Title: **Auditory Cortex Learns to Discriminate Audiovisual Cues through Selective Multisensory Enhancement**

Song Chang[1], Beilin Zheng [2], Les Keniston[3], Jinghong Xu[1*], Liping Yu[1*]

[1]Key Laboratory of Brain Functional Genomics (Ministry of Education and Shanghai), School of Life Sciences, East China Normal University, Shanghai 200062, China

[2]College of Information Engineering, Hangzhou Vocational & Technical College, Hangzhou, Zhejiang 310018, China

[3]Department of Biomedical Sciences, Kentucky College of Osteopathic Medicine, University of Pikeville, Pikeville, Kentucky 41501

*Co-corresponding authors

lead contact: Liping Yu, lpyu@bio.ecnu.edu.cn

Professor, School of Life Sciences, East China Normal University, Shanghai 200062, China

Jinghong Xu, jhxu@bio.ecnu.edu.cn

Associate professor, School of Life Sciences, East China Normal University, Shanghai 200062, China

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Abstract

Multisensory object discrimination is essential in everyday life, yet the neural mechanisms underlying this process remain unclear. In this study, we trained rats to perform a two-alternative forced-choice task using both auditory and visual cues. Our findings reveal that multisensory perceptual learning actively engages auditory cortex (AC) neurons in both visual and audiovisual processing. Importantly, many audiovisual neurons in the AC exhibited experience-dependent associations between their visual and auditory preferences, displaying a unique integration model. This model employed selective multisensory enhancement for specific auditory-visual pairings, which facilitated improved multisensory discrimination. Additionally, AC neurons effectively distinguished whether a preferred auditory stimulus was paired with its associated visual stimulus using this distinct integrative mechanism. Our results highlight the capability of sensory cortices to develop sophisticated integrative strategies, adapting to task demands to enhance multisensory discrimination abilities.

Introduction

In our daily lives, the integration of visual and auditory information is crucial for detecting, discriminating, and identifying multisensory objects. When faced with stimuli like seeing an apple while hearing the sound 'apple' or 'peach' simultaneously, our brain must synthesize these inputs to form perceptions and make judgments based on the knowledge of whether the visual information matches the auditory input. To date, it remains unclear exactly where and how the brain integrates across-sensory inputs to benefit cognition and behavior. Traditionally, higher association areas in the temporal, frontal, and parietal lobes were thought to be pivotal for merging visual and auditory signals. However, recent research suggests that even primary sensory cortices, such as auditory and visual cortices, contribute significantly to this integration process[1-7].

44 Studies have shown that visual stimuli can modulate auditory responses in the AC via pathways
45 involving the lateral posterior nucleus of the thalamus[8], and the deep layers of the AC serve as crucial
46 hubs for integrating cross-modal contextual information[9]. Even irrelevant visual cues can affect how the
47 AC perceives sound frequency[10]. Anatomical investigations reveal reciprocal nerve projections between
48 auditory and visual cortices[4,11-14], highlighting the interconnected nature of these sensory systems.
49 Sensory experience has been shown to shape cross-modal presentations in sensory cortices[15-17].
50 However, despite these findings, the precise mechanisms by which sensory cortices integrate cross-
51 sensory inputs for multisensory object discrimination remain unknown.

52 Previous research on cross-modal modulation has predominantly focused on anesthetized or passive
53 animal models[3,5,6,18,19], exploring the influence of stimulus properties and spatiotemporal arrangements
54 on sensory interactions[3,6,19-21]. However, sensory representations, including multisensory processing, are
55 known to be context-dependent[22-24], and can be shaped by perceptual learning [17,25]. Therefore, the way
56 sensory cortices integrate information during active tasks may differ significantly from what has been
57 observed in passive or anesthetized states. Relatively few studies have investigated cross-modal
58 interactions during the performance of multisensory tasks, regardless of the brain region studied [25-27].
59 This limits our understanding of multisensory integration in sensory cortices, particularly regarding: (1)
60 Do neurons in sensory cortices use the same or different strategies to integrate various audiovisual
61 pairings? (2) How does the "match" or "mismatch" between auditory and visual features influence this
62 integration? (3) How does learning to discriminate audiovisual objects affect their representations in
63 sensory cortices?

64 We investigated this by training rats on a multisensory discrimination task involving both auditory and
65 visual stimuli. We then examined cue selectivity and auditory-visual integration in AC neurons of these
66 well-trained rats. Our findings demonstrate that multisensory discrimination training fosters experience-

67 dependent associations between auditory and visual features within AC neurons. During task
68 performance, AC neurons often exhibited multisensory enhancement for the preferred auditory-visual
69 pairing, with no such enhancement observed for the non-preferred pairing. Importantly, this selective
70 enhancement correlated with the animals' ability to discriminate the audiovisual pairings. Furthermore,
71 the degree of auditory-visual integration correlated with the congruence of auditory and visual features.
72 Our findings suggest AC plays a more significant role in multisensory integration than previously thought.

73 **Results**

74 **Multisensory discrimination task in freely moving rats**

75 To investigate how AC neurons integrate visual information into audiovisual processing, we trained ten
76 adult male Long Evans rats on a multisensory discrimination task (Fig. 1a). During the task, the rat
77 initiated a trial by inserting its nose into the central port, which triggered a randomly selected target
78 stimulus from a pool of six cues: two auditory (3 kHz pure tone, A_{3k};10 kHz pure tone, A_{10k}), two visual
79 (horizontal light bar, V_{hz}; vertical light bar, V_{vt}), and two multisensory cues ($A_{3k}V_{hz}$, $A_{10k}V_{vt}$). Based on the
80 cue, the rats had to choose the correct left or right port for a water reward within 3 seconds after the
81 stimulus onset. Incorrect choices or no response resulted in a 5-second timeout. To ensure reliable
82 performance, rats needed to achieve 80% accuracy (including at least 70% correct in each modality) for
83 three consecutive sessions (typically taking 2-4 months to achieve this level of accuracy).

84 Consistent with previous studies [10,26,28,29], rats performed better when responding to combined
85 auditory and visual cues (multisensory trials) compared to trials with only auditory or visual cue (Fig. 1b).
86 This suggests that multisensory cues facilitate decision-making. Reaction time, measured as the time
87 from cue onset to when the rat left the central port, was shorter in multisensory trials (Fig. 1c, d). This
88 indicates that multisensory processing in the brain helps rats discriminate between cues more efficiently

(mean reaction time across rats, multisensory, 367±53 ms; auditory, 426±60 ms; visual, 417±53 ms; multisensory vs. each unisensory, p <0.001, paired t-test. Fig. 1d).

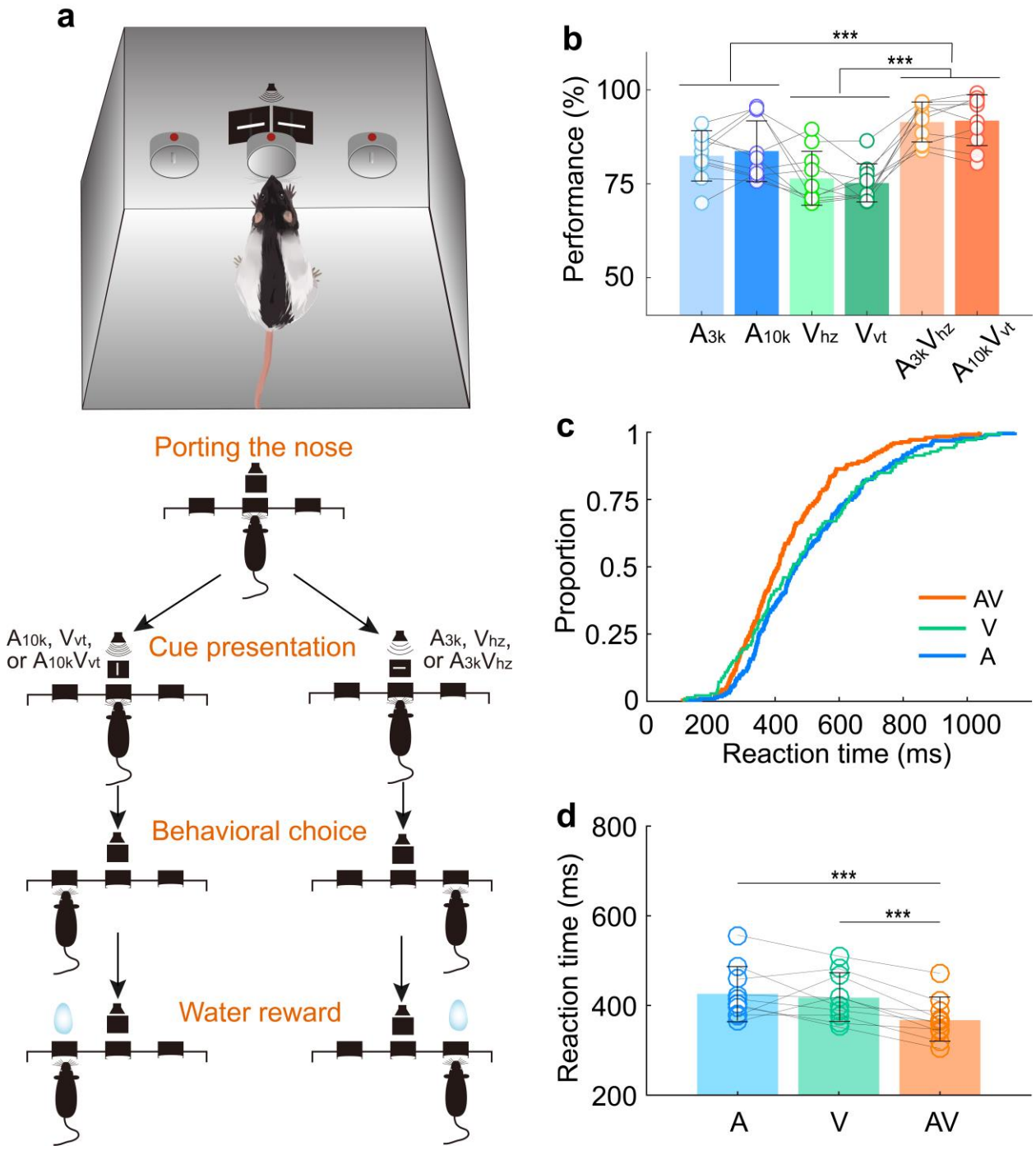

Fig. 1 Multisensory discrimination task for rats

a Schematic illustration of the behavioral task. A randomly selected stimulus was triggered when a rat placed its nose in the central port. If the triggered stimulus was a 10 kHz pure tone (A_{10k}), a

vertical light bar (V_{vt}), or their combination ($A_{10k}V_{vt}$), the rat was rewarded at the left port. Conversely, for other stimuli (a 3 kHz pure tone (A_{3k}), a horizontal light bar (V_{hz}), or $A_{3k}V_{hz}$), the rat was required to move to the right port. Visual stimuli were presented via custom-made LED matrix panels, with one panel for each side. Auditory stimuli were delivered through a centrally located speaker. **b** The mean performance for each stimulus condition across rats. Circles connected by a gray line represent data from one individual rat. **c** Cumulative frequency distribution of reaction time (time from cue onset to leaving the central port) for one representative rat in auditory, visual and multisensory trials. **d** Comparison of average reaction times across rats in auditory, visual, and multisensory trials. ***, p<0.001. Error bars represent SDs.

Auditory, visual, and multisensory discrimination of AC neurons in multisensory discrimination task

To investigate the discriminative and integrative properties of AC neurons, we implanted tetrodes in the right primary auditory cortex of well-trained rats (n=10) (Fig. 2a). Careful implantation procedures were designed and followed to minimize neuron sampling biases. The characteristic frequencies of recorded AC neurons, measured immediately after tetrode implantation, spanned a broad frequency range (Supplementary Fig. 1). We examined a total of 559 AC neurons (56±29 neurons per rat) that responded to at least one target stimulus during task engagement. Interestingly, a substantial proportion of neurons (35%, 196/559) showed visual responses (Fig. 2b), which was notably higher than the 14% (14%, 39/275, $\chi2 = 27.5$, $P < 0.001$) recorded in another group of rats (n=8) engaged in a choice-free task where rats were not required to discriminate triggered cues and could receive water rewards with any behavioral response (Fig. 2b). This suggests multisensory discrimination training enhances visual representation in the auditory cortex. Notably, 27% (150/559) of neurons responded to both auditory and visual stimuli (audiovisual neurons), and a small number (n=7) only responded to the auditory-visual combination (Fig. 2b).

During task engagement, AC neurons displayed a clear preference for one target sound over the other. As exemplified in Fig. 2c-2d, many neurons exhibited a robust response to one target sound while showing a weak or negligible response to the other. We quantified this preference using receiver operating characteristic (ROC) analysis. Since most neurons exhibited their main cue-evoked response

within the initial period of cue presentation (Fig. 2e), our analysis focused on responses within the 0-150ms window after cue onset. We found a significant majority (61%, 307/506) of auditory-responsive neurons exhibited this selectivity during the task (Fig. 2f). Notably, most neurons favored the high-frequency tone (A_{10k} preferred: 261; A_{3k} preferred: 46, Fig. 2f). This aligns with findings that neurons in the AC and medial prefrontal cortex selectively preferred the tone associated with the behavioral choice contralateral to the recorded cortices during sound discrimination tasks [10,30], potentially reflecting the formation of sound-to-action associations[31]. Such pronounced sound preference and bias were absent in the choice-free group (Fig. 2f-2e), suggesting it is directly linked to active discrimination. Anesthesia decreased auditory preference (supplementary Fig. 2a-2b), further supporting its dependence on active engagement.

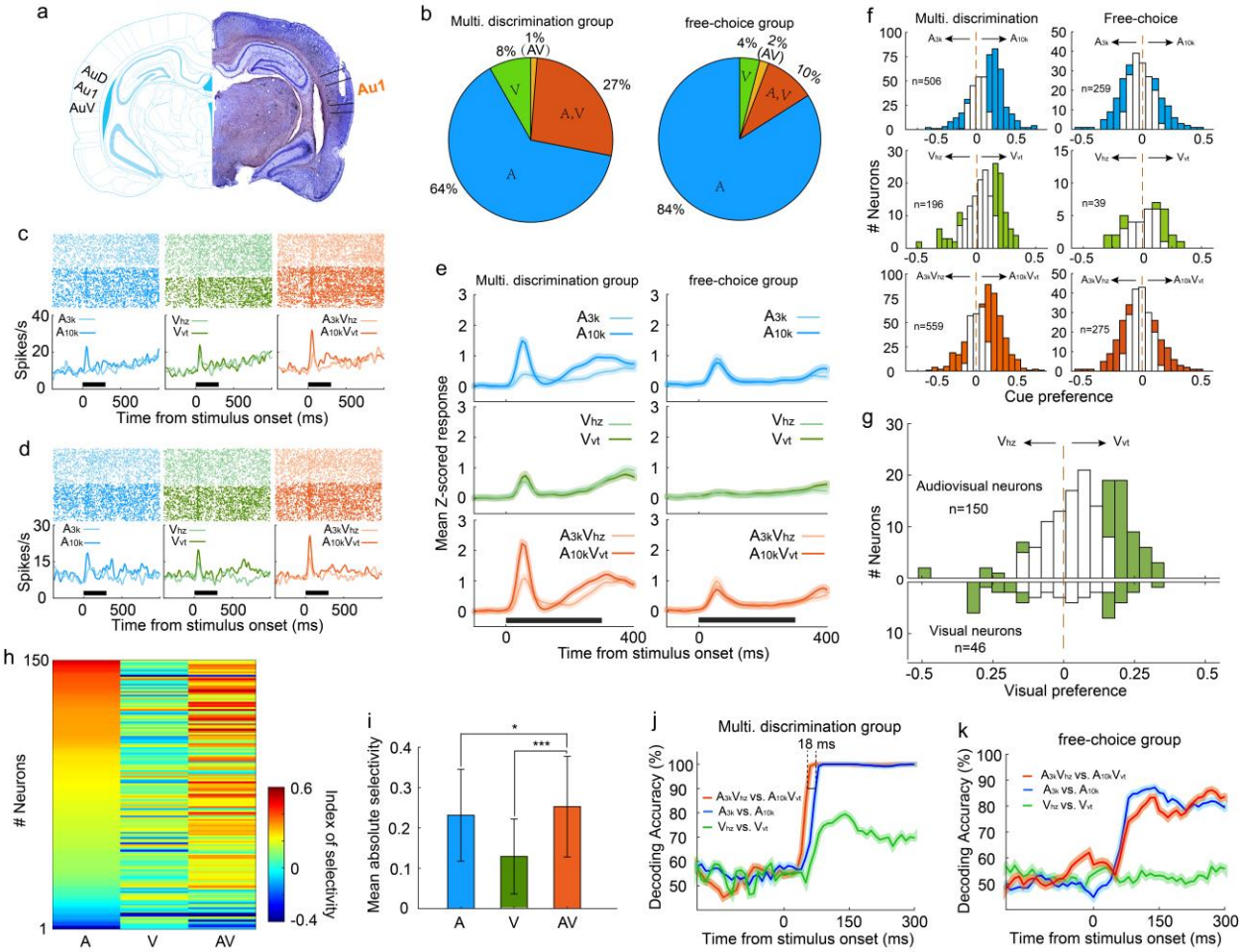

Fig. 2 Auditory, visual and multisensory selectivity in task engagement

a Histological verification of recording locations within the primary auditory cortex (Au1). **b** Proportion of neurons responding to auditory-only (A), visual-only (V), both auditory and visual (A, V), and audiovisual-only (VA) stimuli. **c-d** Rasters (top) and peristimulus time histograms (PSTHs, bottom) show responses of two exemplar neurons in A_{3k} (light blue), A_{10k} (dark blue), V_{hz} (light green), V_{vt} (dark green), $A_{3k}V_{hz}$ (light orange) and $A_{10k}V_{vt}$ (dark orange) trials (correct only). Mean spike counts in PSTHs were computed in 10-ms time windows and then smoothed with a Gaussian kernel (σ = 100ms). Black bars indicate the stimulus onset and duration. **e** Mean normalized response PSTHs across neurons in auditory (top), visual (middle), and multisensory (bottom) trials (correct only) for multisensory discrimination (left) and free-choice (right) groups. Shaded areas represent mean ± SEM. Black bars indicate stimulus onset and duration. **f** Histograms of auditory (top), visual (middle) and multisensory (bottom) selectivity for multisensory discrimination (left) and free-choice (right) groups. Filled bars indicate neurons for which the selectivity index was significantly different from 0 (permutation test, $p<0.05$, bootstrap n = 5000). The dash line represents zero. **g** Comparison of visual selectivity distribution between audiovisual (top) and visual (bottom) neurons. **h** Comparison of auditory (A), visual (V), and multisensory (AV) selectivity of 150 audiovisual neurons, ordered by auditory selectivity. **i** Average absolute auditory, visual and multisensory selectivity across 150 audiovisual neurons. Error bars represent SDs. *, $p<0.05$; ***, $p<0.001$. **j** Decoding accuracy of populations in the multisensory discrimination group. Decoding accuracy (cross-validation accuracy based on SVM) of populations between responses in two auditory (blue), two visual (green) and two multisensory (red) trials. Each decoding value was calculated in a 100ms temporal window moving at the step of 10ms. Shadowing represents the mean ± SD from bootstrapping of 100 repeats. Two dashed lines represent 90% of decoding accuracy for auditory and multisensory conditions. **k** Decoding accuracy of populations in the free-choice group.

Regarding the visual modality, 41% (80/196) of visually-responsive neurons showed a significant visual preference (Fig. 2f). Similar to the auditory selectivity observed, a greater proportion of neurons favored the visual stimulus (V_{vt}) associated with the contralateral choice, with a ratio of 60:20 for V_{vt} preferred vs. V_{hz} preferred neurons. This convergence of auditory and visual selectivity likely results from multisensory perceptual learning. Notably, such patterns were absent in neurons recorded from a separate group of rats performing choice-free tasks (Fig. 2e). Further supporting this conclusion is the difference in visual preference between audiovisual and exclusively visual neurons (Fig. 2g). Among audiovisual neurons with a significant visual selectivity, the majority favored V_{vt} (V_{vt} preferred vs. V_{hz} preferred, 48 vs. 7) (Fig. 2g), aligning with their established auditory selectivity. In contrast, visual neurons did not exhibit this bias (12 preferred V_{vt} vs. 13 preferred V_{hz}) (Fig. 2g). We propose that the dominant auditory input acts as a “teaching signal” that shapes visual processing through the selective reinforcement of specific visual pathways during associative learning. This aligns with Hebbian plasticity,

170 where stronger auditory responses boost the corresponding visual input, ultimately leading visual
171 selectivity to mirror auditory preference.

172 Similar to auditory selectivity, the vast majority of neurons (79%, 270/340) showing significant
173 multisensory selectivity exhibited a preference for the multisensory cue ($A_{10k}V_{vt}$) guiding the
174 contralateral choice (Fig. 2f). To more clearly highlight the influence of visual input on auditory
175 selectivity, we compared auditory, visual, and multisensory selectivity in 150 audiovisual neurons (Fig.
176 2h). We found that pairing auditory cues with visual cues significantly improved the neurons' ability to
177 distinguish between auditory stimuli alone (mean absolute auditory selectivity: 0.23±0.11; mean
178 absolute multisensory selectivity: 0.25±0.12; $p<0.0001$, Wilcoxon Signed Rank Test; Fig. 2i).

179 Our multichannel recordings allowed us to decode sensory information from population activity in AC
180 on a single-trial basis. Using cross-validated support vector machine (SVM) classifiers, we discriminated
181 between auditory, visual, and multisensory cues. While decoding accuracy was similar for auditory and
182 multisensory conditions, the presence of visual cues accelerated the decoding process, with AC neurons
183 reaching 90% accuracy approximately 18ms earlier in multisensory trials (Fig. 2j), aligning with
184 behavioral data. Interestingly, AC neurons could discriminate between two visual targets with around
185 80% accuracy (Fig. 2j), indicating a deeply accurate incorporation of visual processing in the auditory
186 cortex. However, AC neurons in the free-choice group lacked visual discrimination ability and showed
187 lower accuracy for auditory cues (Fig. 2k), suggesting that actively engaging multiple senses is crucial for
188 the benefits of multisensory integration.

189

190 **Audiovisual integration of AC neurons during the multisensory discrimination task**

191 To understand how AC neurons integrate auditory and visual inputs during task engagement, we
192 compared the multisensory response of each neuron to its strongest corresponding unisensory response

193 using ROC analysis to quantify the difference, termed "modality selectivity". A selectivity greater than 0
194 indicates a stronger multisensory response. Over a third (34%, 192 of 559) of AC neurons displayed
195 significant visual modulation of their auditory responses in one or both audiovisual pairings ($p < 0.05$,
196 permutation test), including some neurons with no detectable visual response (104/356). Fig. 3a
197 exemplifies this, where the multisensory response exceeded the auditory response, a phenomenon
198 termed "multisensory enhancement" [32].

199 Interestingly, AC neurons adopted distinct integration strategies depending on the specific auditory-
200 visual pairing presented. Neurons often displayed multisensory enhancement for one pairing but not
201 another (Fig. 3b), or even exhibited multisensory inhibition (Fig. 3c). This enhancement was mainly
202 specific to the A_{10k}-V_{vt} pairing, as shown by population averages (Fig. 3d). Within this pairing,
203 significantly more neurons exhibited enhancement than inhibition (114 vs. 36) (Fig. 3e). In contrast, the
204 A_{3k}-V_{hz} pairing showed a balanced distribution of enhancement and inhibition (35 vs. 33) (Fig. 3e). This
205 resulted in a significantly higher mean modality selectivity for the A_{10k}-V_{vt} pairing compared to the A_{3k}-
206 V_{hz} pairing (0.047 ± 0.124 vs. 0.003 ± 0.096; paired t-test, $p < 0.001$). Among audiovisual neurons, this
207 biasing is even more pronounced (enhanced vs. inhibited: 62 vs. 2 in A_{10k}-V_{vt} pairing, 6 vs. 13 in A_{3k}-V_{hz}
208 pairing; mean modality selectivity: 0.119±0.105 in A_{10k}-V_{vt} pairing vs. 0.020±0.083 A_{3k}-V_{hz} pairing, paired
209 t-test, $p<-0.00001$) (Fig. 3f). A similar but weaker pattern was observed under anesthesia
210 (Supplementary Fig. 2c-2f), suggesting that multisensory perceptual learning may induce plastic changes
211 in AC. In contrast, AC neurons in the choice-free group did not exhibit differential integration patterns
212 (Fig. 3g-3h), indicating that multisensory discrimination and subsequent behavioral reporting are
213 necessary for biased enhancement.

214 To understand how these distinct integration strategies influence multisensory discrimination, we
215 compared the difference in modality selectivity between A_{10k}-V_{vt} and A_{3k}-V_{hz} pairings, and the change in

neuronal multisensory versus auditory selectivity (Fig. 3j). We found a significant correlation between these measures (R=0.65, p<0.001, Pearson correlation test). The greater the difference in modality selectivity, the more pronounced the increase in cue discrimination during multisensory trials compared to auditory trials. This highlights how distinct integration models enhance cue selectivity in multisensory conditions. Additionally, a subset of neurons exhibited multisensory enhancement for each pairing (Fig. 3e), potentially aiding in differentiating between multisensory and unisensory responses. SVM decoding analysis confirmed that population neurons could effectively discriminate between multisensory and unisensory stimuli (Fig. 3i).

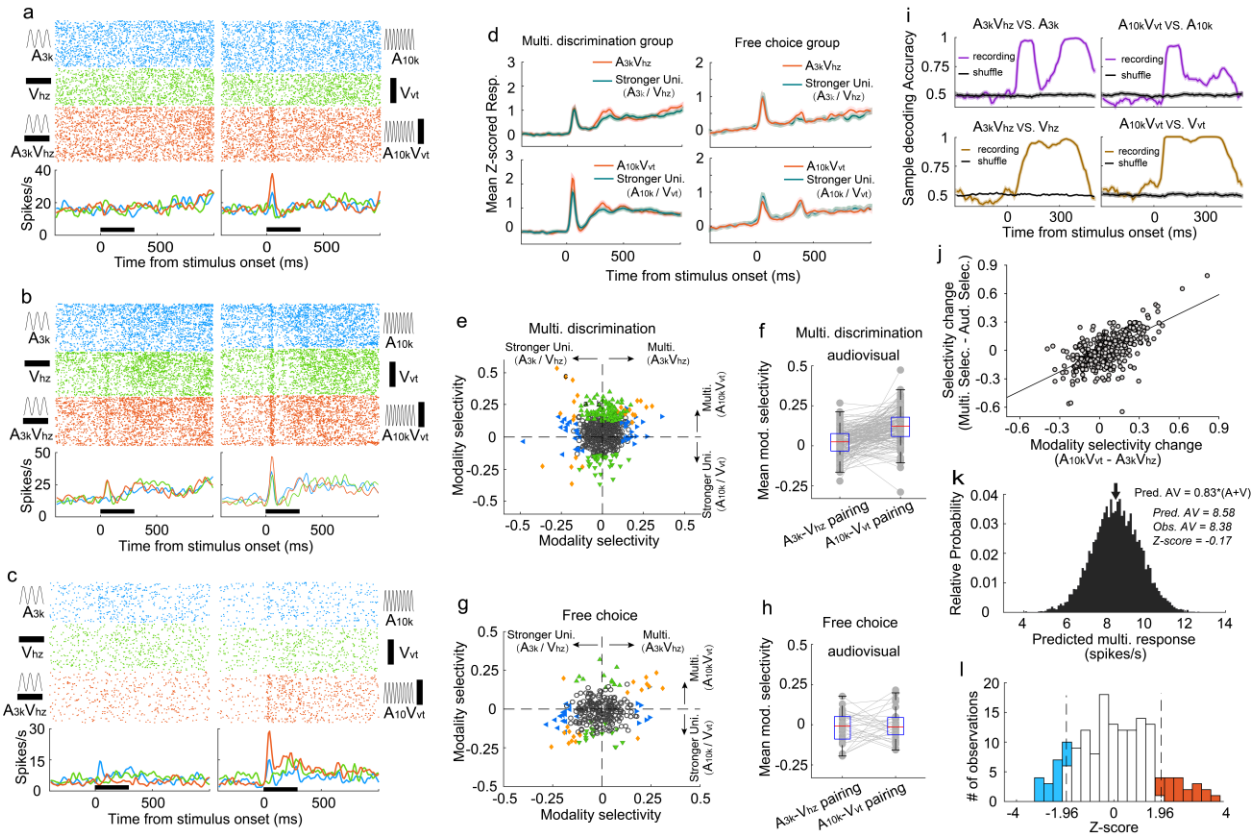

Fig. 3 Auditory and visual integration in multisensory discrimination task

a-c Rasters and PSTHs showing responses of three typical neurons recorded in well-trained rats performing multisensory discrimination tasks. **d** Population-averaged multisensory responses (red) compared to the corresponding stronger unisensory responses (dark blue) in A_{3k}-V_{hz} (top) and A_{10k}-V_{vt} (bottom) pairings for multisensory discrimination (left) and free-choice (right) groups. **e** Comparison of modality selectivity (multisensory vs. stronger unisensory) between A_{3k}-V_{hz} (x-axis) and A_{10k}-V_{vt} (y-axis) conditions. Each point represents values for a single neuron. Open circles: modality selectivity in either condition was not significant (p >

0.05, permutation test); triangles: significant selectivity in either A_{3k}-V_{hz} (blue) or A_{10k}-V_{vt} (green) condition; Diamonds: significant selectivity in both conditions. Dashed lines show zero modality selectivity. Points labeled a-c correspond to the neurons in panels a-c. **f** Mean modality selectivity for A_{10k}-V_{vt} and A_{3k}-V_{hz} pairings across audiovisual neurons in the multisensory discrimination group. **g** Comparison of modality selectivity for the free-choice group. **h** Mean modality selectivity across audiovisual neurons for the free-choice group. **i** SVM decoding accuracy in AC neurons between responses in multisensory vs. corresponding unisensory trials. Black line indicates shuffled control. **j** Positive relationship between the change in modality selectivity (A_{10k}-V_{vt} - A_{3k}-V_{hz}) and the selectivity change (multisensory selectivity - auditory selectivity). **k** Probability density functions of predicted mean multisensory responses (predicted AV) based on 0.83 times the sum of auditory (A) and visual (V) responses (same neuron as in Fig. 2c). The observed multisensory response matches the predicted mean (Z-score = -0.17). **l** Frequency distributions of *Z*-scores. Open bars indicate no significant difference between actual and predicted multisensory responses. Red bars: Z-scores>=1.96; blue bars: Z-scores<=-1.96.

We further explored the integrative mechanisms—whether subadditive, additive, or superadditive—used by AC neurons to combine auditory and visual inputs. Using the bootstrap method, we generated a distribution of predicted multisensory responses by summing mean visual and auditory responses from randomly sampled trials where each stimulus was presented alone. Robust auditory and visual responses were primarily observed in correct contralateral choice trials, so our calculations focused on the A_{10k}-V_{vt} pairing. We found that, for most neurons, the observed multisensory response in contralateral choice trials was below the anticipated sum of the corresponding visual and auditory responses (Supplementary Fig. 3). Specifically, as exemplified in Fig. 3k, the observed multisensory response approximated 83% of the sum of the auditory and visual responses in most cases (Fig. 3i).

Impact of incorrect choices on audiovisual integration

To investigate how incorrect choices affected audiovisual integration, we compared multisensory integration for correct and incorrect choices within each auditory-visual pairing, focusing on neurons with a minimum of 9 trials per choice per cue. Our findings demonstrated a significant reduction in the magnitude of multisensory enhancement during incorrect choice trials in the A_{10k}-V_{vt} pairing. Fig. 4a illustrates a representative case. The mean modality selectivity for incorrect choices was significantly lower than that for correct choices (correct vs. wrong: 0.059±0.137 vs. 0.006±0.207; p=0.005, paired t-

262 test, Fig. 4 b-c). This suggests that strong multisensory integration is crucial for accurate behavioral
263 performance. In contrast, the A_{3k}-V_{hz} pairing showed no difference in modality selectivity between
264 correct and incorrect trials (correct vs. wrong: 0.011±0.081 vs. 0.003±0.199; p=0.542, paired t-test, Fig.
265 4d-e). Interestingly, correct choices here likely correspond to ipsilateral behavioral selection, while
266 incorrect choices correspond to contralateral behavioral selection. This indicates that contralateral
267 behavioral choice alone does not guarantee stronger multisensory enhancement. Overall, these findings
268 suggest that the multisensory perception reflected by behavioral choices (correct vs. incorrect) might be
269 shaped by the underlying integration strength.

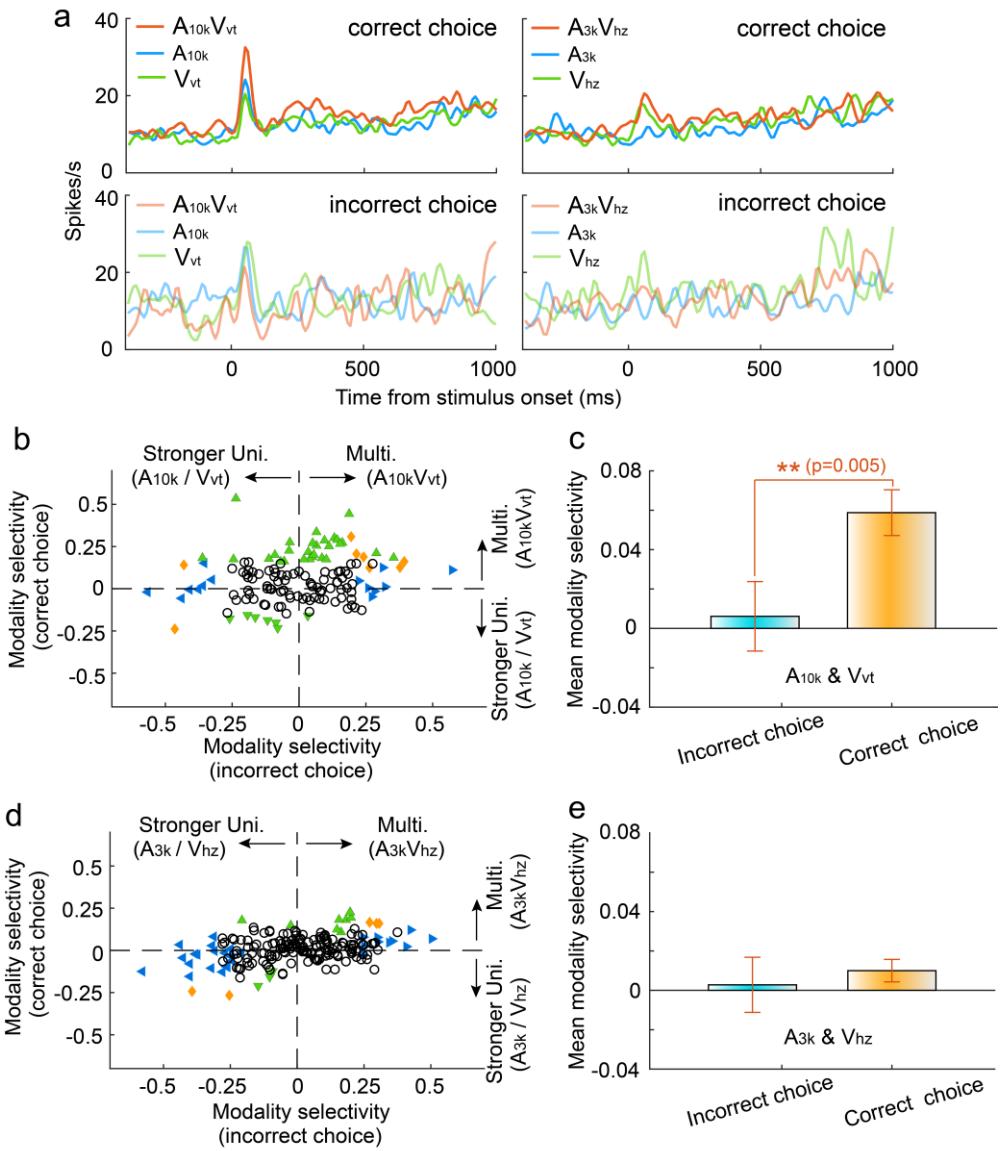

Fig. 4 Impact of Choice selection on Audiovisual Integration

a PSTHs show mean responses of an exemplar neuron for different cue and choice trials. **b** Mean modality selectivity across neurons for correct (orange) and incorrect (blue) choices in the A_{10k}-V_{vt} pairing. **c** Comparison of modality selectivity between correct and incorrect choices for the A_{10k}-V_{vt} pairing. Error bars, SEM. **d, e** Similar comparisons of modality selectivity for correct and incorrect choices in the A_{3k}-V_{hz} pairing.

Impact of informational match on audiovisual integration

A pivotal factor influencing multisensory integration is the precise alignment of informational content conveyed by distinct sensory cues. To explore the impact of the association status between auditory and

visual cues on multisensory integration, we introduced two new multisensory cues ($A_{10k}V_{hz}$ and $A_{3k}V_{vt}$) into the target cue pool during task engagement. These cues were termed 'unmatched multisensory cues' as their auditory and visual components indicated different behavioral choices. Rats received water rewards with a 50% chance in either port when an unmatched multisensory cue was triggered.

We recorded from 280 AC neurons in well-trained rats performing a multisensory discrimination task with matched and unmatched audiovisual cues. We analyzed the integrative and discriminative characteristics of these neurons for matched and unmatched auditory-visual pairings. The results revealed distinct integrative patterns in AC neurons when an auditory target cue was paired with the matched visual cue as opposed to the unmatched visual cue. Neurons typically showed a robust response to A_{10k}, which was enhanced by the matched visual cue but was either unaffected (Fig. 5a) or inhibited (Fig. 5b) by the unmatched visual cue. In some neurons, both matched and unmatched visual cues enhanced the auditory response but matched cues provided a greater enhancement (Fig. 5c). We compared the modality selectivity for different auditory-visual pairings (Fig. 5d). Unlike V_{vt}, the unmatched visual cue, V_{hz}, generally failed to significantly enhance the response to the preferred sound (A_{10k}) in most cases (mean modality selectivity: 0.052 ± 0.097 for A_{10k}-V_{vt} pairing vs. 0.016 ± 0.123 for A_{10k}-V_{hz} pairing, $p<0.0001$, paired t-test). This suggests that consistent information across modalities strengthens multisensory integration. In contrast, neither associative nor non-associative visual cue could boost neurons' response to the nonpreferred sound (A_{3k}) overall (mean modality selectivity: 0.003 ± 0.008 for A_{3k}-V_{hz} pairing vs. 0.003± 0.006 for A_{3k}-V_{vt} pairing, $p=0.19$, paired t-test).

Although distinct AC neurons exhibited varying integration profiles for different auditory-visual pairings, we explored whether, as a population, they could distinguish these pairings. We trained a linear classifier to identify each pair of stimuli and employed the classifier to decode stimulus information from the population activity of grouped neurons. The resulting decoding accuracy matrix for

304 discriminating pairs of stimuli was visualized (Fig. 5e). We found that the population of neurons could
305 effectively discriminate two target multisensory cues. While matched and unmatched visual cues failed
306 to differentially modulate the response to the nonpreferred sound (A_{3k}) at the single-neuron level,
307 grouped neurons still could discriminate them with a decoding accuracy of 0.79 (Fig. 5e), close to the
308 accuracy of 0.81 for discriminating between A_{10k}-V_{vt} and A_{10k}-V_{hz} pairings. This suggests that associative
309 learning experiences enabled AC neurons to develop multisensory discrimination abilities. However, the
310 accuracy for discriminating matched vs. unmatched cues was lower compared to other pairings (Fig. 5e).

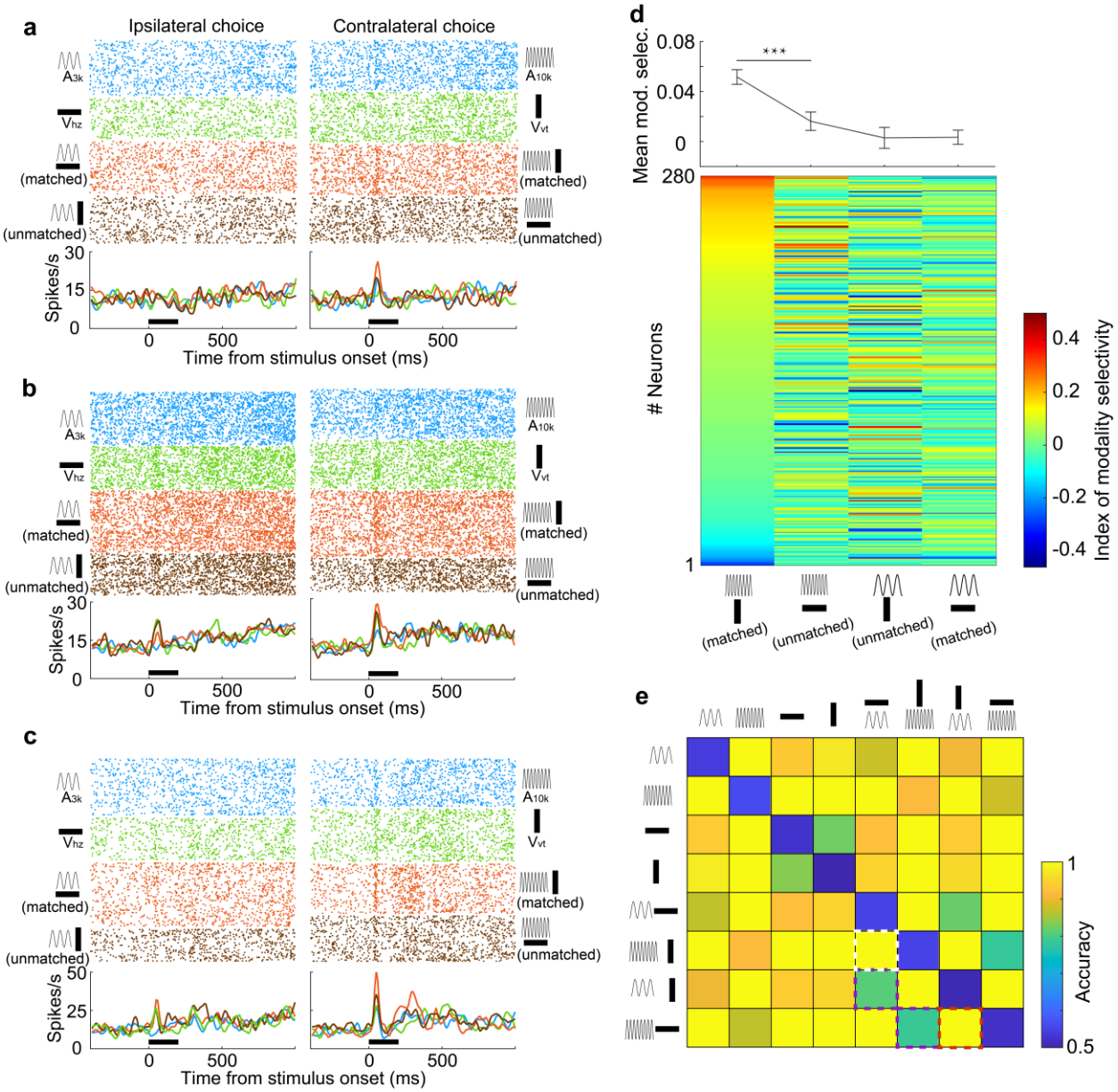

Fig. 5 Information-dependent integration and discrimination of audiovisual cues

a-c Responses of three example neurons to six target stimuli and two unmatched multisensory cues ($A_{3k}V_{vt}$ and $A_{10k}V_{hz}$) presented during task engagement. **d** Lower panel: Modality selectivity for different auditory-visual pairings. Neurons are sorted by their modality selectivity for the $A_{10k}V_{vt}$ condition. Upper panel: Mean modality selectivity across the population for each pairing. Error bar, SEM. ***, $p<0.001$. **e** Population decoding accuracy for all stimulus pairings (8×8) within a 150ms window after stimulus onset. White, purple, and red dashed squares denote the decoding accuracy for discriminating two target multisensory cues (white), discriminating two unmatched auditory-visual pairings (red) and matched versus unmatched audiovisual pairings (purple), respectively. Conventions are consistent with Fig. 2.

323

324 **Cue preference and multisensory integration in left AC**

325 Our data showed that most neurons in the right AC preferred the cue directing the contralateral
326 choice, regardless of whether it was auditory or visual. However, this could simply be because these
327 neurons were naturally more responsive to those specific cues, not necessarily because they learned an
328 association between the cues and the choice. To address this, we trained another 4 rats to perform the
329 same discrimination task but recorded neuronal activity in left AC (Fig. 6a). We analyzed 193 neurons, of
330 which about a third (31%, 60/193) responded to both auditory and visual cues (Fig. 6b). Similar to the
331 right AC, the average response across neurons in the left AC preferred cues guiding the contralateral
332 choice (Fig. 6c). However, in this case, the preferred cues are A_{3k}, V_{hz} and the audiovisual pairing $A_{3k}V_{hz}$.
333 As shown in Fig. 6d, more auditory-responsive neurons favored the sound denoting the contralateral
334 choice. The same was true for visual selectivity in visually responsive neurons (Fig. 6e). This strongly
335 suggests that the preference wasn't simply due to a general bias towards a specific cue, but rather
336 reflected the specific associations the animals learned during the training.

337 Consistent with the cue biasing, differential multisensory integration was observed, with multisensory
338 enhancement biased toward the A_{3k}-V_{hz} pairing (Fig. 6f). This mirrors the finding in the right AC, where
339 multisensory enhancement was biased toward the auditory-visual pairing guiding the contralateral
340 choice. In audiovisual neurons, mean modality selectivity in the A_{3k}-V_{hz} pairing is substantially higher
341 than in the A_{10k}-V_{vt} pairing (0.135±0.126 vs. -0.039±0.138; $p<0.0001$, paired t-test) (Fig. 6g). These
342 findings suggest that AC neurons exhibit cue preference and biased multisensory enhancement based
343 on the learned association between cues and behavioral choice. This mechanism could enable the brain
344 to integrate cues of different modalities into a common behavioral response.

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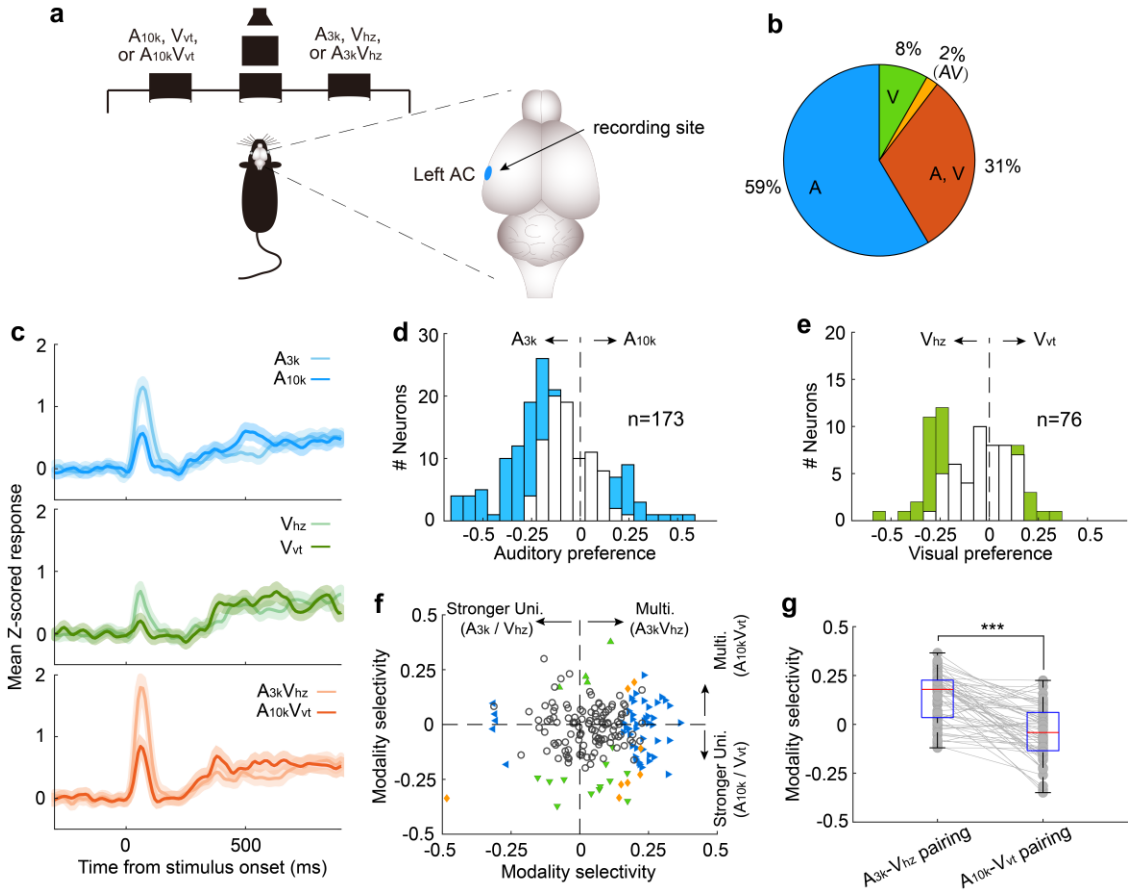

Fig. 6 Multisensory integration and cue preferences of neurons recorded in left AC

a The behavioral task and the recording site within the brain (the left AC). **b** The proportion of neurons responsive to auditory-only (A), visual-only (V), both auditory and visual (A, V), and audiovisual-only (VA) stimuli based on their spiking activity. **c** The mean PSTHs of normalized responses across neurons for different cue conditions. **d** A histogram of auditory selectivity index. Filled bars represent neurons with a significant selectivity index (permutation test, $P < 0.05$, bootstrap n = 5000). **e** Similar to panel **d**, this panel shows a histogram of the visual selectivity index. **f** Comparison of modality selectivity between A_{3k}-V_{hz} and A_{10k}-V_{vt} pairings. **g** A boxplot shows the comparison of modality selectivity for audiovisual neurons. ***, $P<0.001$. The conventions used are consistent with those in Fig. 2 and Fig. 3.

Unisensory training does not replicate multisensory training effects

Our data suggest that most AC audiovisual neurons exhibited a visual preference consistent with their auditory preference following consistent multisensory discrimination training. Additionally, these neurons developed selective multisensory enhancement for a specific audiovisual stimulus pairing. To investigate whether these properties stemmed solely from long-term multisensory discrimination

363 training, we trained a new group of animals (n=3) first on auditory and then on visual discriminations
364 (Fig. 7a). These animals did not receive task-related multisensory associations during the training period.
365 We then examined the response properties of neurons recorded in the right AC when well-trained
366 animals performed auditory, visual and audiovisual discrimination tasks.

367 Among recorded AC neurons, 28% (49/174) responded to visual target cues (Fig. 7b). Unlike the
368 multisensory training group, most visually responsive neurons in the unisensory training group lacked a
369 visual preference, regardless of whether they were visually-only responsive or audiovisual (Fig. 7c).
370 Interestingly, similar to the multisensory training group, nearly half of the recorded neurons (47%,
371 75/159) demonstrated clear auditory discrimination, with most (80%, 60 out of 75) favoring the sound
372 guiding the contralateral choice (Fig. 7d). Furthermore, the unisensory training group did not exhibit
373 population-level multisensory enhancement (n=174, Fig. 7f). The mean modality selectivity for the A_{3k}-
374 V_{Hz} and A_{10k}-V_{Hz} pairs showed no significant difference (p = 0.327, paired t-test), with values of -
375 0.02±0.12 and -0.01±0.13, respectively (Fig. 7g). Even among audiovisual neurons (n=37), multisensory
376 integration did not differ significantly between the two pairings (A_{3k}-V_{Hz} vs A_{10k}-V_{Hz}: 0.002±0.126 vs
377 0.017±0.147; p=0.63; Fig. 7g). These findings suggest that the development of multisensory
378 enhancement for specific audiovisual cues and the alignment of auditory and visual preferences likely
379 depend on the association of auditory and visual stimuli with the corresponding behavioral choice
380 during multisensory training. Unisensory training alone cannot replicate these effects.

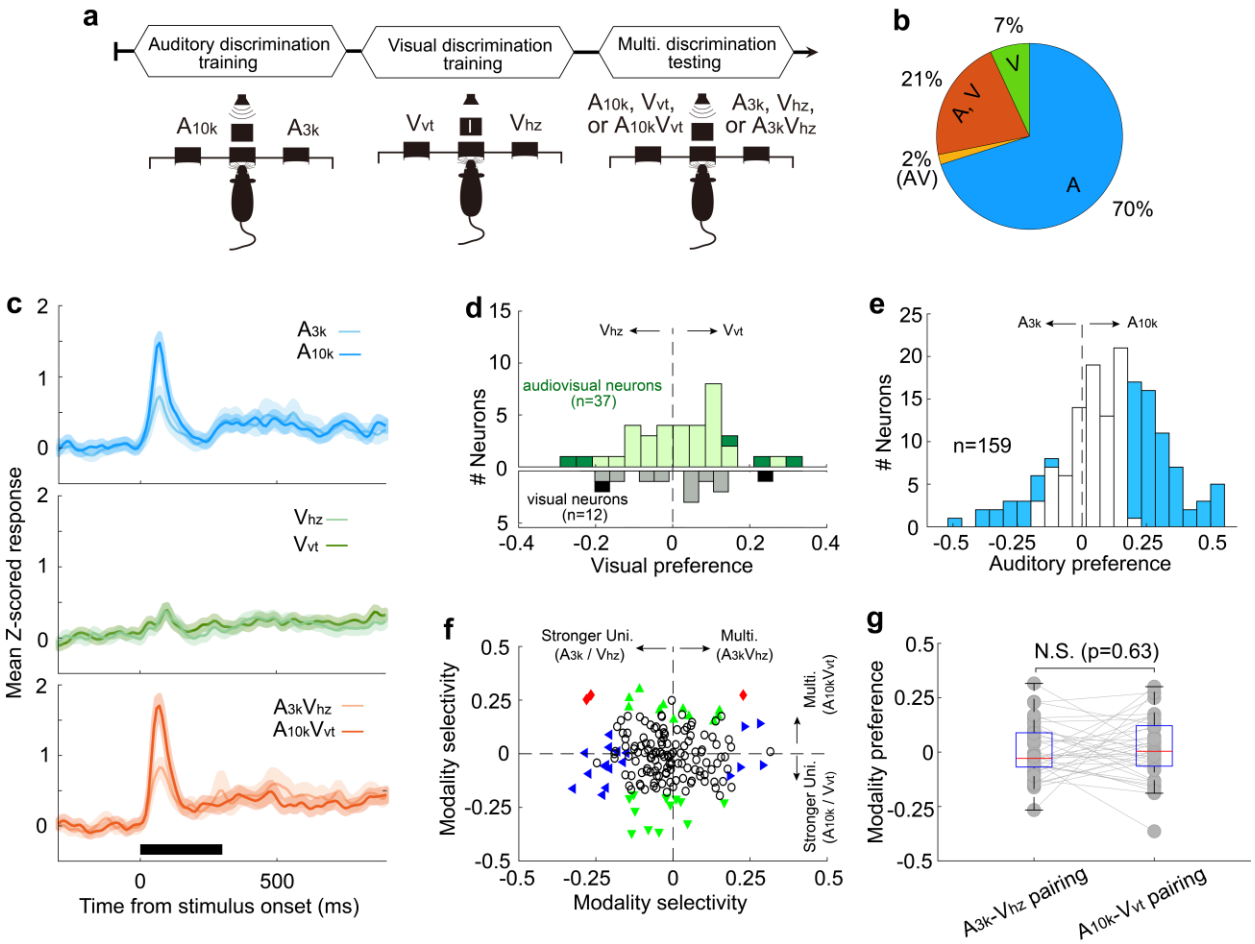

Fig. 7 Multisensory integration and cue preferences in right AC neurons after unisensory training

a This panel depicts the training stages for the rats: auditory discrimination training followed by visual discrimination training. **b** The proportion of neurons of different types. **c** Mean normalized responses across neurons for different cue conditions. **d-e** Histograms of visual (**d**) and auditory (**e**) selectivity. **f** Comparison of neuronal modality selectivity between A_{3k}-V_{hz} and A_{10k}-V_{vt} pairings. **g** Modality selectivity comparison in audiovisual neurons. N.S., no significant difference. The conventions used are consistent with those in Fig. 2, Fig. 3 and Fig. 6.

Discussion

In this study, we investigated how AC neurons integrate auditory and visual inputs to discriminate multisensory objects and whether this integration reflects the congruence between auditory and visual cues. Our findings reveal that most AC neurons exhibited distinct integrative patterns for different auditory-visual pairings, with multisensory enhancement observed primarily for favored pairings. This

394 indicates that AC neurons show significant selectivity in their integrative processing. Furthermore, AC
395 neurons effectively discriminated between matched and mismatched auditory-visual pairings,
396 highlighting the crucial role of the AC in multisensory object recognition and discrimination. Interestingly,
397 a subset of auditory neurons not only developed visual responses but also exhibited congruence
398 between auditory and visual selectivity. This suggests that multisensory perceptual training leads to
399 auditory-visual binding and establishes a memory trace of the trained audiovisual experiences within the
400 AC. Sensory cortices, Like AC, may act as a vital bridge for communicating sensory information across
401 different modalities.

402 Numerous studies have explored the cross-modal interaction of sensory cortices[1,3,4,6,19,33]. Recent
403 research has highlighted the critical role of cross-modal modulation in shaping the stimulus
404 characteristics encoded by sensory cortical neurons. For instance, sound has been shown to refine
405 orientation tuning in layer 2/3 neurons of the primary visual cortex[7], and a cohesive visual stimulus can
406 enhance sound representation in the AC[5]. Despite these findings, the functional role and patterns of
407 cross-modal interaction during perceptual discrimination remain unclear. In this study, we made a
408 noteworthy discovery that AC neurons employed a nonuniform mechanism to integrate visual and
409 auditory stimuli while well-trained rats performed a multisensory discrimination task. This differentially
410 integrative pattern improved multisensory discrimination. Notably, this integrative pattern did not
411 manifest during a free-choice task. These findings indicate that multisensory integration in sensory
412 cortices is not static but rather task-dependent. We propose that task-related differentially integrative
413 patterns may not be exclusive to sensory cortices but could represent a general model in the brain.

414 Consistent with prior research[10,30], the majority of AC neurons exhibited a selective preference for the
415 cue linked to the contralateral choice, irrespective of the sensory modality. We propose that such cue-
416 preference biasing helps animals learn associations between cues and actions. This learning may involve

417 the formation of new connections between sensory and motor areas of the brain (cortico-cortical
418 pathways) driven by sensorimotor association learning[34]. This biasing was not observed in the choice-
419 free group. Similar biasing was also reported in a previous study, where auditory discrimination learning
420 preferentially potentiated corticostriatal synapses from neurons representing either high or low
421 frequencies associated with contralateral choices [31]. These findings underscore the significant impact of
422 associative learning on cue encoding mechanisms, specifically highlighting the integration of stimulus
423 discrimination and behavioral choice.

424 Prior work has investigated how neurons in sensory cortices discriminate unisensory cues in
425 perceptual tasks[35-37]. It is well-established that when animals learn the association of sensory features
426 with corresponding behavioral choices, cue representations in early sensory cortices undergo significant
427 changes[24,38-40]. For instance, visual learning shapes cue-evoked neural responses and increases visual
428 selectivity through changes in the interactions and correlations within visual cortical neurons [41,42].
429 Consistent with these previous studies, we found that auditory discrimination in the AC of well-trained
430 rats substantially improved during the task.

431 In this study, we extended our investigation to include multisensory discrimination. We discovered
432 that when rats performed the multisensory discrimination task, AC neurons exhibited robust
433 multisensory selectivity, responding strongly to one auditory-visual pairing and showing weak or
434 negligible responses to the other, similar to their behavior in auditory discrimination. Audiovisual
435 neurons demonstrated higher selectivity in multisensory trials compared to auditory trials, driven by the
436 differential integration pattern. Additionally, more AC neurons were involved in auditory-visual
437 discrimination compared to auditory discrimination alone, suggesting that the recruitment of additional
438 neurons may partially explain the higher behavioral performance observed in multisensory trials. A

439 previous study indicates that cross-modal recruitment of more cortical neurons also enhances
440 perceptual discrimination[43].

441 Our study explored how multisensory discrimination training influences visual processing in the AC.
442 We observed well-trained rats exhibited a higher number of AC neurons responding to visual cues
443 compared to untrained rats. This suggests that multisensory training enhances AC's ability to process
444 visual information. Our result is in line with an increasing number of studies showing that multisensory
445 perceptual learning can effectively drive plastic change in both sensory and association cortices[44,45]. For
446 instance, a calcium imaging study in mice showed that a subset of multimodal neurons in mouse visual
447 cortex develops enhanced auditory responses to the paired auditory stimulus following coincident
448 auditory–visual experience[17]. A study conducted on the gustatory cortex of alert rats has shown that
449 cross-modal associative learning was linked to a dramatic increase in the prevalence of neurons
450 responding to nongustatory stimuli [16].

451 Among neurons responding to both auditory and visual stimuli, a congruent visual and auditory
452 preference emerged during multisensory discrimination training, as opposed to unisensory
453 discrimination training. These neurons primarily favored visual cues that matched their preferred sound.
454 Interestingly, this preference was not observed in neurons solely responsive to visual targets. The
455 strength of a visual response seems to be contingent upon the paired auditory input received by the
456 same neuron. This aligns with known mechanisms in other brain regions, where learning strengthens or
457 weakens connections based on experience[17,46]. This synchronized auditory-visual selectivity may be a
458 way for AC to bind corresponding auditory and visual features, potentially forming memory traces for
459 learned multisensory objects. These findings suggest that multisensory training may foster the
460 formation of specialized neural circuits within AC. These circuits enable neurons to process related

461 auditory and visual information together. However, further research is needed to determine if AC is the
462 initial site for these circuit modifications.

463 There is ongoing debate about whether cross-sensory responses in sensory cortices predominantly
464 reflect sensory inputs or are influenced by behavioral factors, such as cue-induced body movements. A
465 recent study shows that sound-clip evoked activity in visual cortex have a behavioral rather than sensory
466 origin and is related to stereotyped movements[47]. Several studies have demonstrated sensory neurons
467 can encode signals associated with whisking[48], running[49], pupil dilation [50] and other movements[51]. In our
468 study, however, we believe that the activity evoked by the flash of light bar in the AC reflects sensory
469 inputs rather than behavioral modulation through visual-evoked body or orofacial movement. The
470 observed responses to visual targets occurred mainly within a 100 ms temporal window following cue
471 onset, which precedes the onset of rat body movement. This temporal dissociation strengthens the case
472 for a sensory origin, aligning with other research showing motor-related activity triggered by sound
473 begins much later in the visual cortex[52]. Additionally, movement evoked response are generally
474 stereotyped, while the visual responses we observed are discriminative.

475 Additionally, our study sheds light on the role of semantic-like information in multisensory integration.
476 During training, we created an association between specific auditory and visual stimuli, as both signaled
477 the same behavioral choice. This setup mimics real-world scenarios where visual and auditory cues
478 possess semantic coherence, such as an image of a cat paired with the sound of a "meow." Previous
479 research has shown that semantically congruent multisensory stimuli enhance behavioral performance,
480 while semantically incongruent stimuli either show no enhancement or result in decreased
481 performance[53]. Intriguingly, our findings revealed a more nuanced role for semantic information. While
482 AC neurons displayed multisensory enhancement for the preferred congruent audiovisual pairing, this
483 wasn't observed for the other congruent pairing. This suggests that simple semantic similarity may not

be enough for enhanced multisensory integration. However, the strength of multisensory enhancement itself served as a key indicator in differentiating between matched and mismatched cues. These findings provide compelling evidence that the nature of the semantic-like information plays a vital role in modifying multisensory integration at the neuronal level.

Methods

Animals. The animal procedures conducted in this study were ethically approved by the Local Ethical Review Committee of East China Normal University and followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals of East China Normal University. Twenty-five adult male Long-Evans rats, weighing approximately 250g, were obtained from the Shanghai Laboratory Animal Center (Shanghai, China) and used as subjects for the experiments. Of these rats, 14 were assigned to the multisensory discrimination task experiments, 3 to the unisensory discrimination task, and 8 to the control free-choice task experiments. The rats were group-housed with no more than four rats per cage and maintained on a regular light-dark cycle. They underwent water deprivation for two days before the start of behavioral training, with water provided exclusively inside the training box on training days. Training sessions were conducted six days per week, each lasting between 50 to 80 minutes, and were held at approximately the same time each day to minimize potential circadian variations in performance. The body weight of the rats was carefully monitored throughout the study, and supplementary water was provided to those unable to maintain a stable body weight from task-related water rewards.

Behavioral apparatus. All experiments were conducted in a custom-designed operant chamber, measuring 50×30×40 cm (length × width × height), with an open-top design. The chamber was placed in a sound-insulated double-walled room, and the inside walls and ceiling were covered with 3 inches of sound-absorbing foam to minimize external noise interference. One sidewall of the operant chamber was equipped with three snout ports, each monitored by a photoelectric switch (see Fig. 1A).

507 Automated training procedures were controlled using a real-time control program developed in
508 MATLAB (Mathworks, Natick, MA, USA). The auditory signals generated by the program were sent to an
509 analog-digital multifunction card (NI USB 6363, National Instruments, Austin, TX, USA), amplified by a
510 power amplifier (ST-601, SAST, Zhejiang, China), and delivered through a speaker (FS Audio, Zhejiang,
511 China). The auditory stimuli consisted of 300ms-long (15ms ramp-decay) pure tones with a frequency of
512 3kHz or 10kHz. The sound intensity was set at 60 dB sound pressure level (SPL) against an ambient
513 background of 35-40 dB SPL. SPL measurements were taken at the position of the central port, which
514 served as the starting position for the rats.

515 The visual cue was generated using two custom-made devices located on each side in front of the
516 central port. Each device consisted of two arrays of closely aligned light-emitting diodes arranged in a
517 cross pattern. The light emitted by the vertical or horizontal LED array passed through a ground glass,
518 resulting in the formation of the corresponding vertical or horizontal light bar, each measuring 6×0.8 cm
519 (Fig. 1). As stimuli, the light bars were illuminated for 300ms at an intensity of 2~3cd/m^2. The audiovisual
520 cue (multisensory cue) was the simultaneous presentation of both auditory and visual stimuli.

521 **Multisensory discrimination task.** The rats were trained to perform a cue-guided two-alternative
522 forced-choice task, modified from previously published protocols [10,30]. Each trial began with the rat
523 placing its nose into the center port. Following a short variable delay period (500-700ms) after nose
524 entry, a randomly selected stimulus signal was presented. Upon cue presentation, the rats were allowed
525 to initiate their behavioral choice by moving to either the left or right port (Fig. 1A). The training
526 consisted of two stages. In the first stage, which typically lasted 3-5 weeks, the rats were trained to
527 discriminate between two audiovisual cues. In the second stage, an additional four unisensory cues
528 were introduced, training the rats to discriminate a total of six cues (two auditory, two visual, and two
529 audiovisual). This stage also lasted approximately 3-5 weeks.

530 During the task, the rats were rewarded with a drop (15-20μl) of water when they moved to the left
531 reward port following the presentation of a 10 kHz pure tone sound, a vertical light bar, or their
532 combination. For trials involving a 3 kHz tone sound, a horizontal light bar, or their combination, the rats
533 were rewarded when they moved to the right reward port. Incorrect choices or failures to choose within
534 3 seconds after cue onset resulted in a timeout punishment of 5-6 seconds. Typically, the rats completed
535 between 300 and 500 trials per day. They were trained to achieve a competency level of more than 80%
536 correct overall and >70% correct in each cue condition in three consecutive sessions before the surgical
537 implantation of recording electrodes.

538 The correct rate was calculated as follows:

539 Correct rate (%) = 100*(the number of correct trials) / (total number of trials);

540 The reaction time was defined as the temporal gap between the cue onset and the time when the rat
541 withdrew its nose from the infrared beam in the cue port.

542 **Unisensory discrimination task.** Similar to the multisensory discrimination task, the rats first learned to
543 discriminate between two auditory cues. Once their performance in the auditory discrimination task
544 exceeded 75% correct, the rats then learned to discriminate between two visual cues. The auditory and
545 visual cues used were the same as those in the multisensory discrimination task.

546 **No cue discrimination choice-free task.** In this task, the rats were not required to discriminate the cues
547 presented. They received a water reward at either port after the onset of the cue. The six cues used
548 were the same as those in the multisensory discrimination task. One week of training was sufficient for
549 the rats to learn this no cue discrimination choice-free task.

550 **Assembly of tetrodes.** The tetrodes were constructed using Formvar-Insulated Nichrome Wire (bare
551 diameter: 17.78 μm, A-M systems, WA, USA) twisted in groups of four. Two 20 cm-long wires were
552 folded in half over a horizontal bar to facilitate twisting. The ends of the wires were clamped together

553 and manually twisted in a clockwise direction. The insulation coating of the twisted wires was then fused
554 using a heat gun at the desired twist level. Subsequently, the twisted wire was cut in the middle to
555 produce two tetrodes. To enhance the longitudinal stability of each tetrode, it was inserted into
556 polymide tubing (inner diameter: 0.045 inches; wall: 0.005 inches; A-M systems, WA, USA) and secured
557 in place using cyanoacrylate glue. An array of 2×4 tetrodes was assembled, with an inter-tetrode gap of
558 0.4-0.5 mm. After assembly, the insulation coating at the tip of each wire was gently removed, and the
559 exposed wire was soldered to a connector pin. For the reference electrode, a Ni-Chrome wire with a
560 diameter of 50.8 µm (A-M systems, WA, USA) was used, with its tip exposed. A piece of copper wire with
561 a diameter of 0.1 mm served as the ground electrode. Each of these electrodes was also soldered to a
562 corresponding pin on the connector. The tetrodes, reference electrode, and ground electrode, along
563 with their respective pins, were carefully arranged and secured using silicone gel. Immediately before
564 implantation, the tetrodes were trimmed to an appropriate length.

565 **Electrode Implantation.** Prior to surgery, the animal received subcutaneous injections of atropine
566 sulfate (0.01 mg/kg) to reduce bronchial secretions. The animal was then anesthetized with an
567 intraperitoneal (i.p.) injection of sodium pentobarbital (40–50 mg/kg) and securely positioned on a
568 stereotaxic apparatus (RWD, Shenzhen, China). An incision was made in the scalp, and the temporal
569 muscle was carefully recessed. Subsequently, a craniotomy and durotomy were performed to expose
570 the target brain region. Using a micromanipulator (RWD, Shenzhen, China), the tetrode array was slowly
571 advanced and implanted in the right primary auditory cortex at coordinates 3.5 - 5.5 mm posterior to
572 bregma and 6.4 mm lateral to the midline. The craniotomy was then sealed with tissue gel (3 M,
573 Maplewood, MN, USA). The tetrode array was secured to the skull using stainless steel screws and
574 dental acrylic. Following the surgery, animals received a 4-day prophylactic course of antibiotics (Baytril,
575 5 mg/kg, body weight, Bayer, Whippany, NJ, USA). They were allowed a recovery period of at least 7
576 days (typically 9-12 days) with free access to food and water.

577 **Neural recordings.** After rats had sufficiently recovered from the surgery, they resumed performing the
578 same behavioral task they were trained to accomplish before surgery. Recording sessions were initiated
579 once the animals' behavioral performance had returned to the level achieved before the surgery
580 (typically within 2-3 days). Wideband neural signals in the range of 300-6000 Hz were recorded using the
581 AlphaOmega system (AlphaOmega Instruments, Nazareth Illit, Israel). The amplified signals (×20) were
582 digitized at a sampling rate of 25 kHz. These neural signals, along with trace signals representing the
583 stimuli and session performance information, were transmitted to a PC for online observation and data
584 storage.

585 Additionally, we recorded neural responses from well-trained rats under anesthesia. Anesthesia was
586 induced with an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight) and
587 maintained throughout the experiment by continuous intraperitoneal infusion of sodium pentobarbital
588 (0.004 ~ 0.008 g/kg/h) using an automatic microinfusion pump (WZ-50C6, Smiths Medical, Norwell, MA,
589 USA). The anesthetized rats were placed in the behavioral training chamber, and their heads were
590 positioned in the cue port to mimic the cue-triggering as observed during task engagement. To maintain
591 body temperature, a heating blanket was used to maintain a temperature of 37.5 °C. The same auditory,
592 visual, and audiovisual stimuli used during task engagement were randomly presented to the
593 anesthetized rats. For each cue condition, we recorded 40-60 trials of neural responses.

594 **Analysis of electrophysiological data.** The raw neural signals were recorded and saved for subsequent
595 offline analysis. Spike sorting was performed using Spike 2 software (CED version 8, Cambridge, UK).
596 Initially, the recorded raw neural signals were band-pass filtered in the range of 300-6000 Hz to
597 eliminate field potentials. A threshold criterion, set at no less than three times the standard deviation
598 (SD) above the background noise, was applied to identify spike peaks. The detected spike waveforms
599 were then subjected to clustering using template-matching and principal component analysis.

Waveforms with inter-spike intervals of less than 2.0 ms were excluded from further analysis. Spike trains corresponding to an individual unit were aligned to the onset of the stimulus and grouped based on different cue and choice conditions. Units were included for further analysis only if their overall mean firing rate within the session was at least 2 Hz. To generate peristimulus time histograms (PSTHs), the spike trains were binned at a resolution of 10 ms, and the average firing rate in each bin was calculated. The resulting firing rate profile was then smoothed using a Gaussian kernel with a standard deviation (σ) of 100 ms. In order to normalize the firing rate, the mean firing rate and SD during a baseline period (a 400-ms window preceding cue onset) were used to convert the averaged firing rate of each time bin into a Z-score.

Population decoding. To evaluate population discrimination for paired stimuli (cue_A vs. cue_B), we trained a Support Vector Machine (SVM) classifier with a linear kernel to predict cue selectivity. The SVM classifier was implemented using the "fitcsvm" function in Matlab. In this analysis, spike counts for each neuron in correct trials were grouped based on the triggered cues and binned into a 100 ms window with a 10 ms resolution. To minimize overfitting, only neurons with more than 30 trials for each cue were included. All these neurons were combined to form a pseudo population. The responses of the population neurons were organized into an M × N × T matrix, where M is the number of trials, N is the number of neurons, and T is the number of bins. For each iteration, 30 trials were randomly selected for each cue from each neuron. During cross-validation, 90% of the trials were randomly sampled as the training set, while the remaining 10% were used as the test set. The training set was used to compute the linear hyperplane that optimally separated the population response vectors corresponding to cue_A vs cue_B trials. The performance of the classifier was calculated as the fraction of correctly classified test trials, using 10-fold cross-validation procedures. To ensure robustness, we repeated the resampling process 100 times and computed the mean and standard deviation of the decoding accuracy across the 100 resampling iterations. Decoders were trained and tested independently for each bin. To assess the

significance of decoding accuracy exceeding the chance level, shuffled decoding procedures were conducted by randomly shuffling the trial labels for 1000 iterations.

Cue selectivity. To quantify cue (auditory, visual and multisensory) selectivity between two different cue conditions (e.g., low tone trials vs. high tone trials), we employed a receiver operating characteristic (ROC) based analysis, following the method described in a previous study[54]. This approach allowed us to assess the difference between responses in cue_A and cue_B trials. Firstly, we established 12 threshold levels of neural activity that covered the range of firing rates obtained in both cue_A and cue_B trials. For each threshold criterion, we plotted the proportion of cue_A trials where the neural response exceeded the criterion against the proportion of cue_B trials exceeding the same criterion. This process generated an ROC curve, from which we calculated the area under the ROC curve (auROC). The cue selectivity value was then defined as 2 * (auROC - 0.5). A cue selectivity value of 0 indicated no difference in the distribution of neural responses between cue_A and cue_B, signifying similar responsiveness to both cues. Conversely, a value of 1 or -1 represented the highest selectivity, indicating that responses triggered by cue_A were consistently higher or lower than those evoked by cue_B, respectively. To determine the statistical significance of the observed cue selectivity, we conducted a two-tailed permutation test with 2000 permutations. We randomly reassigned the neural responses to cue_A and cue_B trials and recalculated a cue selectivity value for each permutation. This generated a distribution of values from which we calculated the probability of our observed result. If our actual value fell within the top 5% of this distribution, it was deemed significant (i.e., $p < 0.05$).

Comparison of actual and predicted multisensory responses. We conducted a comprehensive analysis to compare the observed multisensory responses with predicted values. The predicted multisensory response is calculated as either the sum of visual and auditory responses or as a coefficient multiplied by this sum. To achieve this, we first computed the mean observed multisensory response by averaging

647 across audiovisual trials. We then created a benchmark distribution of predicted multisensory responses
648 by iteratively calculating all possible predictions. In each iteration, we randomly selected (without
649 replacement) the same number of trials as used in the actual experiment for both auditory and visual
650 conditions. The responses from these selected auditory and visual trials were then averaged to obtain
651 mean predicted auditory and visual responses, which were used to create a predicted multisensory
652 response. This process was repeated 5,000 times to generate a comprehensive reference distribution of
653 predicted multisensory responses. By comparing the actual mean multisensory response to this
654 distribution, we expressed their relationship as a Z-score. This method allowed us to quantitatively
655 assess multisensory interactions, providing valuable insights into neural processing and enhancing our
656 understanding of the mechanisms underlying multisensory integration.

657 **Histology.** Following the final data recording session, the precise tip position of the recording electrode
658 was marked by creating a small DC lesion (-30 μA for 15 s). Subsequently, the rats were deeply
659 anesthetized with sodium pentobarbital (100 mg/kg) and underwent transcardial perfusion with saline
660 for several minutes, followed immediately by phosphate-buffered saline (PBS) containing 4%
661 paraformaldehyde (PFA). The brains were carefully extracted and immersed in the 4% PFA solution
662 overnight. To ensure optimal tissue preservation, the fixed brain tissue underwent cryoprotection in PBS
663 with a 20% sucrose solution for at least three days. Afterward, the brain tissue was coronally sectioned
664 using a freezing microtome (Leica, Wetzlar, Germany) with a slice thickness of 50 μm. The resulting
665 sections, which contained the auditory cortex, were stained with methyl violet to verify the lesion sites
666 and/or the trace of electrode insertion within the primary auditory cortex.

667 **Statistical analysis.**

668 We also use ROC analysis to calculate the modality selectivity to denote the difference between
669 multisensory and the corresponding stronger unisensory responses. All statistical analyses were

conducted in MATLAB, and statistical significance was defined as a P value of < 0.05. To determine the responsiveness of AC neurons to sensory stimuli, neurons exhibiting evoked responses greater than 2 spikes/s within a 0.3 s window after stimulus onset, and significantly higher than the baseline response ($P < 0.05$, Wilcoxon signed-rank test), were included in the subsequent analysis. For behavioral data, such as mean reaction time differences between unisensory and multisensory trials, cue selectivity and mean modality selectivity across different auditory-visual conditions, comparisons were performed using either the Wilcoxon signed-rank test or paired t-test, as appropriate. We performed a Chi-square test to analyze the difference in the proportions of neurons responding to visual stimuli between the multisensory discrimination and free-choice groups. Correlation values were computed using Pearson's correlation. All data are presented as mean ± SD for the respective groups.

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Acknowledgements

This work was supported by grants from the “STI2030-major projects” (2021ZD0202600), Natural Science Foundation of China (32371046, 31970925, 32271057).

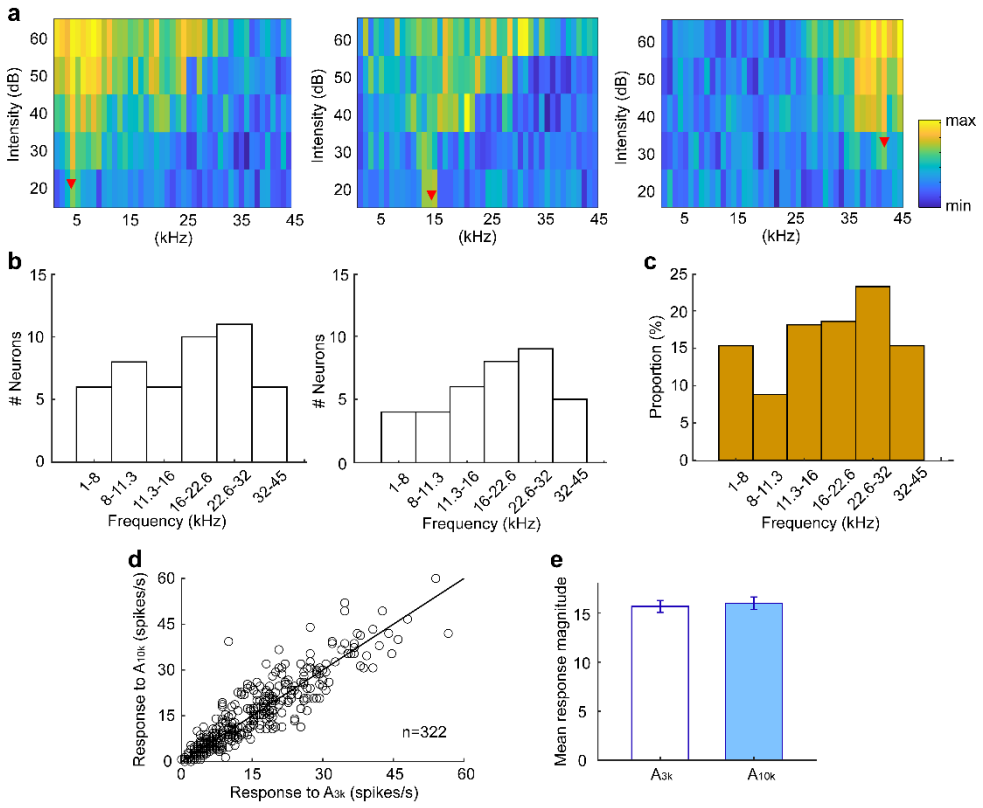
Author contributions

L.Y. and J.X. supervised and directed this project. L.Y. wrote the manuscript and revision. S.C. performed the experimental studies. J.X., L.K. and P.Z. helped with discussion of the study and contributed to the writing of the manuscript.

Competing interests

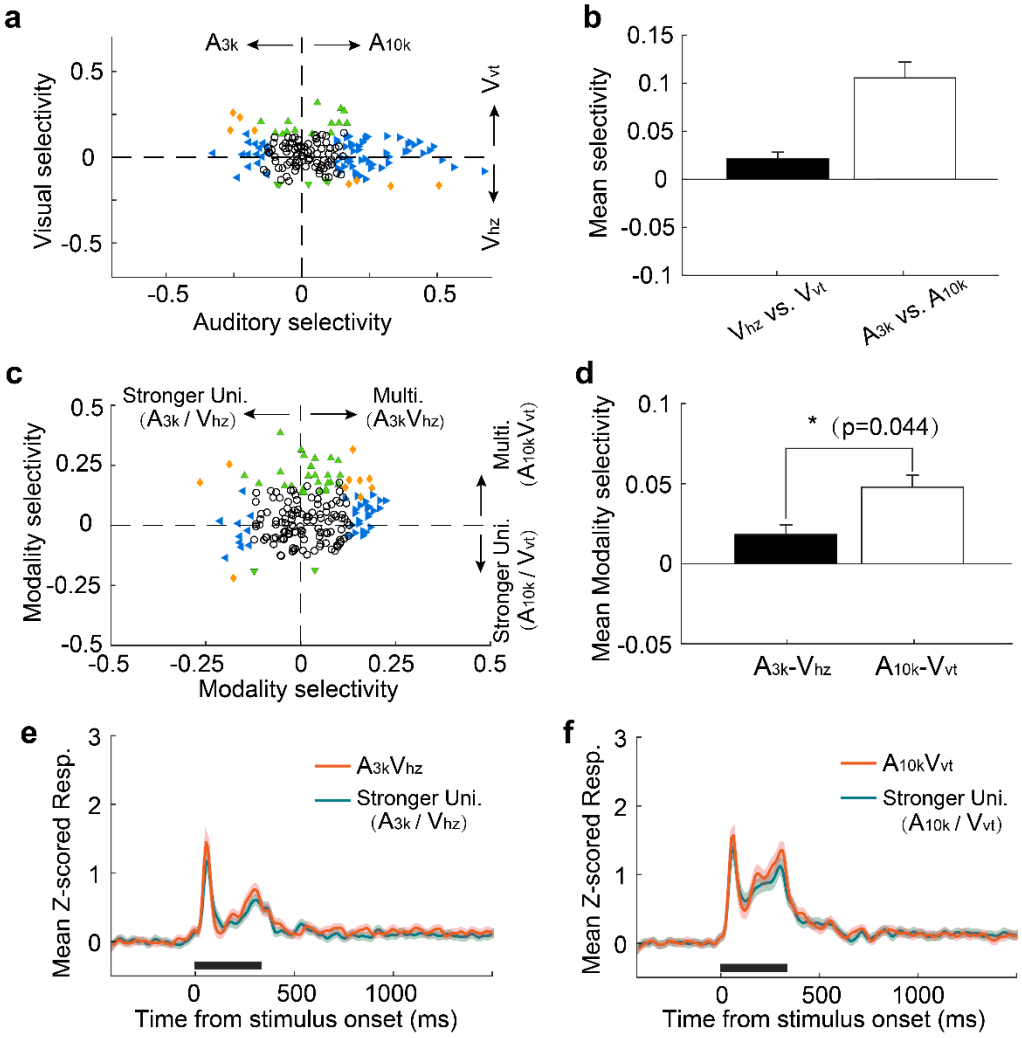
The authors declare no competing interests.

Supplemental figures



Supplementary Fig. 1 Characteristic Frequency (CF) and response to target sound stimuli of AC neurons recorded in well-trained rats under anesthesia

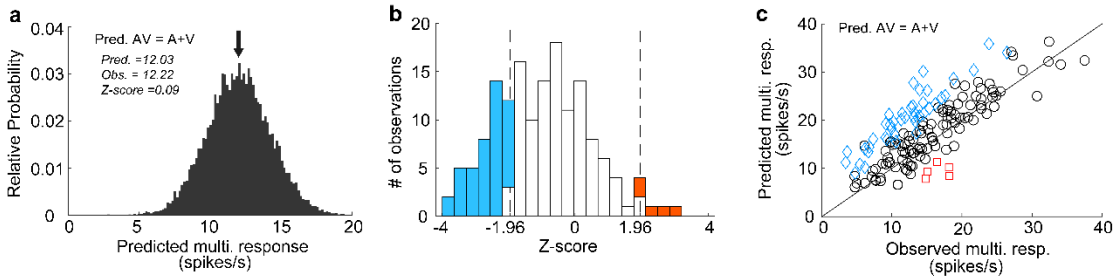
a Frequency-intensity tonal receptive fields (TRFs) of three representative AC neurons. The red triangle denotes CF. To create the TRF, we measured responses to a series of pure tone sounds (1-45 kHz in 1 kHz steps) at different intensities (20-60 dB in 10 dB increments). The color bar indicates the range of maximum and minimum spike response counts observed within the TRFs. **b** CF distributions of neurons recorded from two representative rats. **c** Overall distribution of all recorded neurons. **d** Comparison of responses to 3 kHz (A_{3k}) and 10 kHz (A_{10k}) pure tones. Each circle represents one neuron. **e** Comparison of mean response magnitude across populations for A_{3k} and A_{10k} conditions.



Supplementary Fig. 2 Cue and modality selectivity of AC neurons in well-trained rats under anesthesia

a Comparison of auditory and visual selectivity. Each point represents values for a single neuron. Open circles indicate neurons where neither auditory nor visual selectivity was significant ($p > 0.05$, permutation test). Triangles indicate significant selectivity in either auditory (blue) or visual (green) conditions, while diamonds represent significant selectivity in both auditory and visual conditions. Dashed lines represent zero cue selectivity. **b** Mean auditory (filled) and visual (unfilled) selectivity. Error bars represent SEMs. **c** Modality selectivity in A_{3k}-V_{hz} and A_{10k}-V_{vt} pairings. **d** Mean modality selectivity in A_{3k}-V_{hz} (unfilled) and A_{10k}-V_{vt}

(filled) pairings. Error bars represent SEMs. **e, f** Comparison of mean multisensory responses (red) with mean corresponding largest unisensory responses (dark blue) across populations for A_{3k}-V_{hz} (**e**) and A_{10k}-V_{vt} (**f**) pairings. The conventions used are consistent with those in Fig. 2 and Fig. 3.



Supplementary Fig. 3 The observed versus the predicted multisensory response

a Probability density functions of the predicted mean multisensory responses (predicted AV) based on summing the modality-specific visual (V) and auditory (A) responses (see *Methods* for details). Black arrow, the mean of the predicted distribution. The same neuron is shown in Fig. 2B and only responses in correct contralateral choice trials are involved. The predicted mean, actual mean observed, and their difference expressed in SDs (*Z*-score) are shown. **b** Frequency distributions of *Z*-scores. Open bars represent Z-scores between -1.96 and 1.96, indicating that the actual observed multisensory response is not significantly different from the predicted multisensory response (additive integration). Red bars, *Z*-score>=1.96 (superadditive integration); blue bars, *Z*-score<=-1.96 (subadditive integration). **c** Comparison between the mean predicted multisensory response and the actual observed multisensory response for all audiovisual neurons. Note that the predicted multisensory response is greater than the observed in most cases. Red squares and blue diamonds represent neurons with observed multisensory responses that are significantly greater (red squares) or less (blue diamonds) than the predicted mean multisensory responses.