Meijer et al., 2018). Therefore, we hypothesized that sound reduces the latency of the light-evoked response at a neuronal level as well. For each neuron, we calculated the response latency as the first time bin after stimulus onset at which the firing rate exceeded 1 standard deviation above baseline (Fig 2 Sup 2A), and found that sound reduced the response latency across contrast levels (Fig 2 Sup 2B; p(vis)=6.9e-4, p(aud)=6.8e-15, p(interact)=0.045, paired 2-way ANOVA; $p_{c=0.25}$=2.3e-4, $p_{c=0.5}$=7.1e-12, $p_{c=0.75}$=4.6e-5, $p_{c=1}$=9.9e-4, post hoc Bonferroni-corrected paired t-test, Table 1). We additionally calculated the slope of the onset response of light-responsive sound-modulated neurons, measured from trial onset until the time at which each neuron achieved its peak firing rate (Fig 2 Sup 2C). We found that sound increased the slope of the onset response (Fig 2 Sup 2D; p(vis)=3.5e-121, p(aud)=2.7e-15, p(interact)=0.038, paired 2-way ANOVA; $p_{c=0.25}$=1.4e-4,

$p_{c=0.5}$=8.9e-13, $p_{c=0.75}$=3.6e-12, $p_{c=1}$=5.5e-8, post hoc Bonferroni-corrected paired t-test, Table 1), both indicating that the response latency was reduced in the audiovisual condition compared to the visual condition. Additionally, the duration of the light-evoked response, defined as the full width at half maximum of the peak onset firing rate, increased in the presence of sound (Fig 2 Sup 2E,F; p(vis)=1.3e-10, p(aud)=8.7e-98, p(interact)=0.23, paired 2-way ANOVA). Both of these timing effects were relatively constant across contrast levels. Therefore, the latency and onset duration of light-evoked responses in V1 neurons is enhanced by sound.

Having observed changes in response magnitude and timing, we next investigated the effect of sound on the variability of light-evoked responses. If individual neurons encode the visual stimulus using changes in their firing rate, a more consistent response would entail less spread in the response magnitude relative to the mean response across trials of a single stimulus type. We quantified this relationship using the coefficient of variation (CV) defined as the ratio of the standard deviation to the response mean (Gur et al., 1997). We hypothesized that sound reduces the CV of light-evoked responses, corresponding to reduced response variability and higher SNR. Fig 2 Sup 2G depicts the relationship between response magnitude and CV in an example sound-modulated light-responsive neuron, demonstrating that increased response magnitude correlates with reduced CV. Consistent with sound increasing the visual response magnitude in the majority of sound-modulated light-responsive neurons (Figure 2), we observed a reduction of CV in the audiovisual condition relative to the visual condition when averaged across these neurons (Fig 2 Sup 2H; p(vis)=0.28, p(aud)=4.2e-103, p(interact)=0.38, paired 2-way ANOVA). Taken together, these results indicate that sound not only modulates the magnitude of the visual response (Figure 2), but also improves the timing and consistency of individual neurons' responses (Fig 2 Sup 2).

**Sound-induced movement does not account for sound's effect on visual responses**

It is known that whisking and locomotive behaviors modulate neuronal activity in mouse visual cortex (Niell and Stryker, 2010) and auditory cortex (Nelson et al., 2013; Schneider and Mooney, 2018; Bigelow et al., 2019). Therefore, having established that sound robustly modulates visual responses (Figure 2), we tested whether these observed changes were more accurately attributable to sound-induced movement. In an additional cohort of mice, we performed V1 extracellular recordings with the same audiovisual stimuli described above while recording movement activity of the mice throughout stimulus presentation. We found that sound did evoke whisking and locomotive behavior in mice, leading to increased movement on audiovisual trials compared to visual trials (Figure 3A; p=9.1e-5, paired t-test). However, there were many visual trials in which substantial movement occurred, as well as audiovisual trials in which little movement was detected (Figure 3B). Because of this large variability in sound-induced movement, we were able to control for movement when comparing visual and audiovisual activity in the recorded neurons.

Similar to above, we used a GLM to classify each neuron as light-, sound-, and/or motion-responsive based on the neuron's firing rate and mouse's movement activity during the onset (0-300ms) of the trial. The vast majority of light-responsive neurons, 71.1% (249/350), displayed both sound- and motion-modulated visual responses (Figure 3C). 11.1% (39/350) and 5.2% (18/350) of light-responsive neurons were purely sound- or motion-modulated, respectively. An additional 12.6% (44/350) were invariant to sound or motion. We then compared the visually and audiovisually evoked firing rates of neurons when controlling for movement. Among sound- and motion-modulated light-responsive neurons, the firing rate was higher on audiovisual trials than visual trials when movement was held constant (Figure 3D), especially when mice showed limited movement.

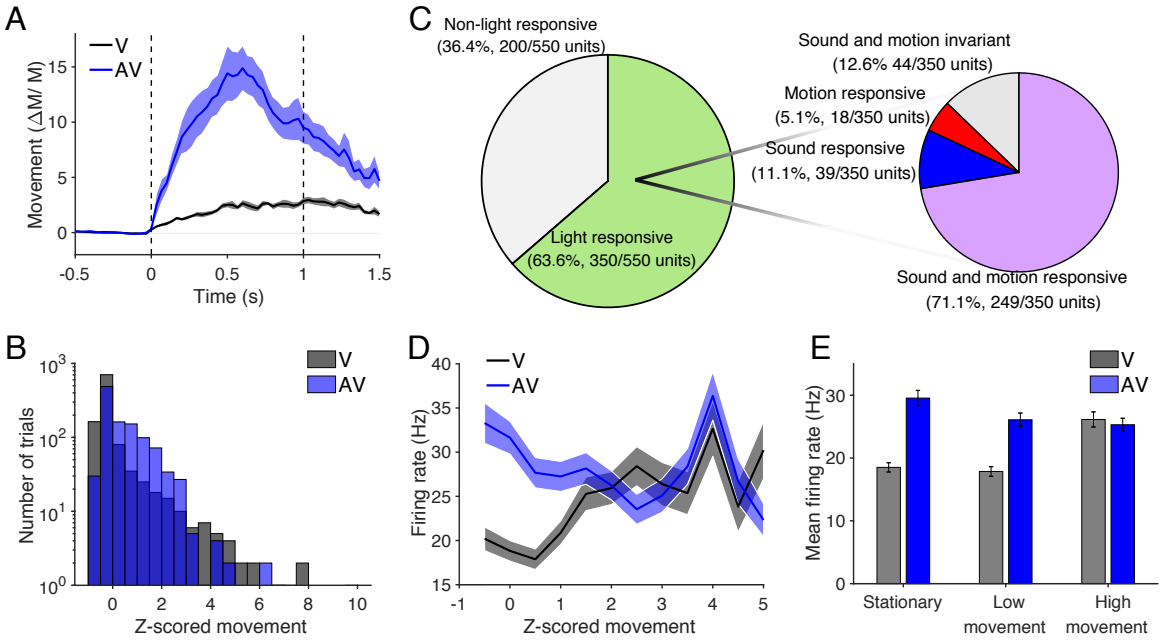


**Figure 3 | Sound modulates visual activity when controlling for stimulus-induced movement** (A) Mice displayed more movement response to audiovisual trials than in visual trials (n=9 recording sessions; p=9.1e-5, paired t-test). (B) Histogram of trials' z-scored movements show a range of levels of movement during both visual and audiovisual trials. (C) Venn diagram demonstrating that 87% of light-responsive neurons exhibited some combination of sound- and movement-responsiveness. (D) Comparison of firing rate of sound- and motion-modulated light-responsive neurons across trials with a range of z-scored movement. (E) Responses to audiovisual stimuli evoke larger magnitude responses than visual stimuli when mice were stationary (z-score<-0.5) or displayed low to moderate movement (-0.5<z-score<1.5), but responses were not significantly different when mice displayed the highest amount of movement (z-score>1.5; p(motion)=0.001, p(aud)=1.4e-13, p(interact)=1.8e-8, 2-way ANOVA, post hoc Bonferroni-corrected two-sample t-test)

On trials in which the mice were largely stationary (z-score<-0.5, 43% of visual trials, 32% of audiovisual trials) or displayed moderate levels of movement (-0.5<z-score<1.5, 51% of visual trials, 57% of audiovisual trials), the mean firing rate of neurons was 54-62% higher when sound was presented than when sound was absent. The firing rates under the two stimulus conditions converged on trials in which the mice displayed high movement activity (z-score>1.5, 4.8% of visual trials, 11% of audiovisual trials; Figure 3D,E; p(move)=0.010, p(aud)=1.4e=13, p(interact)=1.8e-8, unbalanced 2-way ANOVA; $p_{stationary}$=1.5e-14, $p_{low\ motion}$=7.1e-10, $p_{high\ motion}$=0.6, post hoc Bonferroni-corrected two-sample t-test, Table 1). Notably, increasing movement activity was correlated with increased firing rates on visual trials, but was correlated with decreasing firing rates among audiovisual trials (Figure 3E). These results indicate that sound modulated visually evoked neuronal activity even when accounting for sound-induced movement in awake mice.

**Sound and movement have distinct and complementary effects on visual responses**

To further parse out the role of sound and movement on audiovisual responses, we used a separate GLM to capture the time course of these parameters' effects on visual activity. For each neuron, we used a GLM with a sliding 10ms window to reconstruct the PSTH based on the visual contrast level, sound presence, and movement during that time window (Figure 4A). Figure 4B shows an example neuron in which the GLM accurately captures the light-evoked, sound-evoked, and audiovisually evoked PSTHs using the average movement for each trial type. Across neurons, the GLM-estimated PSTHs accurately reconstructed observed PSTHs, with the highest

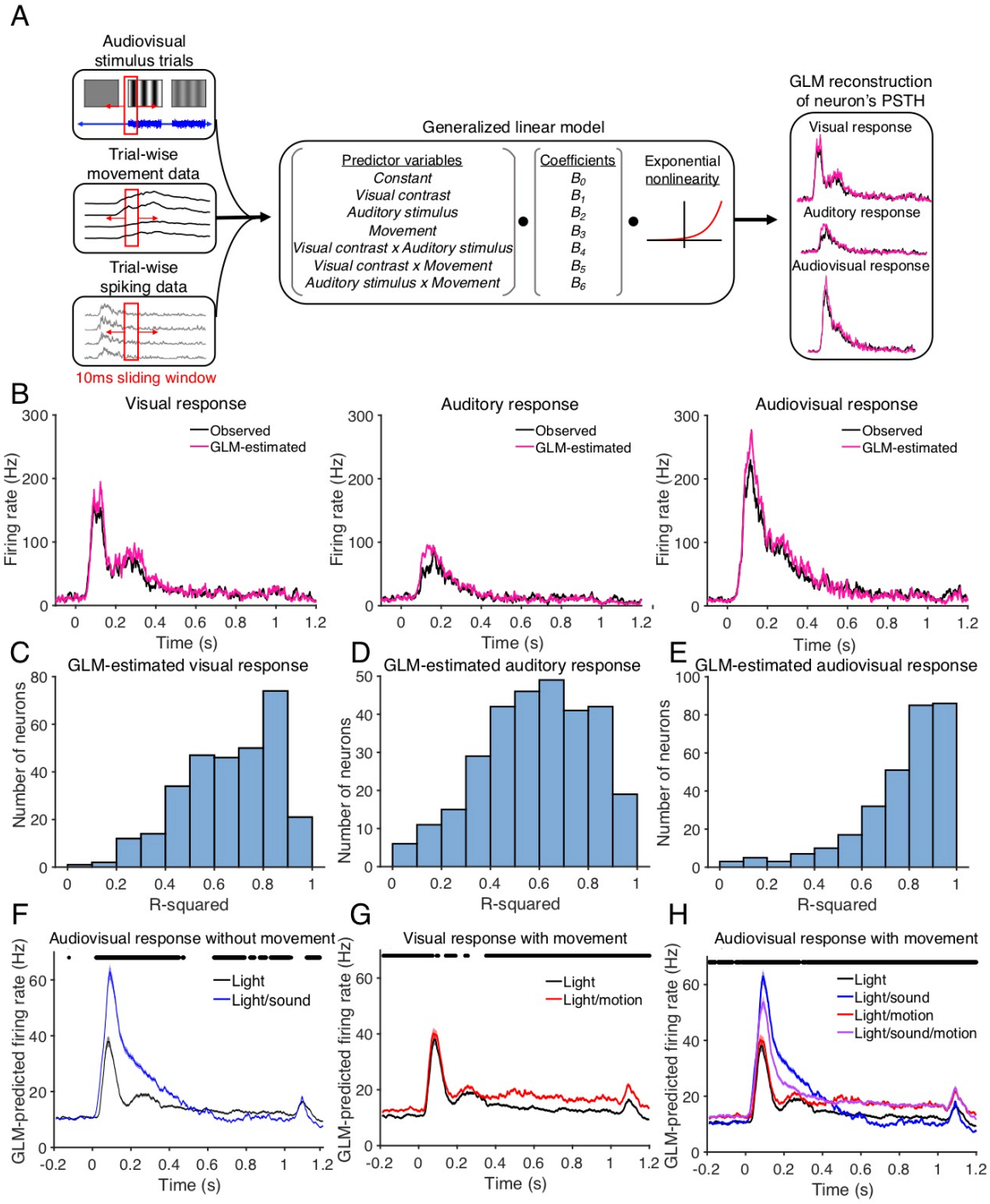


**Figure 4 | Sound and movement modulate visual responses in distinct but complementary ways** (A) Diagram illustrating the use of a GLM to reconstruct individual neurons' PSTHs based on neuronal responses and mouse movement during stimulus presentation. The GLM was then used to predict the time course of neuronal responses audiovisual stimuli with and without movement. (B) Observed trial-averaged PSTHs for visual-only (left), auditory-only (middle), and audiovisual (right) trials overlaid with GLM estimates based on the selected stimulus features. (C-E) Histograms demonstrating $R^2$ values of the GLM-estimated PSTHs, averaged across sound- and motion-modulated light-responsive neurons. Moderate to high $R^2$ values across the population indicate a good ability for the GLM to estimate neuronal firing rates. (F-H) GLM-predicted visually evoked PSTHs with and without sound and motion. Asterisks indicate time windows in which there was a significant difference between the *light* prediction and the

*light+sound*, *light+motion*, and *light+sound+motion* predictions, respectively. (F) Excluding motion highlights that sound primarily enhances the onset response. Asterisks indicate time windows in which there was a significant difference (n=295 fitted neurons; paired t-test, $\alpha$=3.6e-5). (G) Excluding sound highlights that motion primarily enhances the sustained portion of the response. Asterisks indicate time windows in which there was a significant difference (n=295 fitted neurons; paired t-test, $\alpha$=3.6e-5). (H) Sound and motion together enhance both the onset and sustained periods of the visually evoked response. (n=295 fitted neurons; paired t-test, $\alpha$=3.6e-5).

correlation when all parameters were included in the estimate (Figure 4C-E). We leveraged the coefficients fit to each neuron (Figure 4A) to estimate the unique contribution of each predictor to the firing rates as a function of time (see Materials and Methods). In the absence of movement, sound predominantly enhanced neuronal activity at the onset of the visual response and suppressed activity during the response's sustained period (Figure 4F; n=295 fitted neurons, paired t-test at each time window [1391], $\alpha$=3.6e-5). Conversely, movement had little effect on the onset activity in the absence of sound, but rather enhanced firing rates during the response's sustained period (Figure 4G; n=295 fitted neurons, paired t-test at each time window [1391], $\alpha$=3.6e-5). Together, sound and movement have complementary effects in which both the onset and sustained portions of the visual response are enhanced (Figure 4H; n=295 fitted neurons, paired t-test at each time window [1391], $\alpha$=3.6e-5). Again notably, the peak onset response under the audiovisual condition was lower when movement was included in the estimate (Figure 4H). These findings indicate not only that movement is unable to account for the changes in onset response reported above, but also that sound and motion have distinct and complementary effects on the time course of visually evoked activity in V1.

**Decoding of the visual stimulus from individual neurons is improved with sound**

Behaviorally, sound can improve the detection and discriminability of visual responses, however whether that improved visual acuity is reflected in V1 audiovisual responses is unknown. Despite many studies reporting neuronal correlates of audiovisual integration in V1, whether sound improves neuronal encoding of the visual stimulus has yet to be demonstrated. The increase in response magnitude and decrease in CV suggest that sound may improve visual stimulus discriminability in individual V1 neurons. Consistent with these changes in response magnitude and variability, we observed sound-induced improvements in the *d'* sensitivity index between responses to low contrast drifting grating directions among orientation- and direction-selective neurons (Fig 5 Sup 1), further indicating improved orientation and directional discriminability in individual neurons. To directly test this hypothesis, we used the neuronal responses of individual neurons to estimate the visual stimulus drifting grating orientation and direction. We trained a maximum likelihood estimate (MLE)-based decoder (Montijn et al., 2014; Meijer et al., 2017) on trials from the preferred and orthogonal orientations in orientation-selective neurons and on trials from the preferred and anti-preferred directions in direction-selective neurons. We used leave-one-out cross-validation and cycled the probe trial through the repeated trials of the stimulus condition in order to calculate the mean decoding performance. The MLE decoder's output was the orientation or direction with the maximum posterior likelihood based on the training data (Figure 5A). This decoding technique achieves high decoding accuracy (Figure 5B). When averaged across sound-modulated orientation-selective neurons, decoding performance was improved on audiovisual trials compared to visual trials (Figure 5C; p(vis)=4.8e-112, p(aud)=7.8e-4, p(interact)=0.71, paired 2-way ANOVA), with the greatest improvements at low to intermediate contrast levels (Figure 5D). We applied this approach to sound-modulated direction-selective units and found similar trends towards improvements at low contrast levels (Figure 5E,F; p(vis)=2.1e-4, p(aud)=0.18, p(interact)=0.78, paired 2-way ANOVA), limited by fewer and weaker direction-selective neurons in V1. These results demonstrate that sound-induced changes in

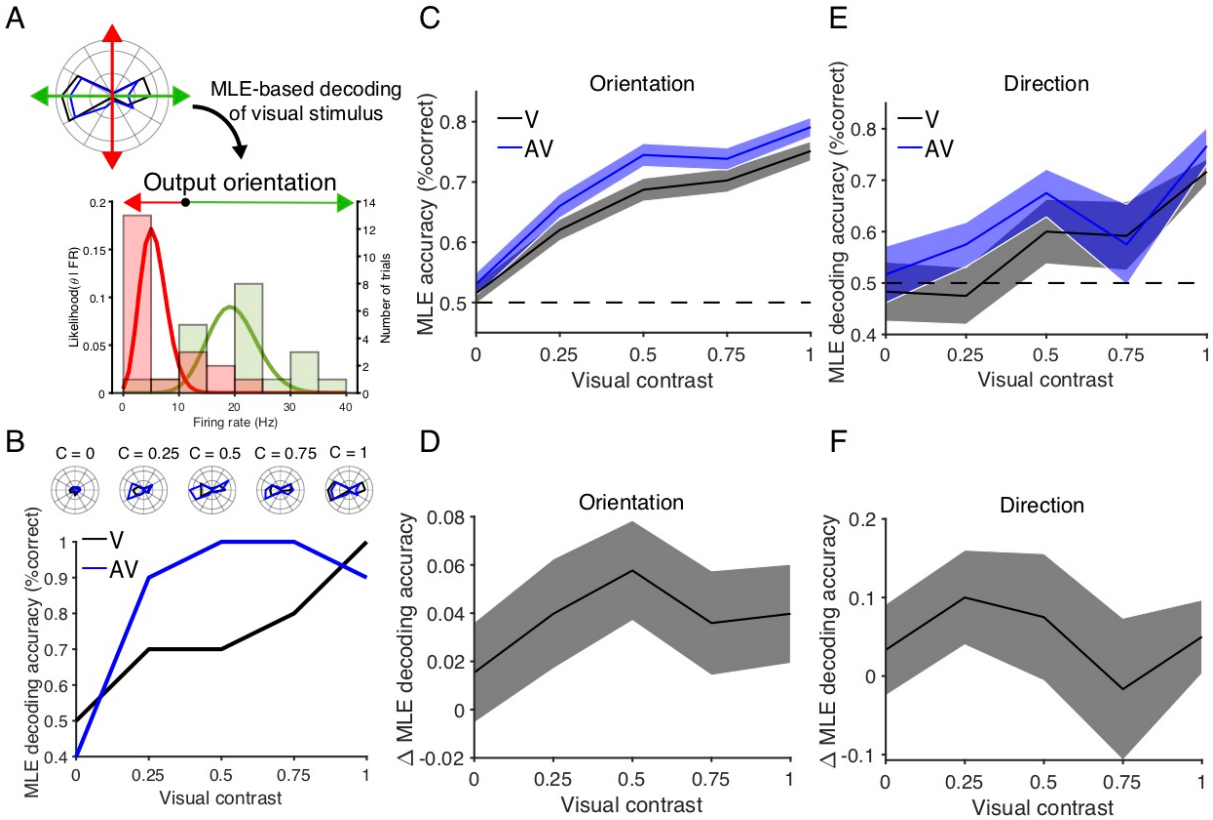


**Figure 5 | Sound improves decoding of drifting grating direction and orientation in individual neurons** (A) Diagram illustrating MLE-based decoding of an individual neuron's preferred versus orthogonal orientations. (B) Performance of the MLE decoder, trained on an example orientation-selective neuron, in decoding the neuron's preferred versus orthogonal orientations. The neuron's polar plots are shows in the above inset. (C-D) Absolute (C) and difference (D) in decoding accuracy of preferred versus orthogonal orientations, averaged across sound-modulated orientation-selective neurons, demonstrating higher performance in the audiovisual condition (n=78, p(vis)=4.8e-112, p(aud)=7.8e-4, p(interact)=0.71, paired 2-way ANOVA). (E-F) Absolute (E) and difference (F) in decoding accuracy of preferred versus anti-preferred directions, averaged across sound-modulated direction-selective neurons. No significant effect of sound on decoding accuracy was observed (n=12, p(vis)=2.1e-4, p(aud)=0.18, p(interact)=0.78, paired 2-way ANOVA).

response magnitude and consistency interact in order to improve neuronal representation of the visual stimulus in individual neurons.

## Population-based decoding of the visual stimulus improves with sound

V1 uses population coding to relay information about the various stimulus dimensions to downstream visual areas (Montijn et al., 2014, Berens et al., 2012), so we next tested whether these improvements in visual stimulus encoding in individual neurons extended to the population level. We began by training a support vector machine (SVM) to perform pairwise classification of visual drifting grating directions based on neuronal population activity. We again used a leave-one-out cross-validation approach when training and testing the SVM (Figure 6A). Decoding accuracy improved as more neurons were included in the population (Fig 6 Sup 1A), achieving an accuracy of ~90% when averaged across all pairwise orientation comparisons. At full visual contrast, there was little difference between the performance on visual and audiovisual trials.

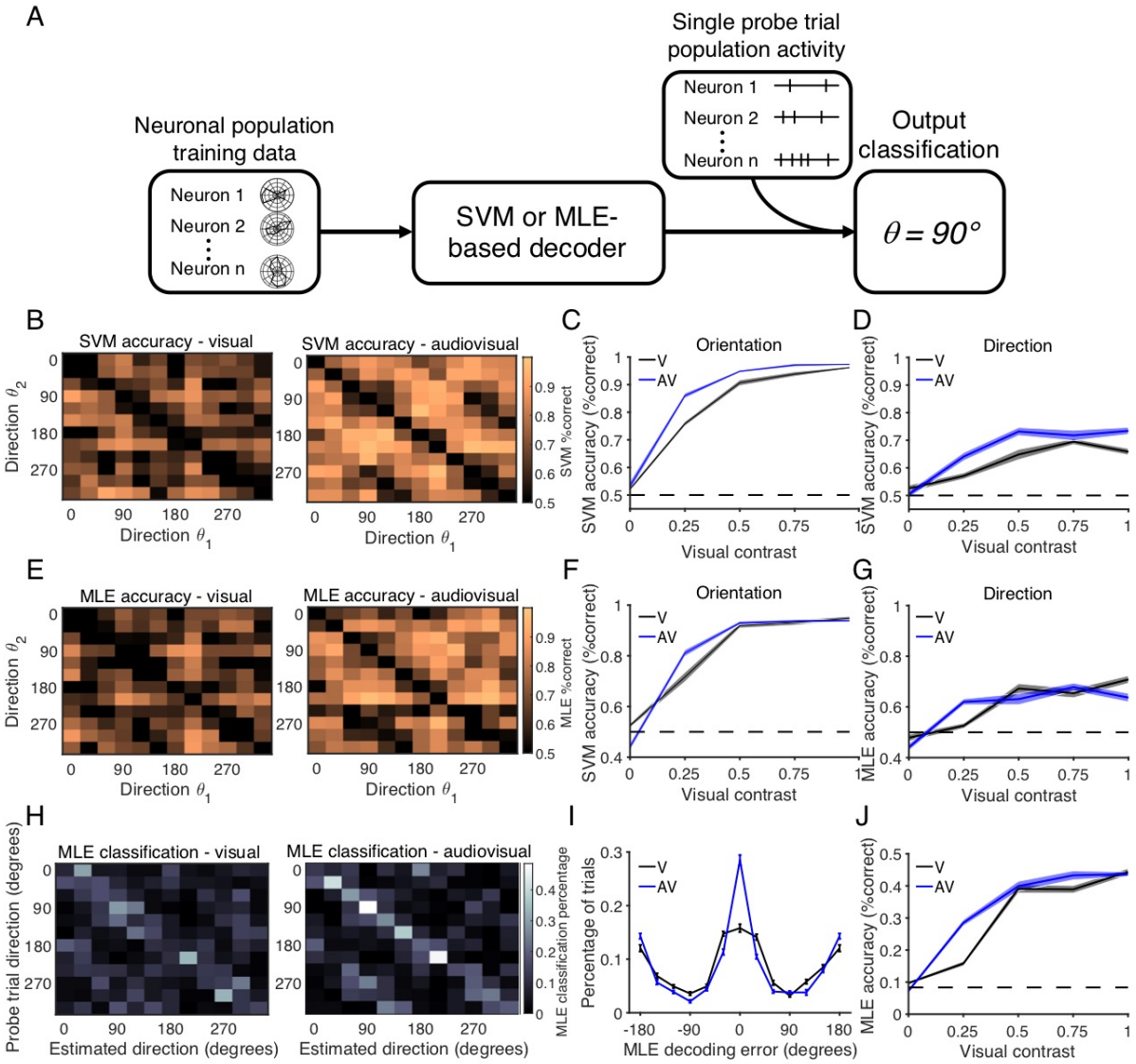


**Figure 6 | Sound improves accuracy of population-based visual stimulus decoding** (A) Schematic illustrating the decoding of the drifting grating direction using either an SVM or MLE decoder trained on neuronal population activity. (B) Accuracy of SVM pairwise classification of drifting grating directions on visual (left) and audiovisual (right) trials, contrast 0.25. (C) SVM decoding accuracy improved with sound when classifying orthogonal drifting grating orientations (n=10 randomizations, p(vis)=1.8e-61, p(aud)=1.9e-8, p(interact)=2.4e-4, 2-way ANOVA, post hoc Bonferroni-corrected paired t-test). (D) SVM decoding accuracy when classifying opposite drifting grating directions, demonstrating improved performance with sound (n=10 randomizations, p(vis)=1.1e-21, p(aud)=9.0e-9, p(interact)=0.0019, 2-way ANOVA, post hoc Bonferroni-corrected paired t-test). (E) Accuracy of MLE pairwise classification of drifting gratings on visual (left) and audiovisual (right) trials, contrast 0.25. (F) MLE decoding accuracy when classifying orthogonal drifting grating orientations improved with sound (n=10 randomizations, p(vis)=2.3e-66, p(aud)=0.61, p(interact)=9.6e-11, 2-way ANOVA, post hoc Bonferroni-corrected paired t-test). (G) MLE decoding accuracy when classifying opposite drifting grating directions, demonstrating less effect of sound on performance (n=10 randomizations, p(vis)=4.6e-26, p(aud)=0.51, p(interact)=4.1e-6, 2-way ANOVA, post hoc Bonferroni-corrected paired t-test). (H) Heat map of actual vs MLE-output directions under visual (left) and audiovisual (right) trials, contrast 0.25. MLE decoder could choose between all 12 drifting grating directions. (I) MLE decoder classification percentage, comparing estimated direction to actual direction. (J) Overall decoding accuracy of MLE decoder when choosing between all 12 drifting grating directions improved with sound (n=20 randomizations, p(vis)=2.2e-92, p(aud)=1.9e-5, p(interact)=2.7e-11, 2-way ANOVA, post hoc Bonferroni-corrected paired t-test).

However, at low to intermediate visual contrast levels, classification performance robustly increased on audiovisual trials as compared to visual trials (Figure 6B). This improvement in performance was greatest when comparing orthogonal drifting grating orientations (Figure 6C; p(vis)=1.8e-61, p(aud)=1.9e-8, p(interact) = 2.4e-4, 2-way ANOVA; $p_{c=0}$=0.12, $p_{c=0.25}$=0.0016, $p_{c=0.5}$,=0.0014, $p_{c=0.75}$=0.0023; $p_{c=1}$=1, post hoc Bonferroni-corrected paired t-test, Table 1). However, a similar improvement was also observed in decoding opposite drifting grating directions (Figure 6D, p(vis)=1.1e-21, p(aud)=9.0e-9, p(interact)=0.0019, 2-way ANOVA; $p_{c=0}$=0.55, $p_{c=0.25}$=5.3e-5, $p_{c=0.5}$=0.0036, $p_{c=0.75}$=0.17, $p_{c=1}$=0.0036, post hoc Bonferroni-corrected paired t-test, Table 1). These results indicate that sound improves neuronal population encoding of grating orientation and drift direction.

Similar performance levels were also observed when decoding drifting grating orientation and direction using an MLE-based population decoder, indicating that the results were not specific to the decoding algorithm. Again, performance improved with increasing population sizes (Fig 6 Sup 1B), and accuracy was higher on audiovisual trials than visual trials (Figure 6E-G; orientation: p(vis)=2.3e-66, p(aud)=0.61, p(interact)=9.6e-11, 2-way ANOVA; $p_{c=0}$-5.8e-4, $p_{c=0.25}$=1.8e-4, $p_{c=0.5}$=0.3, $p_{c=0.75}$=0.53, $p_{c=1}$=0.15, post hoc Bonferroni-corrected paired t-test, Table 1; direction: p(vis)=4.6e-26, p(aud)=0.51, p(interact)=4.1e-6, 2-way ANOVA; $p_{c=0}$=0.037, $p_{c=0.25}$=6.4e-6, $p_{c=0.5}$=0.036, $p_{c=0.75}$-0.16, $p_{c=1}$=0.14, post hoc Bonferroni-corrected paired t-test, Table 1).

Expanding on the SVM approach, the MLE-based decoder allowed us to perform not only pairwise classification, but also classification of 1 out of all 12 drifting grating directions. When trained and tested in this fashion, MLE decoding performance again improved at low to intermediate contrast levels on audiovisual trials (Figure 6H-I), before reaching asymptotic performance of ~45% at full visual contrast (Figure 6J; p(vis)=2.2e-92, p(aud)=1.9e-5, p(interact)=2.7e-11, 2-way ANOVA; $p_{c=0}$=0.012, $p_{c=0.25}$=1.4e=10, $p_{c=0.5}$=0.48, $p_{c=0.75}$=0.0013, $p_{c=1}$=0.5, post hoc Bonferroni-corrected paired t-test, Table 1). Taken together, these results indicate that sound improves neuronal encoding of the visual stimulus both in individual neurons and at a population level, especially at intermediate visual contrast levels.

**Sound improves stimulus decoding when controlling for sound-induced movements**

It is known that locomotion improves visual processing in V1 (Dardalat and Stryker, 2017). We next tested whether the sound-induced improvement in visual stimulus representation (Figure 6) was attributable to sound's effect on visual responses or indirectly via sound-induced movement. We observed previously that sound was primarily responsible for enhancing the visual response onset, whereas motion enhanced the sustained portion (Figure 4). We therefore hypothesized that the improvement on MLE decoding performance, based on the visual response onset, would be present even when accounting for sound-induced uninstructed movements. We tested this hypothesis by expanding on the GLM-based classification of neurons described in Figure 3. Using the same GLM generated for each neuron, we modified the movement variable and its corresponding pairwise predictors to the lowest observed value, and then used the GLM coefficients and the exponential nonlinearity to estimate each neuron's audiovisual response magnitude when regressing out the effect of motion (Figure 7A, Materials and Methods). We then input these estimated trial-wise neuronal responses into the same MLE-based decoder described above. Using this approach, we found that in individual orientation-selective neurons, controlling for the effect of motion on audiovisual trials minimally changed the accuracy of the population decoder across contrast levels (Figure 7B-C; p(vis)=7.7e-93, p(aud)=0.055, p(interact)=0.058, paired 2-way ANOVA, Table 1). However, regressing out both sound and motion from the audiovisual responses resulted in decoding accuracy that resembled that on visual trials (Figure 7B-C; p(vis)=8.1e-95, p(aud) = 0.55, p(interact)=0.24, paired 2-way ANOVA, Table 1). These results in individual neurons indicate that sound and not movement primarily drives the

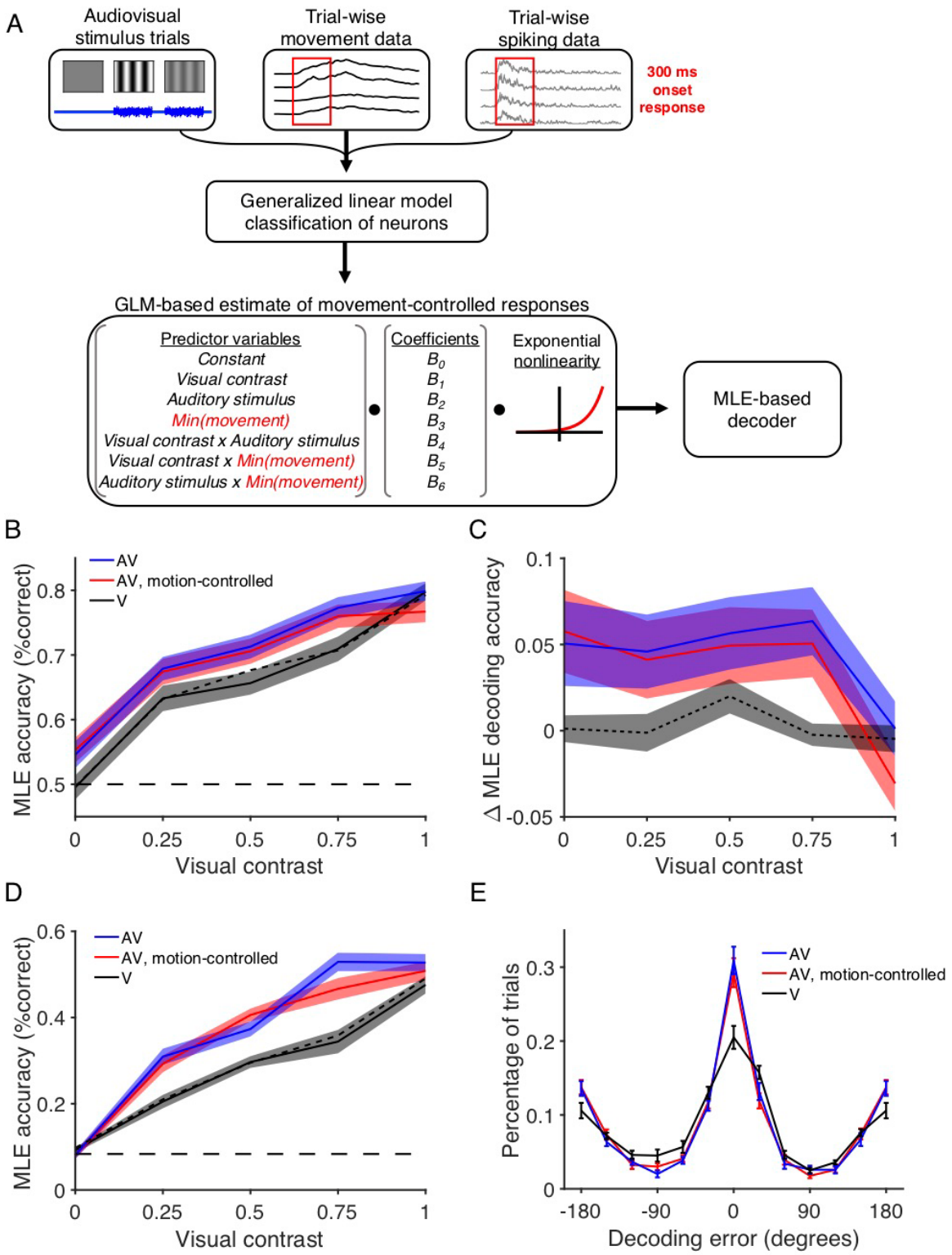


**Figure 7 | Sound improved decoding performance when controlling for motion.** (A) Diagram illustrating the use of a GLM to calculate each predictor variable's coefficient. These are then used when varying the predictor variables to estimate trial-wise neuronal responses, which are then into the MLE-based decoder. (B) Absolute accuracy of decoding orientation among orientation-selective, sound/motion-modulated light-responsive neurons, comparing visual responses (black, solid) to audiovisual responses (blue) and audiovisual responses when regressing out motion (red). The finely dotted line represents audiovisual responses when controlling for the effects of both motion and

sound. (C) Relative decoding accuracy compared to decoding on visual trials. Regressing out motion did not reduce performance compared to audiovisual trials (n=85 neurons, p(vis)=7.7e-93, p(aud)=0.055, p(interact)=0.058, paired 2-way ANOVA), whereas regressing out both motion and sound resulted in comparable performance to visual trials (n=85 neurons, p(vis)=8.1e-95, p(aud)=0.55, p(interact)=0.24, paired 2-way ANOVA). (D) Population decoding accuracy of population-based decoder on audiovisual trials (blue) is preserved even when controlling for motion (red) compared to decoding of visual trials (black; n=10 randomizations, p(vis) = 1.4e-38, p(aud)=6.0e-8, p(interact)=0.0015, 2-way ANOVA; $p_{c=0}$=0.30, $p_{c=0.25}$0.0012, $p_{c=0.5}$=0.0022, $p_{c=0.75}$=0.0044, $p_{c=1}$=0.35, Bonferroni-corrected paired t-test). The finely black dotted line represents decoding accuracy when regressing out both sound and motion. (E) MLE decoder classification percentage, comparing estimated direction to actual direction, contrast 0.25. Little difference is observed between audiovisual trials and audiovisual trials when controlling for motion, whereas both are more accurate than visual trials.

improvements in decoding accuracy in audiovisual trials. We found similar results when implementing this approach in the MLE-based population decoder. We again found that that decoding performance on audiovisual trials when regressing out motion was still significantly improved compared to that on visual trials (Figure 7D-E; p(vis)=1.4e-38, p(aud)=6.0e-8, p(interact)=0.0015, 2-way ANOVA; $p_{c=0}$=0.30, $p_{c=0.25}$=0.0012, $p_{c=0.5}$=0.0022, $p_{c=0.75}$=0.0044, $p_{c=1}$=0.35, Bonferroni-corrected paired t-test). Furthermore, regression of both sound and movement from audiovisual trials resulted in population decoding performance similar to that on visual trials (Figure 7D-E; p(vis)=2.5e-39, p(aud)=0.48, p(interact)=0.99, 2-way ANOVA). These results demonstrate that at both an individual neuron and population level, sound improves visual stimulus decoding on audiovisual trials even when controlling for sound-induced motion.

## Discussion

Audiovisual integration is an essential aspect of sensory processing (Stein et al., 2020). In humans, audiovisual integration is used in everyday behaviors such as speech perception and object recognition (Fujisaki et al., 2014). In animal models, audiovisual integration improves the detection and discriminability of unisensory auditory and visual stimuli (Gleiss and Kayser, 2012; Meijer et al., 2018). However, the neuronal mechanisms underlying these behavioral improvements are still being revealed. Specifically, it remains unclear how sound-induced changes in neuronal activity affect encoding of the visual stimulus. Furthermore, whether the reported audiovisual integration can more accurately be attributed to sound-induced movement has yet to be studied.

The goal of the present study was to test the hypothesis that sound improves neuronal encoding of visual stimuli in V1 independent of sound-induced movement. We performed extracellular recordings in V1 while presenting combinations of visual drifting gratings and auditory white noise and recording movement of awake mice. The drifting gratings were presented at a range of visual contrast levels to determine the threshold levels at which sound is most effective. As in previous studies, we found neurons in V1 whose spontaneous and visually evoked firing rates are modulated by sound (Figure 2). Notably, the effects we observed were stronger and more positive than in previous studies (80.1% of neurons were modulated by sound, with ~95% exhibiting sound-induced increases in firing rate). When accounting for movement in awake animal subjects, we found that the neurons' audiovisual responses actually represented a mixed effect of both sound- and movement-sensitivity (Figure 3), an effect in which sound primarily enhances the onset response whereas movement complementarily enhances the sustained response (Figure 4). We also found that sound-induced changes in response magnitude and consistency combined to improve the discriminability of drifting grating orientation and direction in individual neurons and

at a population level (Figure 5,6). The improvements in neuronal encoding were most pronounced at low to intermediate visual contrast levels, a finding that supports the current understanding that audiovisual integration is most beneficial for behavioral performance under ambiguous unisensory conditions (Gleiss and Kayser, 2012; Meijer et al., 2018; Stein et al., 2020). Importantly, the improvement in neuronal encoding was based on firing at the onset of the visual response, indicating that the auditory signal itself is responsible for improvements in visual encoding and not attributable to uninstructed movements. This was directly demonstrated by the persistence of sound-induced improvements in stimulus decoding, even when controlling for the effect of motion (Figure 7).

**Auditory and locomotive inputs distinctly shape visual responses**

We present the novel finding that sound and movement have distinct and complementary effects on visual response. Specifically, we found that sound primarily enhances the firing rate at the onset of the visual response, whereas motion enhances the firing rate during the sustained period of the visual response (Figure 4F-H). Our initial classification of sound-modulated neurons and the subsequent decoding analyses were based on firing rates during the onset period. Therefore, despite robust differences in movement during visual and audiovisual trials, motion was unable to account for the sound-induced changes in neuronal responses that resulted in improved neuronal encoding (Figure 7). The distinct effects that sound and locomotion have on visual responses also adds nuance to our understanding of how motion affects visual processing, as other groups have predominantly used responses averaged across the duration of the stimulus presentation in categorizing motion responsive neurons in V1 (Neil and Stryker, 2010; Dardalat and Stryker, 2017). Our findings indicate that the timing of cross-sensory interactions is an important factor in the classification and quantification of multisensory effects.

We also observed that motion decreases the magnitude of the enhancing effect that sound has on the onset of the visual response (Figure 3E, 4H). This finding suggests a degree of suppressive effect that motion has on this audiovisual interaction. A potential mechanism for this result may relate to the circuits underlying audiovisual integration in V1. Other groups have shown using retrograde tracing, optogenetics and pharmacology that the AC projects directly to V1 and is responsible for the auditory signal in this region (Falchier et al., 2002; Ibrahim et al., 2016; Deneux et al., 2019). It is currently understood that unlike in V1, in other primary sensory cortical areas including the AC movement suppresses sensory evoked activity (Nelson et al., 2013; Schneider and Mooney, 2018; Bigelow et al., 2019). Therefore, one explanation for this observation is that despite motion enhancing the visual response magnitude in the absence of sound, the suppressive effect that motion has on sound-evoked responses in the AC leads to weaker AC enhancement of visual activity on trials in which the mice move. A detailed experimental approach using optogenetics or pharmacology would be required to test this hypothesis of a tripartite interaction and would also reveal the potential contribution of other auditory regions.

**Enhanced response magnitude and consistency combine to improve neuronal encoding**

Signal detection theory indicates that improved encoding can be mediated both by enhanced signal magnitude as well as reduced levels of noise (von Trapp et al., 2016). When using purely magnitude-based metrics of discriminability, OSI and DSI, we found a small reduction from the visual to audiovisual conditions (Fig 2 Sup 1). However, we also observed that sound reduced the CV of visual responses (Fig 2 Sup 2), a measure of the trial-to-trial variability in response. When we measured the *d'* sensitivity index of neuronal responses, a measure that factors in both the response magnitude and distribution, we found that sound improved the discriminability of drifting grating orientation and direction (Fig 4 Sup 1). These findings indicate that the improved discriminability of visual responses in individual neurons was mediated not only by changes in response magnitude but also by the associated improvement in response consistency between

trials. Therefore, it is important to consider response variability in addition to magnitude-based metrics when quantifying tuning and discriminability in neurons (Churchland et al., 2011).

Prior studies using calcium imaging found equivocal results when investigating whether sound-induced changes in visual responses led to improved population encoding of the visual stimulus (Meijer et al., 2017). The improved discriminability of grating orientation and direction by individual neurons supports our finding that the presence of sound enhances population encoding of the visual stimulus. One explanation for this difference may be the recording modality and analysis parameters. We performed electrophysiological recordings of spiking activity and limited our quantification to the onset of the stimulus (0-300 ms), the time window in which there was the greatest change in firing rate across neurons. Calcium imaging, on the other hand, may lack the temporal resolution required to detect the trial-by-trial differences in spiking activity associated with improved neuronal discriminability. Additionally, extracellular electrophysiology allowed us to take advantage of large numbers of neurons in awake animals to include in the population analysis, as opposed to patch-clamp approaches with a limited number of neurons (Ibrahim et al., 2016). Finally, presenting a wide range of visual contrast levels allowed use to demonstrate that sound improves neuronal encoding at low to intermediate contrasts, above which further improvement is difficult to demonstrate due to already reliable encoding in the absence of sound.

**Stimulus parameters relevant to audiovisual integration**

Sensory neurons are often tuned to specific features of unisensory auditory and visual stimuli, and these features are relevant to cross-sensory integration of the signals. In the current study we paired the visual drifting gratings with a static burst of auditory white noise as a basic well-controlled stimulus. Previous studies found that temporally congruent audiovisual stimuli, e.g. amplitude-modulated sounds accompanying visual drifting gratings, evoke larger changes in response than temporally incongruent stimuli in the mouse visual cortex (Meijer et al., 2017), and therefore using such stimuli would potentially result in even stronger effects than we observed. Auditory pure tones can also induce changes in V1 visual responses (McClure and Polack, 2019). However, in other brain regions such as the inferior colliculus, audiovisual integration is highly dependent on spatial congruency between the unimodal inputs (Bergan and Knudsen, 2009). Our results show that spatially congruent, static white noise is sufficient to improve the neuronal response magnitude and latency to light-evoked response. However, additional studies are needed to explore the full range of auditory stimulus parameters relevant to visual responses in V1. Additionally, visual drifting gratings are often used to evoke robust responses in V1, but it would be valuable to determine whether sound is also capable of modulating responses to looming stimuli and more complex visual patterns as well.

**Neuronal correlates of multisensory behavior**

Our findings of multisensory improvements in neuronal performance are supported by numerous published behavioral studies in humans and various model organisms (Gleiss and Kayser, 2012; Meijer et al., 2018; Stein et al., 2020). Training mice to detect or discriminate audiovisual stimuli allows the generation of psychometric performance curves in the presence and absence of sound. We would hypothesize that the intermediate visual contrast levels in which we see improvements in neural encoding would align with behavioral detection threshold levels. One could also correlate the trial-by-trial neural decoding of the visual stimulus with the behavioral response on a stimulus discriminability task, an analysis that could provide information about the proximity of the V1 responses to the behavioral perception and decision. Additionally, a behavioral task could allow the comparison of neural responses between passive and active observing, helping to reveal the role of attention on how informative or distracting one stimulus is about the other.

### Multisensory integration in other systems

It is useful to contextualize audiovisual integration by considering multisensory integration that occurs in other primary sensory cortical areas. The auditory cortex contains visually responsive neurons and is capable of binding temporally congruent auditory and visual stimulus features in order to improve deviance detection within the auditory stimulus (Atilgan et al., 2018; Morrill and Hasenstaub, 2018). Additionally, in female mice, pup odors reshape AC neuronal responses to various auditory stimuli and drive pup retrieval behavior (Cohen et al., 2011; Marlin et al., 2015), demonstrating integration of auditory and olfactory signals. However, whether these forms of multisensory integration rest on similar coding principles of improved SNR observed in the current V1 study is unknown. Investigation into this relationship between the sensory cortical areas will help clarify the neuronal codes that support multisensory integration, and the similarities and differences across sensory domains.

### Acknowledgements

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## Materials and methods

### Mice

All experimental procedures were in accordance with NIH guidelines and approved by the IACUC at the University of Pennsylvania. Mice were acquired from Jackson Laboratories (5 male, 6 female, aged 10-18 weeks at time of recording; B6.Cast-*Cdh23*$^{Ahl+}$ mice [Stock No: 018399]) and were housed at 28°C in a room with a reversed light cycle and food provided ad libitum. Experiments were carried out during the dark period. Mice were housed individually after headplate implantation. Euthanasia was performed using $CO_2$, consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. All procedures were approved by the University of Pennsylvania IACUC and the AALAC Guide on Animal Research. We made every attempt to minimize the number of animals used and to reduce pain or discomfort.

### Surgical procedures

Mice were implanted with skull-attached headplates to allow head stabilization during recording, and skull-penetrating ground pins for electrical grounding during recording. The mice were anesthetized with 2.5% isoflurane. A ~1mm craniotomy was performed over the right frontal cortex, where we inserted a ground pin. A custom-made stainless steel headplate (eMachine Shop) was then placed on the skull at midline, and both the ground pin and headplate were fixed in place using C&B Metabond dental cement (Parkell). Mice were allowed to recover for 3 days post-surgery before any additional procedures took place.

### Electrophysiological recordings

All recordings were carried out inside a custom-built acoustic isolation booth. 1-2 weeks following the headplate and ground pin attachment surgery, we habituated the mice to the recording booth for increasing durations (5, 15, 30 minutes) over the course of 3 days. On the day of recording, mice were placed in the recording booth and anesthetized with 2.5% isoflurane. We then

performed a small craniotomy above the left primary visual cortex (V1, 2.5mm lateral of midline, 0-0.5 mm posterior of the lambdoid suture). Mice were then allowed adequate time to recover from anesthesia. Activity of neurons were recorded using a 32-channel silicon probe (NeuroNexus A1x32-Poly2-5mm-50s-177). The electrode was lowered into the primary visual cortex via a stereotactic instrument to a depth of 775-1000μm. Following the audiovisual stimulus presentation, electrophysiological data from all 32 channels were filtered between 600 and 6000 Hz, and spikes belonging to single neurons and multi-units were identified in a semi-automated manner using KiloSort2 (Pachitariu et al., 2016).

**Audiovisual stimuli**

The audiovisual stimuli were generated using MATLAB (MathWorks, USA), and presented to mice on a 12" LCD monitor (Eyoyo) and through a magnetic speaker (Tucker-Davis Technologies) placed to the right of the mouse. The visual stimulus was generated using the PsychToolBox package for MATLAB and consisted of square wave drifting gratings 1 s in duration, 4-Hz temporal frequency, and 0.1 cycles/°. The gratings moved in 12 directions, evenly spaced 0°-360°, and were scaled to a range of 5 different visual contrast levels (0, 0.25, 0.5, 0.75, 1), totaling 60 unique visual stimuli. The auditory stimulus was sampled at 400 kHz and consisted of a 1 s burst of 70 dB white noise. The visual grating was accompanied by the auditory noise on half of trials (120 unique trial types, 10 repeats each), with simultaneous onset and offset. The auditory-only condition corresponded to the trials with a visual contrast of 0. The trial order was randomized and was different for each recording.

**Data analysis and statistical procedures**

Spiking data from each recorded unit was organized by trial type and aligned to the trial onset. The number of spikes during each trial's first 0-300ms was input into a generalized linear model (GLM; predictor variables: visual contrast [continuous variable 0, 0.25, 0.5, 0.75 ,1], sound [0 or 1]; response variable: number of spikes during 0-300ms; Poisson distribution, log link function), allowing the classification of each neuron's responses as having a main effect ($p<0.05$) of light, sound, and/or a light-sound interaction. Neurons that were responsive to both light and sound or had a significant light-sound interaction term were classified as "light-responsive sound-modulated." To quantify the supra- or sub-linear integration of the auditory and visual responses, we calculated the linearity ratio of neurons' audiovisual responses. This ratio was defined as $FR_{AV}$ / ($FR_V$ + $FR_A$), and the sound-only response $FR_A$ was calculated using the trials with a visual contrast of 0.

We quantified changes in response timing by calculating response latency, onset slope, and onset response duration. First, mean peristimulus time histograms (PSTH) were constructed for each trial type using a 10 ms sliding window. The latency was calculated as the first time bin after stimulus onset in which the mean firing rate at full contrast exceeded 1 standard deviation above baseline. The slope Hz/ms slope was calculated from the trial onset to the time of the peak absolute value firing rate. The response duration was calculated using the full width at half maximum of the peak firing rate at stimulus onset (limited to 0-300 ms).

Orientation selectivity and direction selectivity were determined for all light-responsive neurons. The preferred direction of each direction-selective neuron was defined as the drifting grating direction that evoked the largest mean firing rate at the highest contrast level ($FR_{pref}$). We calculated orientation and direction-selective indices (Zhao et al., 2013) for each neuron according to:

$$\mathrm{OSI} = \frac{FR_{pref} - FR_{ortho}}{FR_{pref} + FR_{ortho}} \qquad \mathrm{DSI} = \frac{FR_{pref} - FR_{antipref}}{FR_{pref} + FR_{antipref}}$$

where $FR_{ortho}$ and $FR_{antipref}$ are the mean firing rates in the orthogonal (90°) and anti-preferred (180°) directions, respectively. One-tailed permutation testing was performed by comparing these OSI and DSI values to pseudo OSI and DSI values obtained by 200 random shuffles of the firing rates from the pooled preferred and orthogonal or anti-preferred trials. If a neuron's actual OSI or DSI value was >95% of shuffled OSI or DSI values, the neuron was classified as "orientation-" or "direction-selective," respectively. To determine whether there were statistically significant changes in the preferred direction from the visual to audiovisual conditions, we applied a bootstrapping procedure, subsampling the visual trials for each neuron 1000 times and creating a confidence interval of the mean shift in preferred direction (degrees) for each population randomization.

We assessed and controlled for sound-induced movement as a potential confound for the audiovisual effects observed. During a subset of V1 recordings (9 recordings, 5 mice), mouse movement was tracked throughout stimulus presentation. Video recording was performed using a Raspberry Pi 4 Model B computer system with an 8MP infrared Raspberry Pi NoIR Camera V2 attachment. The video was converted to MP4 format, and motion was quantified by calculating the frame-by-frame difference, an approach that captured both whisking and locomotive behavior. This movement value for each recording was then aligned to the trials of the audiovisual stimulus from the recording trials for further analysis.

Similar to above, a GLM (predictor variables: visual contrast level, sound presence, average motion during each trial; response variable: trial spikes during 0-300ms; Poisson distribution, log link function) classified each neuron as having a main effect ($p<0.05$) of light, sound, or motion, as well as the pairwise interactions of these parameters. Light-responsive sound-modulated neurons, according to the above definition, that additionally displayed either a main effect of motion or significant light-motion or sound-motion interaction terms were classified as "motion-modulated" and were included for further analysis.

In order to reconstruct peristimulus time histograms of light-responsive, sound-modulated, motion-modulated neurons, we used a separate GLM. Using a 10ms sliding window across all trials, we input the visual contrast level, sound presence, and motion during that window (discretized into five bins) as predictor variables, and the number of spikes during that window as response variables, into the GLM (Poisson distribution, log link function) to calculate coefficients for light, sound, motion, and their pairwise interactions. This approach allowed us to reconstruct the mean PSTH of individual neurons observed during each trial type by calculating:

$$\mathrm{Spikes}_t = \exp\left(\sum_i p_{t,i} \cdot c_{t,i}\right)$$

where the spikes in time window *t* are determined by the values *p* and coefficients *c* of predictor variable *i*. From there, we used this same equation to estimate the shape of the PSTHs when varying sound and motion in order to determine differential effects these parameters had on the temporal trajectory of neurons' visual responses.

The *d'* sensitivity index (Stanislaw and Todorov, 1999; von Trapp et al., 2016) was used to calculate the directional discriminability of direction-selective neurons. The *d'* sensitivity index between two directions $\theta_1$ and $\theta_2$ is calculated as:

$$d' = \frac{\mu_{\theta_1} - \mu_{\theta_2}}{\sqrt{\frac{1}{2}(\sigma_{\theta_1}^2 + \sigma_{\theta_2}^2)}}$$

where $\mu_\theta$ and $\sigma_\theta$ are the response mean and standard deviation, respectively, for direction $\theta$. For each neuron, the sensitivity index was calculated in a pairwise manner for preferred direction versus all other directions and then aligned relative to the preferred direction in order to test sensitivity index as a function of angular distance from preferred direction.

We used a maximum likelihood estimate approach (Montijn et al., 2014; Meijer et al., 2017) to decode the visual stimulus direction from the neuronal responses based on Bayes rule:

$$P(\theta|A_{trial}) = \frac{P(A_{trial}|\theta)P(\theta)}{P(A_{trial})}$$

For decoding using individual neurons, the likelihood $P(A_{trial}|\theta)$ for each orientation or direction was computed based on the Poisson response distribution across all trials of that orientation or direction, with a leave-one-out cross-validation technique in which the probe trial ($A_{trial}$) was excluded from the training data. The prior $P(\theta)$ was uniform, and the normalization term $P(A_{trial})$ was similarly applied to all directions. Therefore, the posterior probability $P(\theta|A_{trial})$ was proportional to and based on evaluating the likelihood function at the value of the probe trial. For orientation-selective neurons, decoding was performed between the preferred and orthogonal orientations, and for direction-selective neurons, decoding was performed between the preferred and anti-preferred directions. For decoding using populations of neurons, neurons were pooled across recording sessions. A similar approach was used; however, here, the posterior probability $P(\theta|A_{pop})$ was proportional to the joint likelihood $P(A_{pop}|\theta)$ of the single-trial activity across all N neurons in the population ($A_{pop}$):

$$P(A_{pop}|\theta) = \prod_{neuron\ i}^{N} P(A_{trial}|\theta)_i$$

With this population-based analysis, pairwise decoding was performed between every orientation and its orthogonal orientation (1 of 2 options), as well as decoding one direction from all possible directions (1 of 12 options).

Additionally, we used a support vector machine (SVM) to corroborate the findings of the MLE-based decoder. The SVM was implemented using MATLAB's fitcsvm function with a linear kernel to predict the drifting grating direction based on single-trial population responses. Similarly, a leave-one-out cross-validation technique was used, and pairwise decoding was performed between every combination of two stimulus directions.

**Statistics**

Figure data are displayed as means with standard error of the mean (SEM), unless otherwise noted. Shapiro-Wilk tests were used to assess normality, and the statistical tests performed are indicated in the text, figures, and Table 1. For multi-group and multivariate analysis (e.g., ANOVA and Kruskal-Wallis tests) in which a significant ($p<0.05$) interaction was detected, we subsequently performed a post hoc Bonferroni-corrected test. P-values reported as 0 are too

small to be accurately calculated by Matlab ($p<2.2e-301$), due to characteristically large data sets. See Table 1 for a detailed summary of statistical results and post hoc comparisons.

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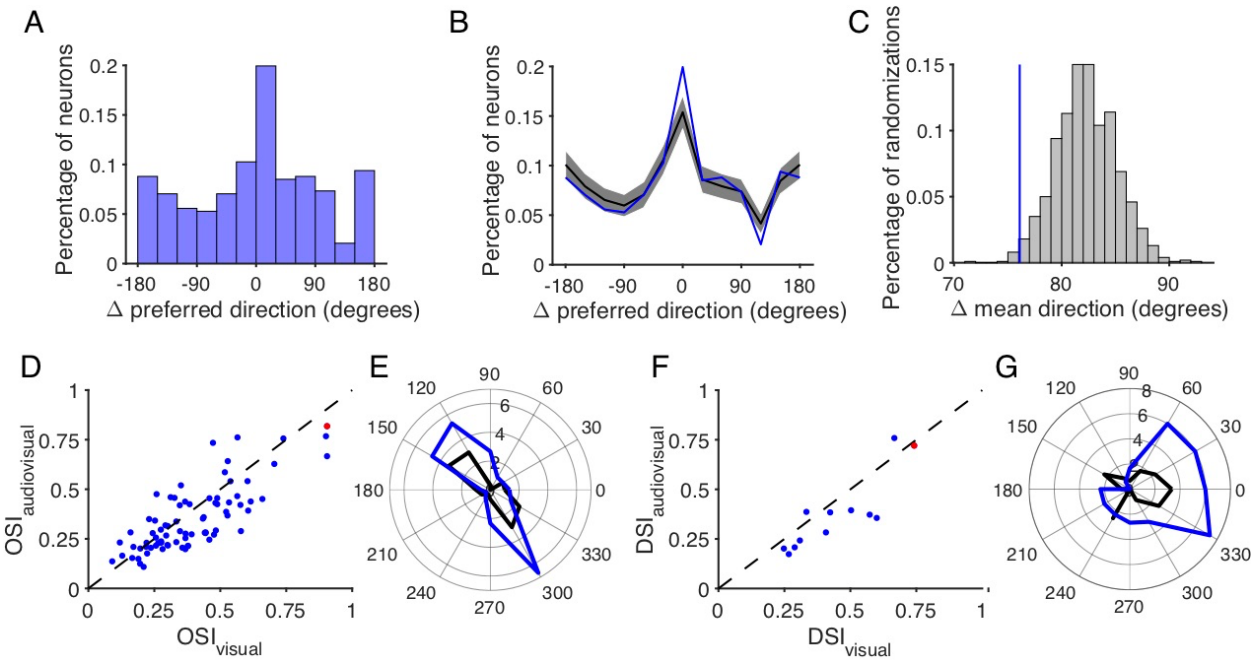


**Figure 2 Supplementary 1 | Sound minimally reduces tuning selectivity in individual neurons** (A) Histogram depiction of changes in preferred drifting grating directions with sound in orientation-selective neuron. (B) Observed changes in preferred direction (blue) compared to shuffled permutations (black) using the mean and standard deviation of observed responses. (C) The observed mean change in preferred direction (blue) is within the expected distribution (gray) based on visual response variability. (D,E) A slight reduction in the orientation selectivity index was observed in orientation-selective neurons (n=78, p=0.0018, paired t-test). The visual tuning of the red data point in *D* is displayed in *E*. (F,G) A slight reduction in the direction selectivity index was also observed in direction-selective neurons (n=12, p=0.021, paired t-test), with the tuning of the red data point in *F* displayed in *G*.

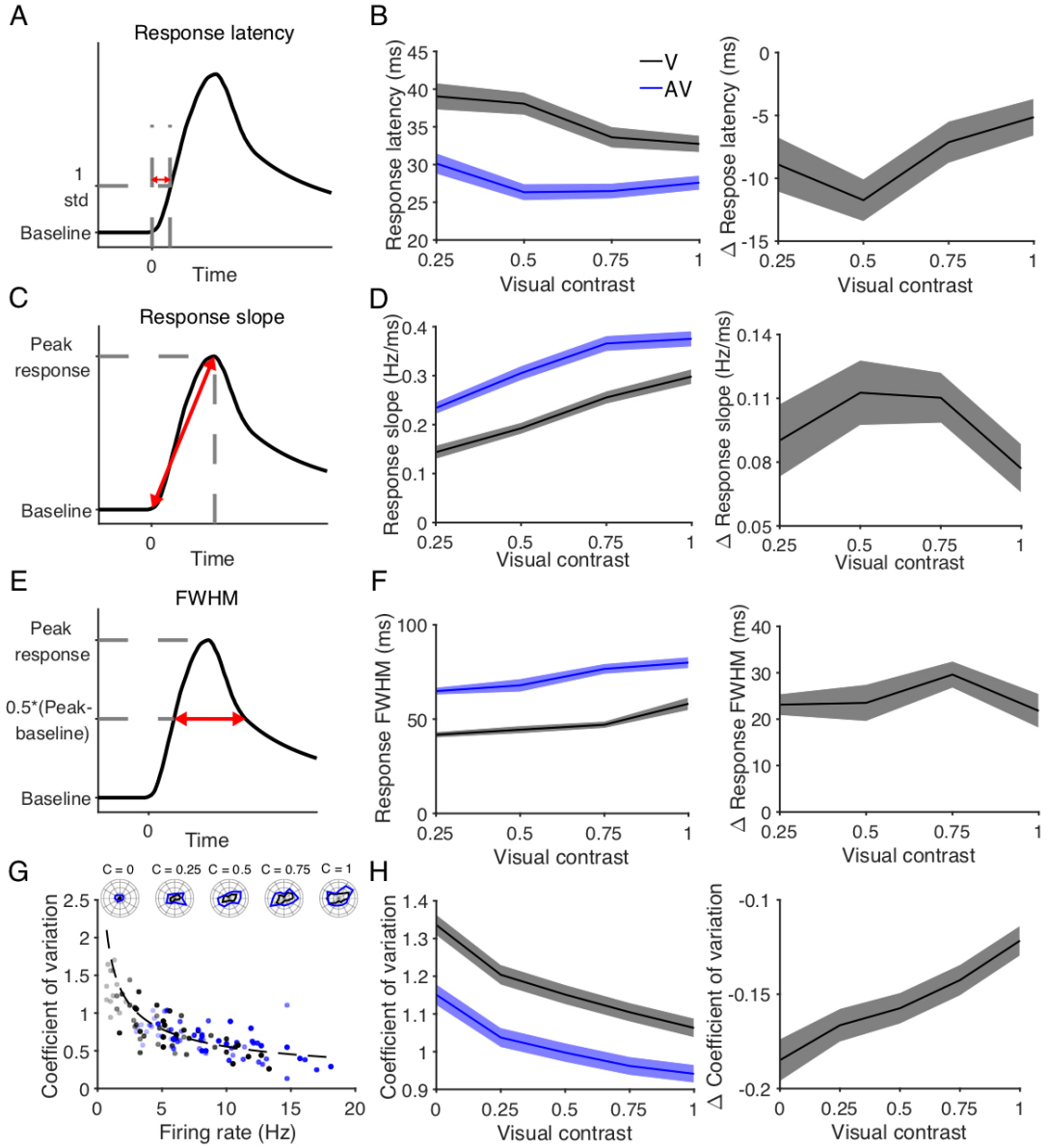


**Figure 2 Supplementary 2 | Sound reduces the latency, increases duration, and reduces variability of light-evoked responses in individual neurons** (A) Diagram of the calculation of response latency, the first time bin in which the FR exceeds 1 std above baseline. (B) Response latency is reduced by sound (left: absolute, right: difference; p(vis)=6.9e-4, p(aud)=6.8e-15, p(interact)=0.045, paired 2-way ANOVA, post hoc Bonferroni-corrected paired t-test, Table 1). (C) Diagram of the calculation of response onset slope, the peak change in FR over the latency to peak response. (D) Sound increases the slope of the onset response (left: absolute, right: difference; n=563, p(vis)=3.5e-121, p(aud)=2.7e-15, p(interact)=0.038, paired 2-way ANOVA, post hoc Bonferroni-corrected paired t-test). (E) Diagram of the calculation of FWHM, the width of the onset response at half maximum FR. (F) Sound increases the FWHM duration of the onset response (left: absolute, right: difference; n=367, p(vis)=1.3e-10, p(aud)=8.7e-98, p(interact)=0.23 paired 2-way ANOVA). (G) An example neuron demonstrating that increased response magnitude corresponds to lower CV according to an inverse square root relationship. The black and blue dots represent visual and audiovisual responses, respectively, and the dot transparency corresponds to visual contrast level. The dotted lines are fitted y=c/sqrt(x) curves, where c is a constant. The above inset is the polar plots corresponding to the example neuron. (H) Lower coefficient of variation indicates reduced response variability in audiovisual compared to visual responses (left: absolute, right: difference; n=563, p(vis)=0.28, p(aud)=4.2e-103, p(interact)=0.38, paired 2-way ANOVA).

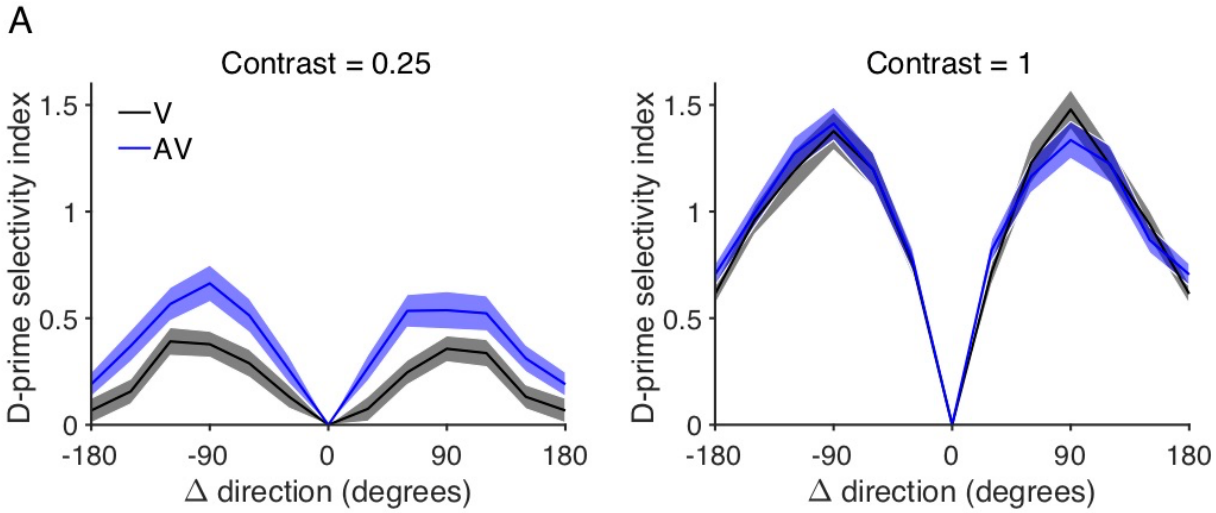


**Figure 5 Supplementary 1 | Sound enhances the *d'* sensitivity index at low contrast levels** (A) The *d'* sensitivity index between neuronal responses to drifting grating directions, averaged across orientation- and direction-selective neurons. Enhancements are observed at low visual contrast (left), whereas minimal changes are present at full contrast (right).

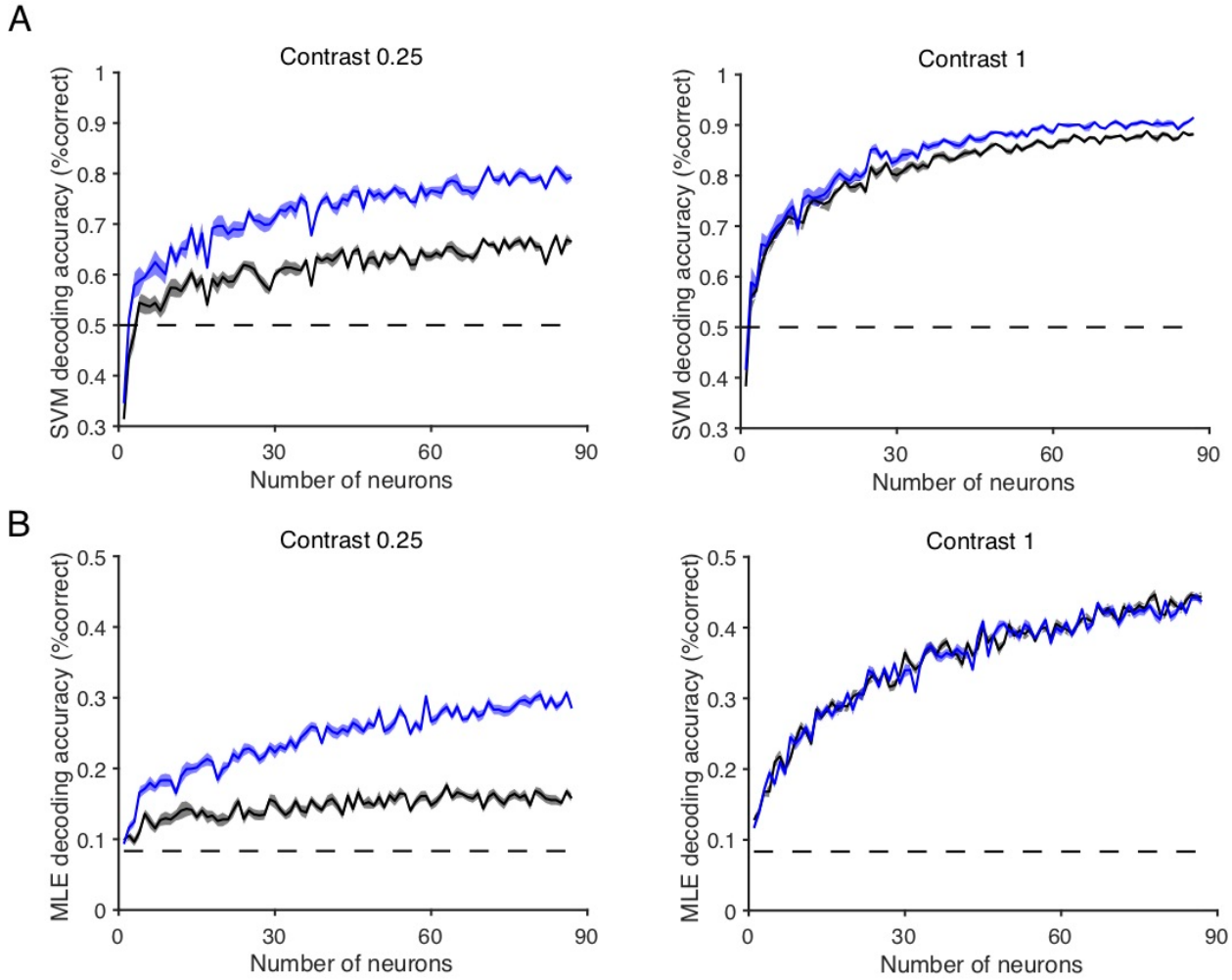


**Figure 6 Supplementary 1 | Decoding accuracy increases with population size** (A) Accuracy of SVM pairwise classification, average across all direction pairs, as the neuronal population size included in the decoder increases. Visual contrast 0.25 is on the left, and full visual contrast is on the right. (B) Accuracy of MLE decoding 1 of 12 drifting grating options, as the neuronal population size increases. Again, visual contrast 0.25 is on the left, and full visual contrast is on the right.

## Table 1: Statistical comparisons

| Comparison | Fig | Test | Test statistic | N | df | p-value | Post hoc test | Post hoc α | Post hoc comparison | Post hoc p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean firing rate, V vs AV | 2C | Paired 2-way ANOVA | F(vis)=340<br>F(aud)=506<br>F(interact)=75 | 565 neurons | vis=4<br>aud=1<br>interact = 4 | p(vis) = 1.2e-100<br>p(aud) = 1.6e-88<br>p(interact) = 5.7e-4 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 2.1e-50 |
| | | | | | | | | | Contrast 0.25, V vs AV | 2.6e-62 |
| | | | | | | | | | Contrast 0.5, V vs AV | 5.7e-75 |
| | | | | | | | | | Contrast 0.75, V vs AV | 1.1e-81 |
| | | | | | | | | | Contrast 1, V vs AV | 2.0e-81 |
| Linearity ratio, V vs AV | 2E | Kruskal-Wallis test | Chi-sq = 61 | 555 neurons | 4 | p = 1.6e-12 | Bonferroni-corrected Wilcoxon signed rank test | 0.0125 | Contrast 0 vs 0.25 | 0.053 |
| | | | | | | | | | Contrast 0 vs 0.5 | 0.0040 |
| | | | | | | | | | Contrast 0 vs 0.75 | 4.6e-8 |
| | | | | | | | | | Contrast 0 vs 1 | 2.1e-5 |
| Sound induced movement | 3A | Paired t-test | t-stat = -7.2 | 9 recording sessions | 8 | p = 9.1e-5 | | | | |
| Firing rate across movement range, V vs AV | 3E | Unbal-anced 2-way ANOVA | F(motion)=6.9<br>F(sound)=55<br>F(interact)=18 | Vari-able trial count | mot=2<br>aud=1<br>Interact=2 | p(motion) = 0.001<br>p(sound) = 1.4e-13<br>p(interact) = 1.8e-8 | Bonferroni corrected two-sample t-test | 0.016 | Stationary, V vs AV | 1.5e-14 |
| | | | | | | | | | Low motion, V vs AV | 7.1e-10 |
| | | | | | | | | | High motion, V vs AV | 0.60 |
| PSTH, light vs light/sound | 4F | Paired t-test | 1391 unique t-stats | 295 neurons | 294 | 1391 unique p-values, α= 0.05/1391= 3.6e-5 | | | | |
| PSTH, light vs light/motion | 4G | Paired t-test | 1391 unique t-stats | 295 neurons | 294 | 1391 unique p-values, α= 0.05/1391= 3.6e-5 | | | | |
| PSTH, light/sound vs light/sound/motion | 4H | Paired t-test | 1391 unique t-stats | 295 neurons | 294 | 1391 unique p-values, α= 0.05/1391= 3.6e-5 | | | | |
| Orientation selectivity index, V vs AV | Fig 2 Sup 1D | Paired t-test | t-stat = 3.2 | 78 neurons | 77 | p = 0.0018 | | | | |
| Direction selectivity index, V vs AV | Fig 2 Sup 1F | Paired t-test | t-stat = 2.7 | 12 neurons | 11 | p = 0.0206 | | | | |
| Onset response latency, V vs AV | Fig 2 Sup 2B | Paired 2-way ANOVA | F(vis)=5.7<br>F(aud)=64<br>F(interact)=2.7 | 517 neurons | vis=3<br>aud=1<br>interact=3 | p(vis)=6.9e-4<br>p(aud)=6.8e-18<br>p(interact)=0.045 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0.25, V vs AV | 2.3e-4 |
| | | | | | | | | | Contrast 0.5, V vs AV | 7.1e-12 |
| | | | | | | | | | Contrast 0.75, V vs AV | 4.6e-5 |
| | | | | | | | | | Contrast 1, V vs AV | 9.9e-4 |
| Onset response slope, V vs AV | Fig 2 | Paired 2-way ANOVA | F(vis)=70<br>F(aud)=66<br>F(interact)=2.8 | 563 neurons | vis=3<br>aud=1 | p(vis)=3.5e-121<br>p(aud) = 2.7e-15<br>p(interact) = 0.038 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0.25, V vs AV | 1.4e-4 |
| | | | | | | | | | Contrast 0.5, V vs AV | 8.9e-13 |
| | | | | | | | | | Contrast 0.75, V vs AV | 3.6e-12 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sup 2D | | | | interact=3 | | | | Contrast 1, V vs AV | 5.5e-8 |
| Onset response duration, V vs AV | Fig 2 Sup 2F | Paired 2-way ANOVA | F(vis)=17<br>F(aud)=129<br>F(interact)=1.4 | 367 neurons | vis=3<br>aud=1<br>Interact=3 | p(vis)=1.3e-10<br>p(aud) = 8.7e-98<br>p(interact) = 0.23 | | | | |
| Response coefficient of variation, V vs AV | Fig 2 Sup 2H | Paired 2-way ANOVA | F(vis)=1.3<br>F(aud)=834<br>F(interact)=1.0 | 564 neurons | vis=4<br>aud=1<br>Interact=4 | p(vis) = 0.28<br>p(aud) = 4.2e-103<br>p(interact) = 0.38 | | | | |
| Orientation decoding accuracy, individual neurons, V vs AV | Fig 5C | Paired 2-way ANOVA | F(vis)=67<br>F(aud)=12<br>F(interact)=0.54 | 78 neurons | vis=4<br>aud=1<br>interact=4 | p(vis)=4.8e-112<br>p(aud)=7.8e-4<br>p(interact) = 0.71 | | | | |
| Direction decoding accuracy, individual neurons, V vs AV | 5E | Paired 2-way ANOVA | F(vis)=6.9<br>F(aud)=2.0<br>F(interact)=0.43 | 12 neurons | vis=4<br>aud=1<br>interact=4 | p(vis)=2.1e-4<br>p(aud)=0.18<br>p(interact)=0.78 | | | | |
| Orientation decoding accuracy, SVM, population, V vs AV | 6C | 2-way ANOVA | F(vis)=526<br>F(aud)=38<br>F(interact)=6 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis) = 1.8e-61<br>p(aud) = 1.9e-8<br>p(interact) = 2.4e-4 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.12 |
| | | | | | | | | | Contrast 0.25, V vs AV | 0.0016 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.0014 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.0023 |
| | | | | | | | | | Contrast 1, V vs AV | 1 |
| Direction decoding accuracy, SVM, population, V vs AV | 6D | 2-way ANOVA | F(vis)=48<br>F(aud)=40<br>F(interact)=4.6 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis) = 1.1e-21<br>p(aud) = 9.0e-9<br>p(interact) = 0.0019 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.55 |
| | | | | | | | | | Contrast 0.25, V vs AV | 5.3e-5 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.0036 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.17 |
| | | | | | | | | | Contrast 1, V vs AV | 0.0036 |
| Orientation decoding accuracy, MLE, population, V vs AV | 6F | 2-way ANOVA | F(vis)=682<br>F(aud)=0.27<br>F(interact)=18 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=2.3e-66<br>p(aud)=0.61<br>p(interact) =9.6e-11 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 5.8e-4 |
| | | | | | | | | | Contrast 0.25, V vs AV | 1.8e-4 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.30 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.53 |
| | | | | | | | | | Contrast 1, V vs AV | 0.15 |
| Direction decoding accuracy, MLE, population, V vs AV | 6G | 2-way ANOVA | F(vis)=67<br>F(aud)=0.43<br>F(interact)=8.9 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=4.6e-26<br>p(aud)=0.51<br>p(interact) =4.1e-6 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.037 |
| | | | | | | | | | Contrast 0.25, V vs AV | 6.4e-6 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.036 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.16 |
| | | | | | | | | | Contrast 1, V vs AV | 0.014 |
| Overall decoding accuracy, MLE, population, V vs AV | 6J | 2-way ANOVA | F(vis)=411<br>F(aud)=19<br>F(interact)=16 | 20 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=2.2e-92<br>p(aud)=1.9e-5<br>p(interact)=2.7e-11 | Bonferroni - corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.012 |
| | | | | | | | | | Contrast 0.25, V vs AV | 1.4e-10 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.48 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.0013 |
| | | | | | | | | | Contrast 1, V vs AV | 0.50 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Orientation decoding accuracy, individual neurons, V vs AV | 7B | Paired 2-way ANOVA | F(vis) = 74<br>F(aud) = 19<br>F(interact) = 1.5 | 85 neurons | vis=4<br>aud=1<br>interact=4 | p(vis) =0<br>p(aud)=3.5e-5<br>p(interact)=0.21 | | | | |
| Orientation decoding accuracy, individual neurons, V vs motion-corrected AV | 7B | Paired 2-way ANOVA | F(vis) = 64<br>F(aud) = 13<br>F(interact) = 3 | 85 neurons | vis=4<br>aud=1<br>interact=4 | p(vis) =0<br>p(aud)=5.9e-4<br>p(interact)=0.019 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.019 |
| | | | | | | | | | Contrast 0.25, V vs AV | 0.071 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.029 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.011 |
| | | | | | | | | | Contrast 1, V vs AV | 0.0602 |
| Orientation decoding accuracy, individual neurons, AV vs motion-corrected AV | 7B | Paired 2-way ANOVA | F(vis) = 34<br>F(aud) = 3.8<br>F(interact) = 2.4 | 85 neurons | vis=4<br>aud=1<br>interact=4 | p(vis) = 7.7e-93<br>p(aud) = 0.055<br>p(interact) = 0.058 | | | | |
| Orientation decoding accuracy, individual neurons, V vs motion/sound-corrected AV | 7B | Paired 2-way ANOVA | F(vis) = 56<br>F(aud) = 0.36<br>F(interact) = 1.4 | 85 neurons | vis=4<br>aud=1<br>interact=4 | p(vis)=8.1e-95<br>p(aud)=0.55<br>p(interact)=0.24 | | | | |
| Population decoding accuracy, V vs AV | 7D | 2-way ANOVA | F(vis) = 166<br>F(aud) = 52<br>F(interact) = 8.2 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=1.1e-40<br>p(aud)=1.6e-10<br>p(interact)=1.1e-5 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.34 |
| | | | | | | | | | Contrast 0.25, V vs AV | 2.2e-5 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.0019 |
| | | | | | | | | | Contrast 0.75, V vs AV | 8.7e-6 |
| | | | | | | | | | Contrast 1, V vs AV | 0.013 |
| Population decoding accuracy, V vs motion-corrected AV | 7D | 2-way ANOVA | F(vis) = 147<br>F(aud) = 35<br>F(interact) = 4.8 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=1.4e-38<br>p(aud)=6.0e-8<br>p(interact)=0.0015 | Bonferroni-corrected paired t-test | 0.01 | Contrast 0, V vs AV | 0.30 |
| | | | | | | | | | Contrast 0.25, V vs AV | 0.0012 |
| | | | | | | | | | Contrast 0.5, V vs AV | 0.0022 |
| | | | | | | | | | Contrast 0.75, V vs AV | 0.0044 |
| | | | | | | | | | Contrast 1, V vs AV | 0.35 |
| Population decoding accuracy, V vs motion/sound-corrected AV | 7D | 2-way ANOVA | F(vis) = 154<br>F(aud) = 0.50<br>F(interact) = 0.088 | 10 repeats | vis=4<br>aud=1<br>interact=4 | p(vis)=2.5e-39<br>p(aud) = 0.48<br>p(interact) = 0.99 | | | | |