Integration of visual information in auditory cortex promotes auditory scene analysis through multisensory binding

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Abstract

How and where in the brain audio-visual signals are bound to create multimodal objects remains unknown. One hypothesis is that temporal coherence between dynamic multisensory signals provides a mechanism for binding stimulus features across sensory modalities in early sensory cortex. Here we report that temporal coherence between auditory and visual streams enhances spiking representations in auditory cortex. We demonstrate that when a visual stimulus is temporally coherent with one sound in a mixture, the neural representation of that sound is enhanced. Supporting the hypothesis that these changes represent a neural correlate of multisensory binding, the enhanced neural representation extends to stimulus features other than those that bind auditory and visual streams. These data provide evidence that early cross-sensory binding provides a bottom-up mechanism for the formation of cross-sensory objects and that one role for multisensory binding in auditory cortex is to support auditory scene analysis.

28 When listening to a sound of interest, we frequently look at the source. However, how auditory and
29 visual information are integrated into a coherent perceptual object is unknown. The temporal
30 properties of a visual stimulus can be exploited to detect correspondence between auditory and visual
31 streams [1-3], can bias the perceptual organisation of a sound scene [4], and can enhance or impair
32 listening performance depending on whether the visual stimulus is temporally coherent with a target
33 or distractor sound stream [5]. Together, these behavioural results suggest that temporal coherence
34 between auditory and visual stimuli can promote binding of cross-modal features to enable the
35 formation of an auditory-visual (AV) object [6].

36 Visual stimuli can both drive and modulate neural activity in primary and non-primary auditory cortex
37 [7-11] but the contribution that visual activity in auditory cortex makes to auditory perception remains
38 unknown. One hypothesis is that the integration of cross-sensory information into early sensory cortex
39 provides a bottom-up substrate for the binding of multisensory stimulus features into a single
40 perceptual object [6]. We have recently argued that binding is a distinct form of multisensory integration
41 that underpins perceptual object formation, and can be separated from other sorts of integration by
42 demonstrating a benefit in the behavioural or neural discrimination of a stimulus feature orthogonal
43 to the features that link crossmodal stimuli (Fig. 1a). Therefore, in order to demonstrate binding an
44 appropriate crossmodal stimulus should elicit not only enhanced neural encoding of the stimulus
45 features that bind auditory and visual streams, but that there should be enhancement in the
46 representation of other stimulus features associated with the source (Fig. 1c).

47 Here we test the hypothesis that the incorporation of visual information into auditory cortex can
48 determine the neuronal representation of an auditory scene through multisensory binding (Fig.1). We
49 demonstrate that when visual luminance changes coherently with the amplitude modulations of one
50 sound stream in a mixture, the neural representation of that sound stream is enhanced in the auditory
51 cortex. Consistent with these effects reflecting cross-modal binding, the encoding of auditory timbre,

52 an orthogonal stimulus dimension, is subsequently enhanced in the temporally coherent auditory
53 stream.

54 Results

55 We recorded neuronal responses in the auditory cortex of awake (n=9 ferrets, 221 single units, 311
56 multi-units) and medetomidine-ketamine anesthetised ferrets (n=5 ferrets, 426 single units, 772 multi
57 units) in response to naturalistic time-varying auditory and visual stimuli adapted from Maddox et al[5].
58 Recordings in anesthetized animals allowed us to isolate bottom-up attention-independent
59 processing, permitted longer recording durations for additional control stimuli and enabled
60 simultaneous characterization of neural activity across cortical laminae. Recordings in awake animals
61 while they held their head at a drinking spout but were not engaged in a behavioral task allowed us
62 to measure neural activity free from any confounds associated with pharmacological manipulation
63 and in the absence of task-directed attention. The auditory streams were two vowels with a distinct
64 pitch and timbre (denoted A1: [u], fundamental frequency (F0) = 175 Hz and A2: [a], F0 = 195 Hz) each
65 of which was independently amplitude modulated with a low-pass (<7 Hz) envelope (Fig. 1d). A full-
66 field visual stimulus accompanied the auditory stimuli, the luminance of which was temporally
67 modulated with a modulation envelope from one of the two auditory streams (Fig. 1e). We tested
68 stimulus conditions in which a single AV stimulus pair was presented ('single stream' stimuli), where
69 the auditory and visual streams could be temporally coherent (A1V1, A2V2) or independently
70 modulated (A1V2, A2V1). We also tested a dual auditory stream condition in which both auditory
71 streams were presented and the visual stimulus was temporally coherent with one of the auditory
72 streams (A12V1 or A12V2, Fig. 1e).

73 Spike patterns in auditory cortex differentiate dynamic auditory-visual stimuli

74 Before exploring the impact of temporal coherence between auditory and visual stimuli on auditory
75 cortical neurons, we first used the responses to single stream stimuli to classify neurons according to
76 whether they were modulated primarily by auditory or visual temporal dynamics. To determine

77 whether the auditory amplitude envelope reliably modulated spiking we used a spike-pattern classifier
78 to decode the auditory stream identity, collapsed across visual stimuli (i.e. we decoded auditory
79 stream identity from the combined responses to A1V1 and A1V2 stimuli and the combination of A2V1
80 and A2V2 responses). An identical approach was taken to determine if neuronal responses reliably
81 distinguished visual modulation (i.e. we decoded visual identity from the combined responses to A1V1
82 and A2V1 stimuli and the combined responses elicited by A1V2 and A2V2). Neuronal responses which
83 were informative about auditory or visual stimulus identity at a level better than chance (estimated
84 with a bootstrap resampling) were classified as auditory-discriminating (Fig. 2a-b) and / or visual-
85 discriminating (Fig. 2c-d) respectively.

86 In awake animals, 39.5% (210/532) of units were auditory-discriminating, 11.1% (59/532) were visual-
87 discriminating, and only 0.38 (2/532) discriminated both auditory and visual stimuli. Overall a smaller
88 proportion of units represented the identity of auditory or visual streams in the anesthetised dataset:
89 20.2% (242/1198) were auditory-discriminating, 6.8% (82/1198) were visual-discriminating, and 0.58%
90 (7/1198) discriminated both.

91 During recordings made under anaesthesia, we also recorded responses to noise bursts and white-
92 light flashes (both 100 ms) presented separately and together to map AV responsiveness in auditory
93 cortex (Bizley et al., 2007). Specifically, responsiveness was defined using a two-way ANOVA (factors:
94 auditory stimulus [on/off] and visual stimulus [on/off]) on spike counts measured during stimulus
95 presentation. We defined units as being sound-driven (main effect of auditory stimulus, no effect of
96 visual stimulus or interaction), light-driven (main effect of visual stimulus, no effect of auditory
97 stimulus or interaction) or both (main effect of both auditory and visual stimuli or significant
98 interaction; $p < 0.05$). Using such stimuli revealed that the classification of units by visual / auditory
99 discrimination of single stream stimuli selected a subset of light or sound driven units and that the
100 proportions of auditory, visual and AV units recorded in our sample were in line with previous studies

101 (Bizley et al, 2007: 65.1% (328/504) of units were driven by noise bursts, 16.1% (81/504) by light
102 flashes and 14.1% (71/504) by both.

103 We hypothesised that temporal coherence between auditory and visual stimuli would enhance the
104 discriminability of neural responses, irrespective of a unit's classification as auditory or visual
105 discriminating. We confirmed this prediction by comparing discrimination of temporally coherent
106 (A1V1 vs. A2V2) and temporally independent (A1V2 vs. A2V1) stimuli (Fig. 2e, f): Temporally coherent
107 AV stimuli produced more discriminable spike patterns than those elicited by temporally independent
108 ones in both awake (Fig. 2e, pairwise t-test, auditory-discriminating $t_{418} = 34.277$, $p<0.001$; visual-
109 discriminating $t_{116} = 13.327$, $p<0.001$; All $t_{540} = 35.196$, $p<0.001$) and anesthetised recordings (Fig.2f,
110 auditory-discriminating $t_{482} = 27.631$, $p<0.001$; visual-discriminating $t_{162} = 22.907$, $p<0.001$; All $t_{664} =$
111 33.149, $p<0.001$).

112 What might underlie the enhanced discriminability observed for temporally coherent cross-modal
113 stimuli? The phase of low frequency oscillations determines the excitability of the surrounding cortical
114 tissue[12-14], is reliably modulated by naturalistic stimulation[15-19] and has been implicated in
115 multisensory processing[20,21]. We hypothesised that sub-threshold visual inputs could modulate spiking
116 activity by modifying the phase of the local field potential such that phase coupling to temporally
117 coherent sounds is enhanced. This in turn would provide a mechanism by which neuronal spiking was
118 enhanced when auditory and visual streams are temporally coherent.

119 **Dynamic visual stimuli elicit reliable changes in LFP phase**

120 Stimulus evoked changes in the local field potential (LFP) were evident from the recorded voltage
121 traces and analysis of cross-trial phase coherence demonstrated that there were reliable changes in
122 phase across repetitions of identical AV stimuli (Fig. 3 a, b). To isolate the influence of visual activity
123 on the LFP for each unit, and address the hypothesis that visual stimuli elicited reliable changes in the
124 LFP, we calculated phase and power dissimilarity functions for stimuli with identical auditory signals
125 but differing visual stimuli[17]. Briefly, this analysis assumes that if the phase (or power) within a

126 particular frequency band differs systematically between responses to two different stimuli, then
127 inter-trial phase coherence (ITPC) across repetitions of a single stimulus will be greater than across
128 randomly selected stimuli. For each frequency band in the LFP, we therefore compared "within-
129 stimulus" ITPC for responses to each stimulus (A1 stream Fig. 3c; A2 stream Fig. 3d) with "across-
130 stimulus" ITPC calculated from stimuli with identical auditory stimuli but randomly selected visual
131 stimuli (e.g. randomly drawn from A1V1 and A1V2; Fig. 3c). The difference between within-stimulus
132 and across-stimulus ITPC was then calculated across frequency and described as the phase
133 dissimilarity index (PDI) (Fig. 3e, f) with positive PDI values indicating reliable changes in phase
134 coherence elicited by the visual component of the stimulus.

135 We calculated PDI values for each of the four single stream stimuli and grouped conditions by
136 coherency (coherent: A1V1 / A2V2, or independent: A1V2 / A2V1). To determine at what frequencies
137 the across-trial phase reliability was significantly positive, we compared within-stimulus and across-
138 stimulus PDI for each frequency band (paired t-test with Bonferroni correction for 43 frequencies, α =
139 0.0012). In awake subjects we identified a restricted range of frequencies between 10 and 20 Hz where
140 visual stimuli enhanced the phase reliability (Fig. 4a, b). In anesthetised animals, average PDI values
141 were larger than in awake animals and all frequencies tested had single stream PDI values that were
142 significantly positive (Fig. 4d, e). We therefore conclude that visual stimulation elicited reliable
143 changes in the LFP phase in auditory cortex. In contrast to LFP phase, a parallel analysis of across trial
144 power reliability showed no significant effect of visual stimuli on LFP power in any frequency band
145 (Supplementary Fig. 1).

146 Next we asked whether there were any frequencies at which phase coherence was increased by AV
147 temporal coherence by performing a pairwise comparison of single stream PDI values, yielded from
148 temporally coherent and independent stimuli, for all frequency points. In anaesthetised animals, the
149 single stream PDI did not differ between coherent and independent stimuli at any frequency (Fig. 4f).
150 In awake animals, PDI values were similar for temporally coherent and temporally independent

151 stimuli, except in the 11-14 Hz band where coherent stimuli elicited significantly greater phase
152 coherence (Fig. 4c). Together these data suggests that visual inputs modulate the phase of the low
153 frequency field potential in auditory cortex independently of temporal coherence with auditory
154 stimuli, and are consistent with auditory cortical neurons integrating visual and auditory information
155 such that discriminability of spiking responses to temporally coherent auditory visual signals are
156 enhanced (Fig.2e, f).

157 **Visual information enhances the representation of the temporally coherent auditory stream in a**
158 **sound mixture**

159 Arguably the greatest challenge for the auditory brain is to reconstruct sound sources in the world
160 from their overlapping cochlear representations. Having demonstrated that temporal coherence
161 between auditory and visual stimuli enhances discriminability of auditory spiking responses, we next
162 asked whether the temporal dynamics of a visual stimulus could enhance the representation of one
163 sound in a mixture. We therefore recorded responses to auditory scenes composed of two sounds
164 (A1 and A2) presented simultaneously with a visual stimulus that was temporally coherent with one
165 or other auditory stream (A12V1 or A12V2). To test if a visual stimulus could enhance the
166 representation of the temporally coherent auditory stream in such dual stream stimuli we then
167 compared dual stream responses with responses to temporally coherent single stream stimuli.

168 Figure 5 illustrates this approach for a single unit: responses to the single stream AV stimuli (Fig. 5a)
169 formed templates against which we judged the similarity of responses to the dual stream stimuli (Fig.
170 5b). Responses to the dual stream stimuli more closely resembled A1V1 when the visual stimulus was
171 V1, and A2V2 when the visual stimulus was V2. In our analysis of single-stream encoding, this unit was
172 classified as visual-discriminating, but many auditory-discriminating units showed similar response
173 properties (e.g. Supplementary Fig. 2). Enhancement of the coherent auditory stimulus representation
174 was visible at the population level (Fig. 5c-f): Auditory cortical responses to dual-stream stimuli most
175 closely resembled responses to the single stream stimulus with the same visual component. This

176 finding was robust in both awake (Fig. 5d, pairwise t-test: $t_{540} = 6.073$, $p<0.001$) and anesthetised
177 animals (Fig. 5f, $t_{660} = 9.5137$, $p<0.001$) suggesting that these effects were not mediated by attention.

178 Modulation of dual stream responses by visual stimulus identity was not simply a consequence of the
179 shared visual component of single stream and dual stream stimuli (Fig. 6). To show this we decoded
180 responses to dual stream stimuli (A12V1 and A12V2) using responses to auditory-only stimuli (Fig. 6a;
181 A1 or A2). We also analysed responses to mixed auditory streams with no visual stimulus (A12) using
182 responses either to coherent single stream stimuli (A1V1, A2V2). A two-way repeated measures
183 ANOVA on the decoder responses with factors of visual stream (V1, V2, no visual), and template type
184 (AV or A) demonstrated a significant effect of visual stream identity on dual stream decoding (Fig. 6d,
185 $F(2, 528) = 19.320$, $p <0.001$), but there was no effect of template type ($F(1, 528) = 0.073$, $p = 0.787$)
186 or interaction between factors ($F(2, 528) = 0.599$, $p = 0.550$). Post-hoc comparison across units
187 revealed that without visual stimulation there was no tendency to respond preferentially to either
188 stream but that visual stream identity significantly influenced classification of dual stream responses.

189 The ability of a visual stimulus to modulate auditory representation in the dual stream condition was
190 observed across all cortical layers (defined by current source density analysis, see online methods,
191 Supplemental Fig. 3a,b) and across three tonotopic fields (Supplemental Fig. 3c,d) of anaesthetized
192 subjects. While present in all effects, the influence of the visual stimulus was strongest in the supra-
193 granular layers (supplemental Fig.3b). Thus cross-modal modulation by temporal coherence was a
194 general phenomenon across auditory cortex. Separating auditory, visual, and auditory-visual units
195 according to responsiveness to classic neurophysiological stimuli (noise bursts and light flashes)
196 revealed that the impact of a visual stimulus on the representation of a sound mixture was present
197 across functional sub-populations but strongest in visual and auditory-visual units (Supplemental Fig.
198 4). Finally, we observed these effects in both single and multi-units (Supplemental Fig.5).

199 **Visual stimuli elicit changes in LFP phase in the context of an auditory scene**

200 Our findings indicate that visual stimuli can shape the representation of auditory mixtures and that
201 temporal coherence between auditory and visual stimuli enhances across-trial phase coherence. To
202 understand whether changes in phase coherence could provide a mechanism for visual modulation of
203 auditory representations, we again generated within-stimulus ITPC for each dual-stream stimulus (Fig.
204 7a, A12V1 and A12V2) and across-stimulus ITPC by randomly selecting responses across visual
205 conditions (Fig. 7b). We then expressed the difference as the dual stream PDI (dual stream phase
206 dissimilarity index, Fig. 7c). Since the auditory components were identical in each dual stream
207 stimulus, the influence of the visual component on LFP phase could be isolated as non-zero dual
208 stream PDI values (paired t-test, bonferoni corrected, $\alpha = 0.0012$). In awake animals, dual stream PDI
209 was significantly greater than zero at 11-14Hz and 16-19 Hz (Fig. 7d, e) whereas in anesthetised
210 animals, we found positive dual stream PDI values across all frequencies tested (Fig. 7f, g). In
211 anesthetised animals where we were able to use the responses of units to noise and light flashes to
212 categorise units as auditory, visual or auditory-visual, we also confirmed significant PDI values in each
213 of these subpopulations of units (Supplemental Fig. 4c). In awake animals, we confirmed the
214 significance of PDI values in the 11-14 Hz range across different rates of amplitude modulation of the
215 auditory stimulus (Supplemental Fig. 6).

216 **Neural responses to auditory timbre deviants are enhanced when changes in visual luminance and**
217 **auditory intensity are temporally coherent**

218 A hall-mark of an object-based rather than feature-based representation is that all stimulus features
219 are bound into a unitary perceptual construct, including those features which do not directly mediate
220 binding[22]. We predicted that binding across modalities would be promoted via synchronous changes
221 in auditory intensity and visual luminance (Fig. 1b) and observed that the temporal dynamics of the
222 visual stimulus enhanced the representation of temporally coherent auditory streams (Fig. 2e-f and
223 5d-f). To determine whether temporal synchrony of visual and auditory stimulus components also
224 enhanced the representation of orthogonal stimulus features (Fig. 1c) and thus fulfil a key prediction

225 of binding, we introduced brief timbre perturbations into our dual stream stimuli (n = 4 deviants, two
226 in A1 and two in A2). Such deviants could be detected by human listeners and were better detected
227 when the auditory stream in which they were embedded was temporally coherent with an
228 accompanying visual stimulus[5]. We hypothesised that, despite containing no information about the
229 occurrence of deviants, a temporally coherent visual stimulus would enhance the representation of
230 changes in timbre in the responses of auditory cortical neurons.

231 To isolate neural responses to the timbre change from those elicited by the on-going amplitude
232 modulation, we extracted the 200ms epochs of the neuronal response during which the timbre
233 deviant occurred and compared these to epochs from responses to otherwise identical stimuli without
234 deviants. We observed that the spiking activity of many units differed between deviant and no-deviant
235 trials (e.g. Fig 8a) and so we used a pattern-classifier approach to estimate the presence/absence of a
236 timbre deviant in a given response window. We first considered the influence of temporal coherence
237 between auditory and visual stimuli on the representation of timbre deviants in the single stream
238 condition (A1V1, A1V2 etc.). We found that a greater proportion of units detected at least one deviant
239 when the auditory stream in which deviants occurred was temporally coherent with the visual
240 stimulus relative to the temporally independent condition. This was true both for awake (Fig. 8b;
241 Pearson chi-square statistic, $\chi^2 = 322.617$, $p < 0.001$) and anesthetised animals (Fig. 8e; $\chi^2 = 288.731$, p
242 < 0.001). For units that discriminated at least one deviant, discrimination scores were significantly
243 higher when accompanied by a temporally coherent visual stimulus (Fig.8c, awake dataset, pairwise
244 t-test $t_{300} = 3.599$ $p<0.001$; Fig. 8f, anesthetised data $t_{262} = 4.444$ $p<0.001$).

245 Across the population of units, we performed a two-way repeated measures ANOVA on discrimination
246 performance with visual condition (V1/V2) and the auditory stream in which the deviants occurred
247 (A1/A2) as factors. We predicted that enhancement of the representation of timbre deviants in the
248 temporally coherent auditory stream would be revealed as a significant interaction term. Significant
249 interactions were seen in both the awake (Fig. 8d, $F(1, 600) = 29.138$, $p<0.001$) and anesthetised

250 datasets (Fig. 8g, $F(1, 524) = 16.652$, $p<0.001$). We also observed significant main effects of auditory
251 and visual conditions in awake (main effect of auditory stream, $F(1, 600) = 4.565$, $p = 0.033$; main effect
252 of visual condition, $F(1, 600) = 2.650$, $p = 0.010$) but not anesthetised animals (main effect of auditory
253 stream, $F(1,524) = 0.004$, $p = 0.948$; main effect of visual condition, $F(1, 524) =1.355$, $p = 0.245$). Thus
254 we concluded a temporally coherent visual stimulus can enhance the representation of features (here
255 auditory timbre) orthogonal to those that promote binding between auditory and visual streams. This
256 finding is consistent with our model of cross-modal binding (Fig. 1a, c) and so these data fulfil our
257 definition of binding.

258 Discussion

259 Here we provide mechanistic insight into how auditory and visual information could be bound
260 together to form coherent perceptual objects. Visual stimuli elicit reliable changes in the phase of the
261 local field potential in auditory cortex that result in an enhanced spiking representation of auditory
262 information. These results are consistent with the binding of cross-modal information to form a
263 multisensory object. When two sounds are presented together within an auditory scene, the
264 representation of the stream that is temporally coherent with the visual stimulus is enhanced.
265 Importantly, this enhancement is not restricted to the encoding of the amplitude changes that bind
266 auditory and visual information but extends to the encoding of auditory timbre, a stimulus dimension
267 orthogonal to the dimensions that link auditory and visual stimuli. Thus our results meet the
268 requirements for a strict neural test of cross-modal binding that was laid out in Bizley et al.[6]. These
269 data provide a physiological underpinning for the pattern of performance observed in human listeners
270 performing an auditory selective attention task in which detection of a pertubation in a stimulus
271 stream is enhanced or suppressed, when a visual stimulus is temporally coherent with the target or
272 masker auditory stream respectively[5]. The electrophysiological data presented here suggest that the
273 temporally coherent auditory stream would be represented more effectively, making the task easier
274 when this stream was the target and making the task more challenging when it was the masker.

275 Surprisingly, the effects of the visual stimulus on the representation of an auditory scene can be
276 observed in anesthetised animals ruling out any top-down effect of attentional modulation.

277 Previous investigations of the impact of visual stimuli on auditory scene analysis have frequently used
278 speech stimuli. In order to probe more general principles that might relate to both speech and non-
279 speech processing we chose to employ non-speech stimuli, but utilized modulation rates that fell
280 within the range of syllable rates in human speech [23]. Previous work has demonstrated that a visual
281 stimulus can enhance the neural representation of the speech envelope both in quiet and in noise [3,
282 24, 25]. Being able to see a talker's mouth provides listeners with rhythm information and information
283 about the amplitude of the speech waveform which may help listeners by cueing them to pay
284 attention to the auditory envelope[26] as well as information about the place of articulation that can
285 disambiguate different consonants[27]. Visual speech information is hypothesised to be relayed in
286 parallel to influence the processing of auditory speech: Our data support the idea that early
287 integration of visual information occurs[26,28-30] and is likely to reflect a general phenomenon whereby
288 visual stimuli can cause phase-entrainment in the local field potential. Our data support the
289 contention that such early integration is unlikely to be specific to speech. Indeed low-frequency
290 entrainment to modulations in an on-going stimulus are observed in the human brain and have been
291 shown to optimize listening performance for non-speech stimuli [31]. In contrast, later integration is
292 likely to underlie information about speech gestures that might be used to constrain lexical identity[26].

293 Consistent with previous studies, our analysis of local field potential activity revealed that visual
294 information reliably modulated the phase of oscillatory activity in auditory cortex independently of
295 the modulation frequency of the stimulus[8-11]. Neuronal excitability varies with LFP phase[32-35] and may
296 be the physiological mechanism through which cross-sensory information is integrated. Our analysis
297 allowed us to isolate changes in LFP phase that were directly attributable to the visual stimulus and
298 identified reliable changes in the LFP phase irrespective of whether the visual stimulus was temporally
299 coherent with the auditory stimulus. Such a finding is consistent with the idea that the LFP phase

300 synchronization arises from fluctuating inputs to cortical networks[14,21,36]. Our finding that visual
301 stimulation elicited reliable phase modulation in both awake and anesthetised animals suggests that
302 bottom-up cross-modal integration interacts with selective attention, which also modulates phase
303 information in auditory cortex[20]. While our data suggest that cross-modal binding can occur in the
304 absence of attention, it is likely that the effects we observe in auditory cortex are the substrates on
305 which selective attention acts to further boost the representation of cross-modal objects.

306 In the awake animal the impact of visual stimulation on LFP phase reliability was smaller than in the
307 anesthetised animal and was restricted to a narrower range of frequencies, consistent with a
308 dependence of oscillatory activity on behavioural state[37-39]. Since the neural correlates of multisensory
309 binding are evident in the anesthetised animal, the specific increase in alpha phase reliability that
310 occurred in awake animals in response to temporally coherent auditory-visual stimulus pairs (Fig. 4c
311 & 7e) may indicate an attention-related signal triggered by temporal coherence between auditory and
312 visual signals. Phase resetting or synchronisation of alpha phase has been associated both with
313 enhanced functional connectivity[38] and as a top-down predictive signal for upcoming visual
314 information[40]. Disambiguating these possibilities would require simultaneous recordings in auditory
315 and visual cortex and/or recording during the performance of a task designed to explicitly manipulate
316 attention.

317 Temporal coherence between sound elements has been proposed as a fundamental organising
318 principle for auditory cortex[41, 42] and here we extend this principle to the formation of cross-modal
319 constructs. Our data provide evidence that one role for the early integration of visual information into
320 auditory cortex is to resolve competition between multiple sound sources within an auditory scene.
321 While previous studies have demonstrated a role for visual information in conveying lip movement
322 information to auditory cortex [3, 8, 9, 20], here we suggest a more general phenomenon whereby visual
323 temporal cues facilitate auditory scene analysis through the formation of cross-sensory objects. The
324 origin of the visual inputs is an open question but both visual cortical and sub-cortical structures

325 innervate tonotopic auditory cortex[7, 43]. Identifying which of these inputs is responsible for the
326 physiological effects we observe requires experiments that manipulate defined neural circuits.

327 In summary, activity in auditory cortex was reliably affected by visual stimulation in a manner that
328 enhanced the representation of temporally coherent auditory information. Enhancement of auditory
329 information was observed for sounds presented alone or in a mixture and for sound features that
330 were related to (amplitude) and orthogonal to (timbre) variation in visual input. Such processes
331 provide mechanistic support for a coherence based model of cross-modal binding in object formation.

Acknowledgments

This work was funded by grants to each author: JKB: Wellcome Trust / Royal Society WT098418MA; Biotechnology and Biological Sciences Research Council (BB/H016813/1), and an Action on Hearing Loss Studentship (596: UEI: JB); RKM: NIH K99DC014288 and Hearing Health Foundation Emerging Research Grant; AKCL: NIH R01DC013260; and an International Exchanges Scheme award from the Royal Society to JKB and AKCL.

Author contributions

HA, RKM, AKCL, JKB Conception and design, HA, SMT, KCW, GPJ, JKB Acquisition of data, HA, JKB Analysis and interpretation of data, HA, SMT, RKM, AKCL, JKB Drafting or revising the article.

Figures

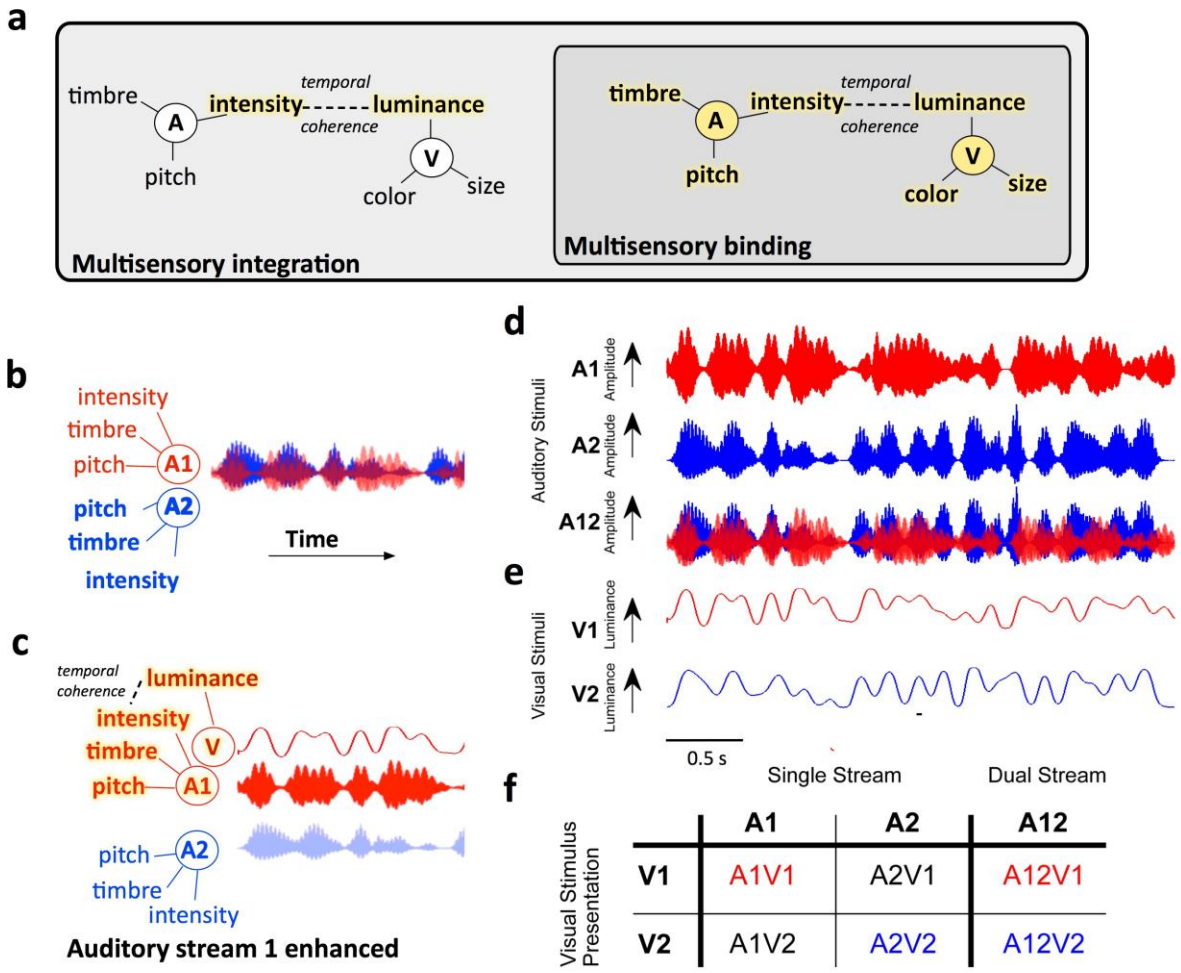


Figure 1: Hypothesis and experimental design

a Conceptual model illustrating how binding can be identified as a distinct form of multisensory integration. Multisensory binding is defined as a subset of multisensory integration which results in the formation of a crossmodal object. During binding, all features of the audio-visual object are linked and enhanced including both those features that bind the stimuli across modalities (here temporal coherence between auditory (A) intensity and visual (V) luminance) and orthogonal features such as auditory pitch and timbre and visual color and size. Other forms of multisensory integration would result in only the features that promote binding being enhanced - here auditory intensity and visual luminance. To identify binding therefore requires a demonstration that non-binding features (e.g. here pitch, timbre, color or size) are enhanced **b** When two competing sounds (red and blue

waveforms) are presented they can be separated on the basis of their features, but may elicit overlapping neuronal representations in auditory cortex. **c** Hypothesised enhancement in auditory stream segregation when a temporally coherent visual stimulus enables multisensory binding. When the visual stimulus changes coherently with the red sound (A1, top) this sound is enhanced and the two sources are better segregated. Perceptually this would result in enhanced auditory scene analysis and an enhancement of the non-binding features. **d** Stimuli design: Auditory stimuli were two artificial vowels (denoted A1 and A2), each with distinct pitch and timbre and independently amplitude modulated with a noisy low pass envelope. **e** Visual stimulus: a luminance modulated white light was presented with one of two temporal envelopes derived from the amplitude modulations of A1 and A2. **f** illustrates the stimulus combinations that were tested experimentally in *single stream* (a single auditory visual pair) and *dual stream* (two sounds and one visual stimulus) conditions.

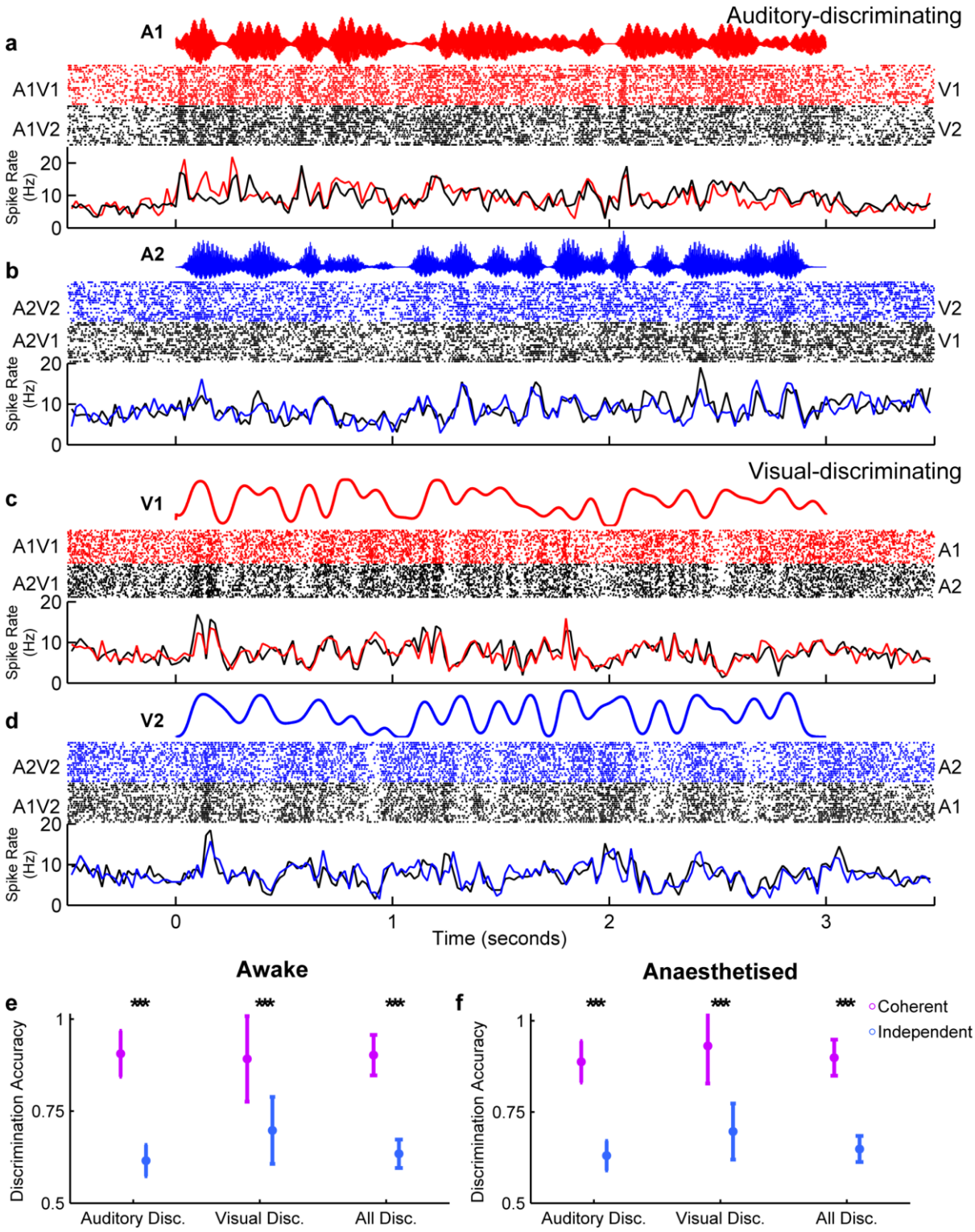
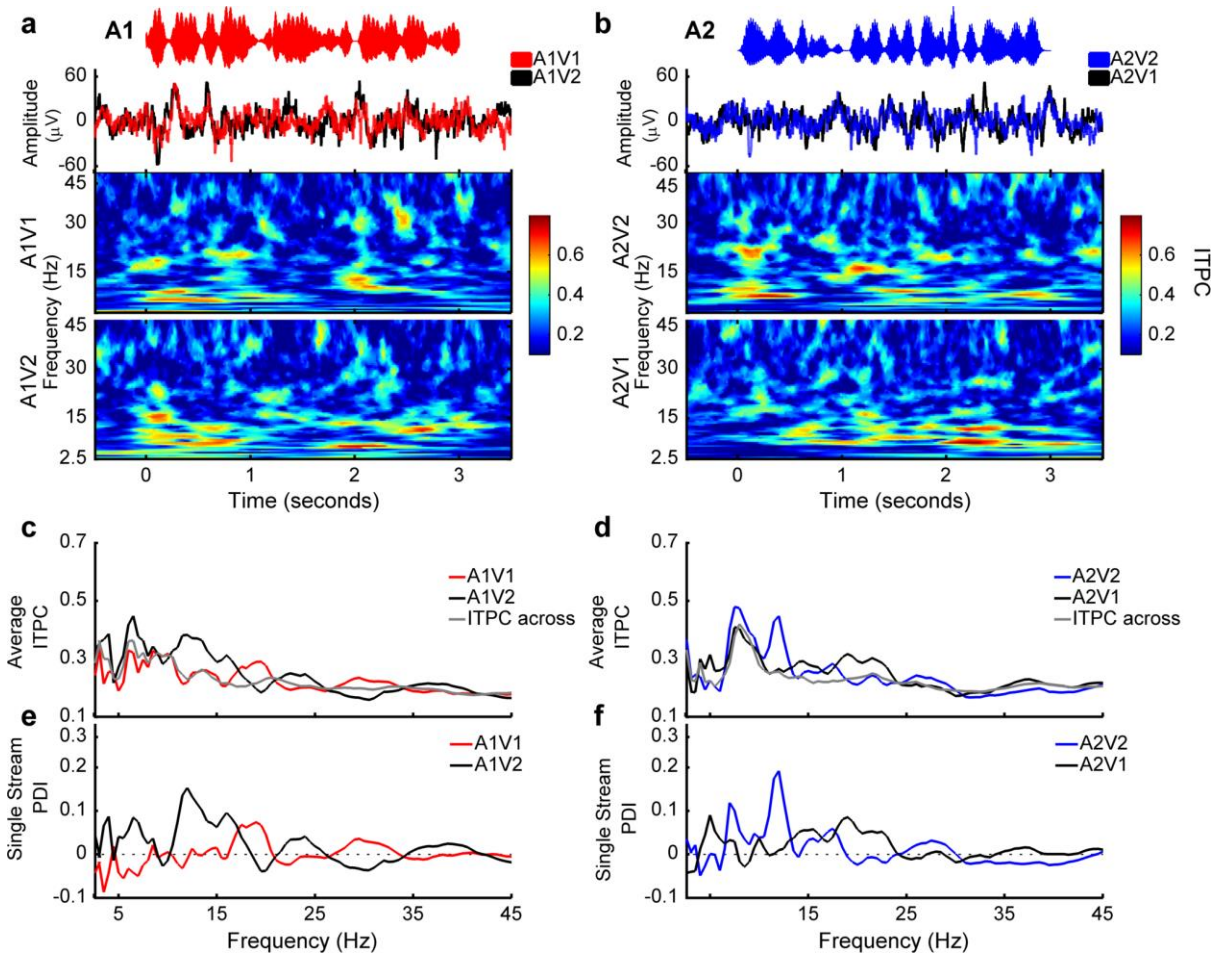


Figure 2: Auditory-visual temporal coherence enhances neural coding in auditory cortex.

A pattern classifier was used to determine whether neuronal responses were informative about auditory or visual stimuli. The responses to single stream stimuli are shown for two example units, with responses grouped according to the identity of the auditory (**a, b**, auditory discriminating unit)

370 or visual stream (**c, d**, visual discriminating unit). In each case the stimulus amplitude (a,b) / luminance
371 (c,d) waveform is shown in the top panel with the resulting raster plots (20 trials per condition) and
372 peri-stimulus time histogram (20 ms bin) below. **e, f**: Decoder performance (mean ± SEM) for
373 discriminating stimulus identity (coherent: A1V1 vs. A2V2; independent: A1V2 vs. A2V1) in auditory
374 and visual classified units recorded in awake (e) and anaesthetised (f) ferrets. Pairwise comparisons
375 for decoding of coherent versus independent stimuli: p<0.001 (***, see results).



376

377 **Figure 3: Visual stimuli elicit reliable changes in the phase of the local field potential**

378 **a, b** Example LFP responses to single stream stimuli across visual conditions. Data obtained from the
379 recording site at which multiunit spiking activity discriminated auditory stream identity in Fig. 2 a and
380 b. The amplitude waveforms of the stimuli are shown in the top panel, with the evoked LFP
381 underneath (mean across 21 trials). The resulting inter-trial phase coherence (ITPC) values are shown

in the bottom two panels. **c, d** ITPC was calculated for coherent and independent AV stimuli separately and compared to a null distribution (ITPC across). Single stream phase dissimilarity values (PDI) were calculated by comparing ITPC values to the ITPC across condition (**e, f**).

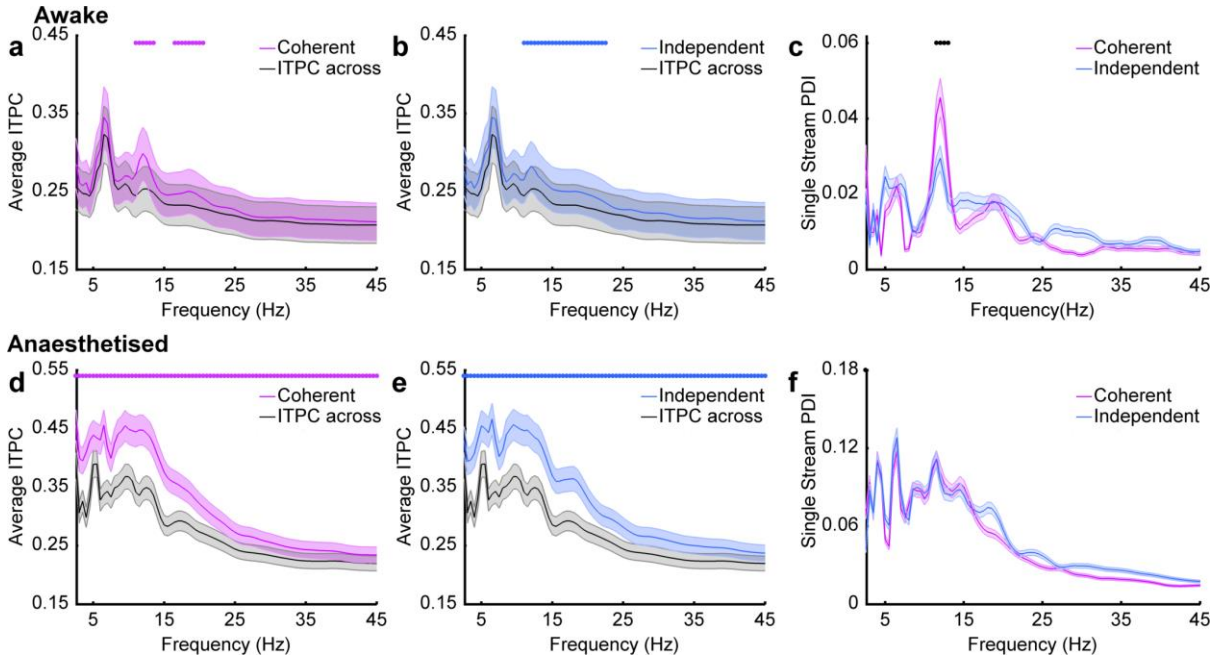


Figure 4: Visual stimuli elicit reliable changes in LFP phase in awake and anesthetised animals. Mean inter-trial phase coherence (ITPC) values across frequency for coherent (**a, d)** and independent (**b, e**) conditions. Dots indicate frequencies at which the ITPC values were significantly greater than chance (Pairwise ttest, α = 0.0012, Bonferroni corrected for 43 frequencies). **c f:** Mean (±SEM) single stream phase dissimilarity index (PDI) values for coherent and independent stimuli in awake (**c**) and anaesthetised (f) animals. Black dots indicate frequencies at which the coherent stream PDI is significantly greater than in the independent conditions ($p<0.001$).

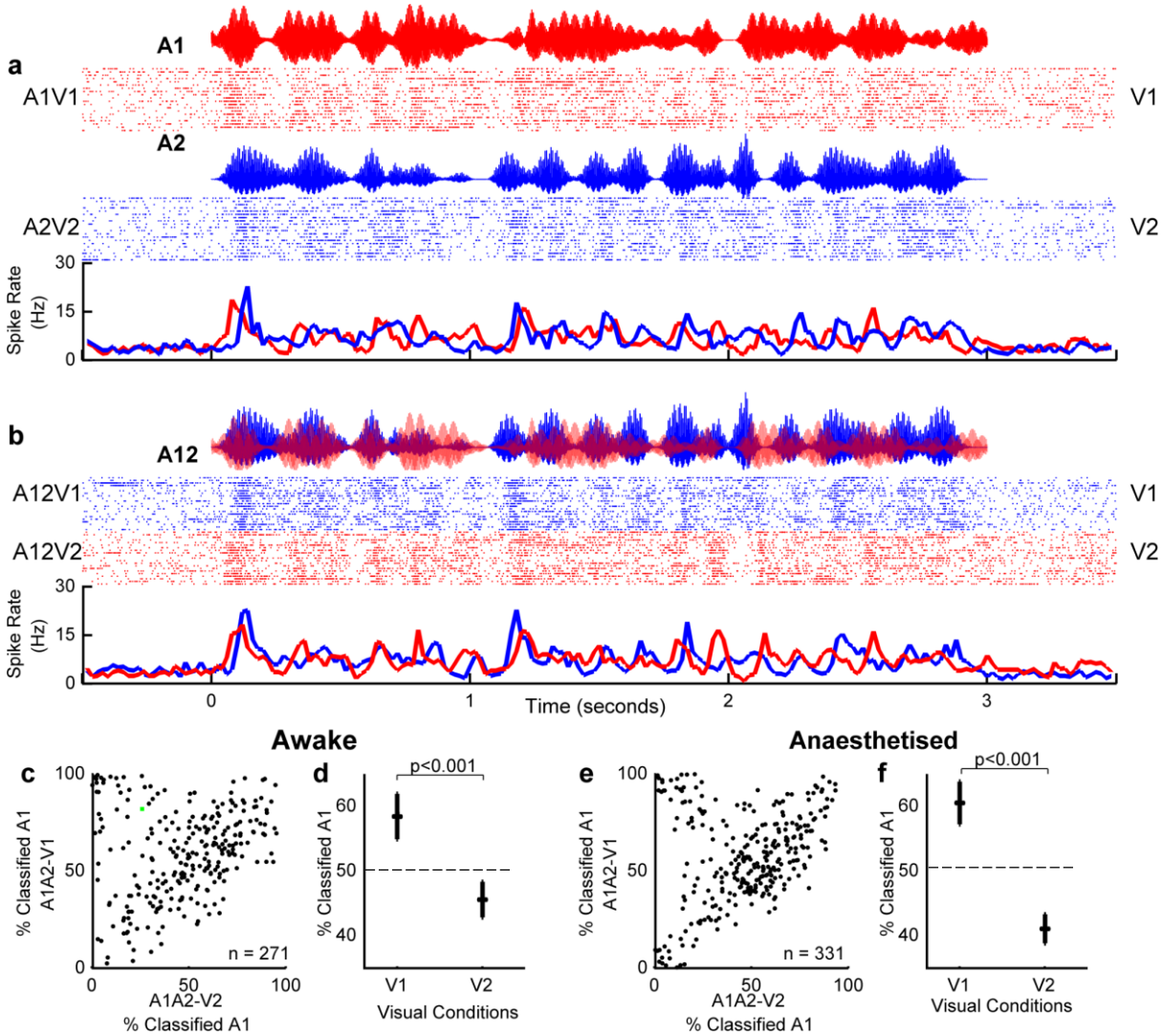
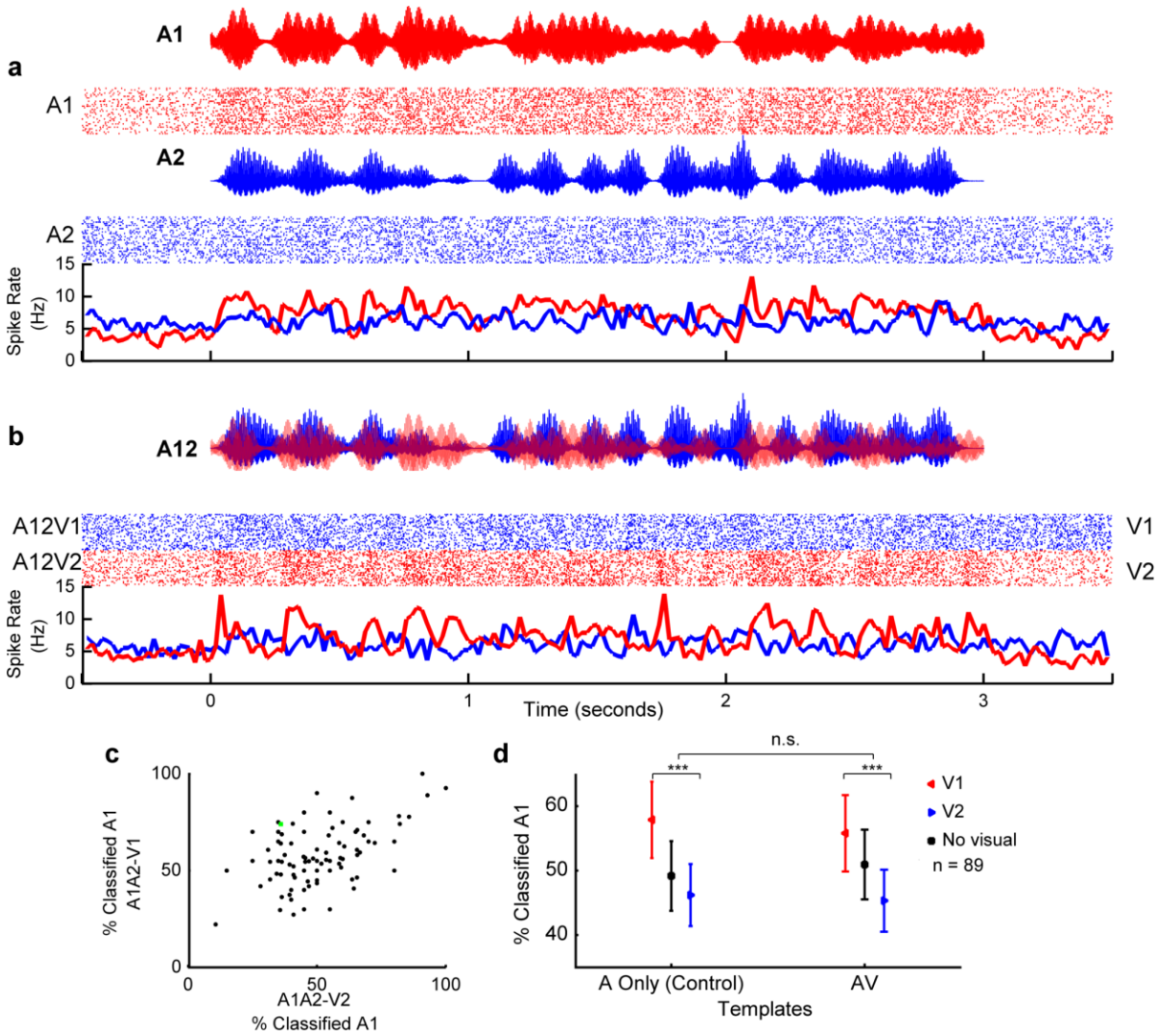


Figure 5: Visual stimuli can determine which sound stream auditory cortical neurons follow in a mixture.

Spiking responses from an example unit (visual classified unit from the awake dataset) in response to **a,** single stream AV stimuli used as decoding templates and **b**, dual stream stimuli, rasters and PSTH responses stimuli from which single trial responses were classified. When the visual component of the dual stream was V1, the majority of trials were classified as A1V1 (82% (19/23) of trials), and A2V2 when the visual stimulus was V2 (26% (6/23) of responses classified as A1V1 (see also green datapoint in c).). **c-f** data for awake (c,d) and anesthetised (e,f) datasets. In each case the left panel (c,e) shows the distribution of decoding values according to the visual condition and the right panel (d,f) shows

405 the population mean (± SEM) Pairwise comparisons revealed significant effect of visual conditionI on

406 decoding in both datasets (p<0.001).



407

408 **Figure 6: Visual stimuli shape the neural representation of an auditory scene.**

409 In an additional control experiment (n=89 units recorded in awake animals), the responses to coherent

410 AV and auditory-only (A Only) single stream stimuli were used as templates to decode dual stream

411 stimuli either accompanied by visual stimuli (V1/V2) or in the absence of visual stimulation (no visual).

412 Spiking responses from an example unit in response to **a,** single stream stimuli (A only, no visual) used

413 as decoding templates and **b**, dual stream stimuli, in each case the auditory waveform, rasters and

414 PSTHs are shown. A two-way ANOVA with template type and visual condition as factors revealed

415 significant effects of visual condition but not template type. Post-hoc comparisons demonstrated that

classification was significantly influenced by visual stimulus identity when both A only and AV templates were used. The proportion of responses classified as A1 when the visual stimulus was V1 or V2 are shown in **c** and **d,** Mean (± SEM) values for these units. Pairwise comparisons revealed significant effect across visual conditions in both datasets (p<0.001).

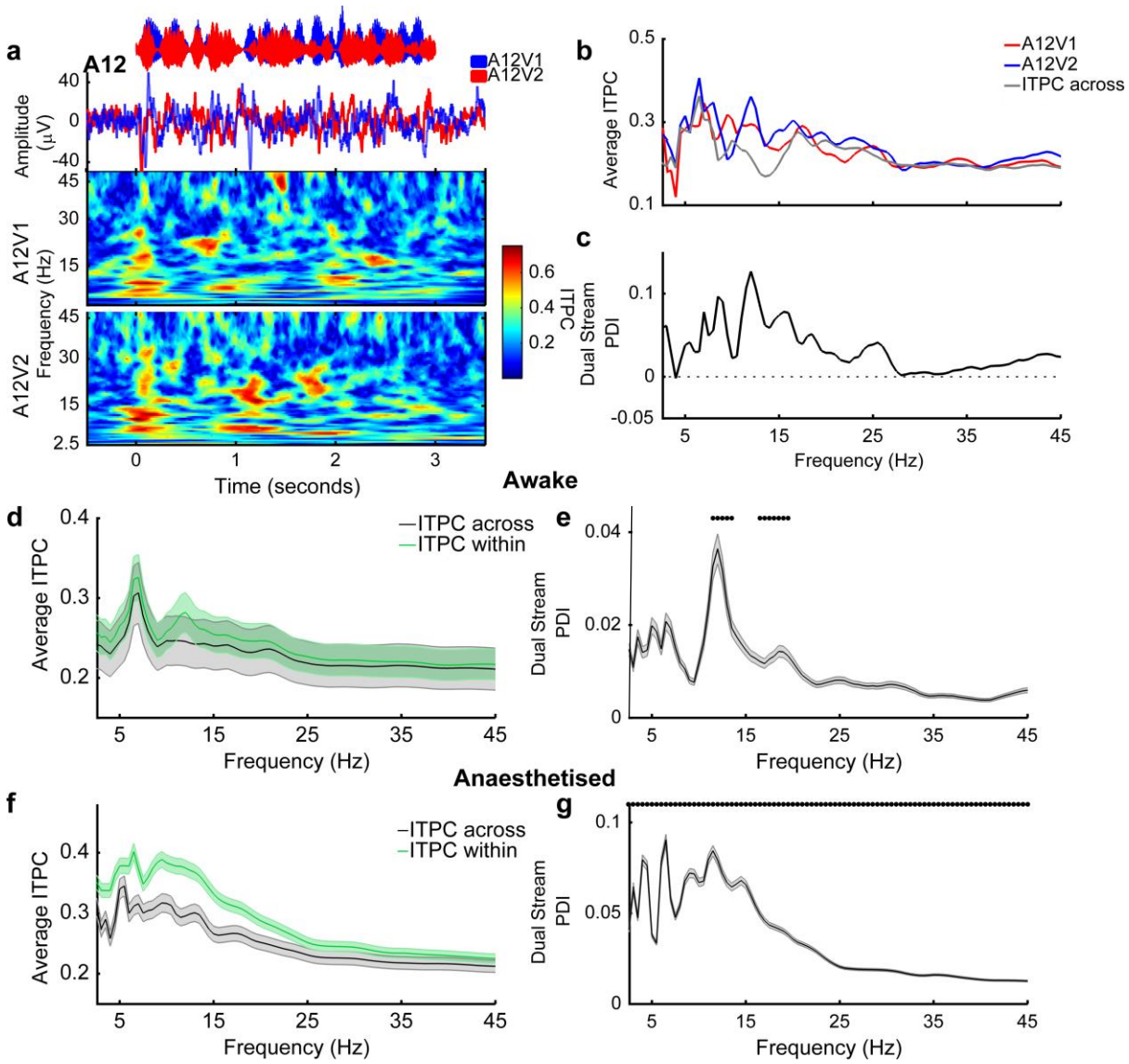


Figure 7: Visual stimuli elicits reliable changes in LFP phase to shape auditory scene analysis

a, Auditory stimulus waveform (top), evoked LFP (middle) and resulting inter-trial phase coherency plots (bottom) for a typical recording site in response to dual stream stimuli. **b**, ITPC values were calculated across frequency for responses to dual stream stimuli with identical visual stimuli ('ITPC within') and across randomly drawn visual stimuli ('ITPC-across', grey). Dual stream phase selectivity

index (PDI) values were then calculated as the difference between shuffled and dual stream ITPC (**c**)**.** **d, f** Average ITPC (± SEM) for dual stream ITPC-within and ITPC-across for awake and anaesthetised animals. Symbols indicate where the dual stream PDI was significant (pairwise t-test for ITPC within versus ITPC across, α = 0.0012 with correction). **e, g** Mean (± SEM) dual stream PDI values for awake and anaesthetised animals.

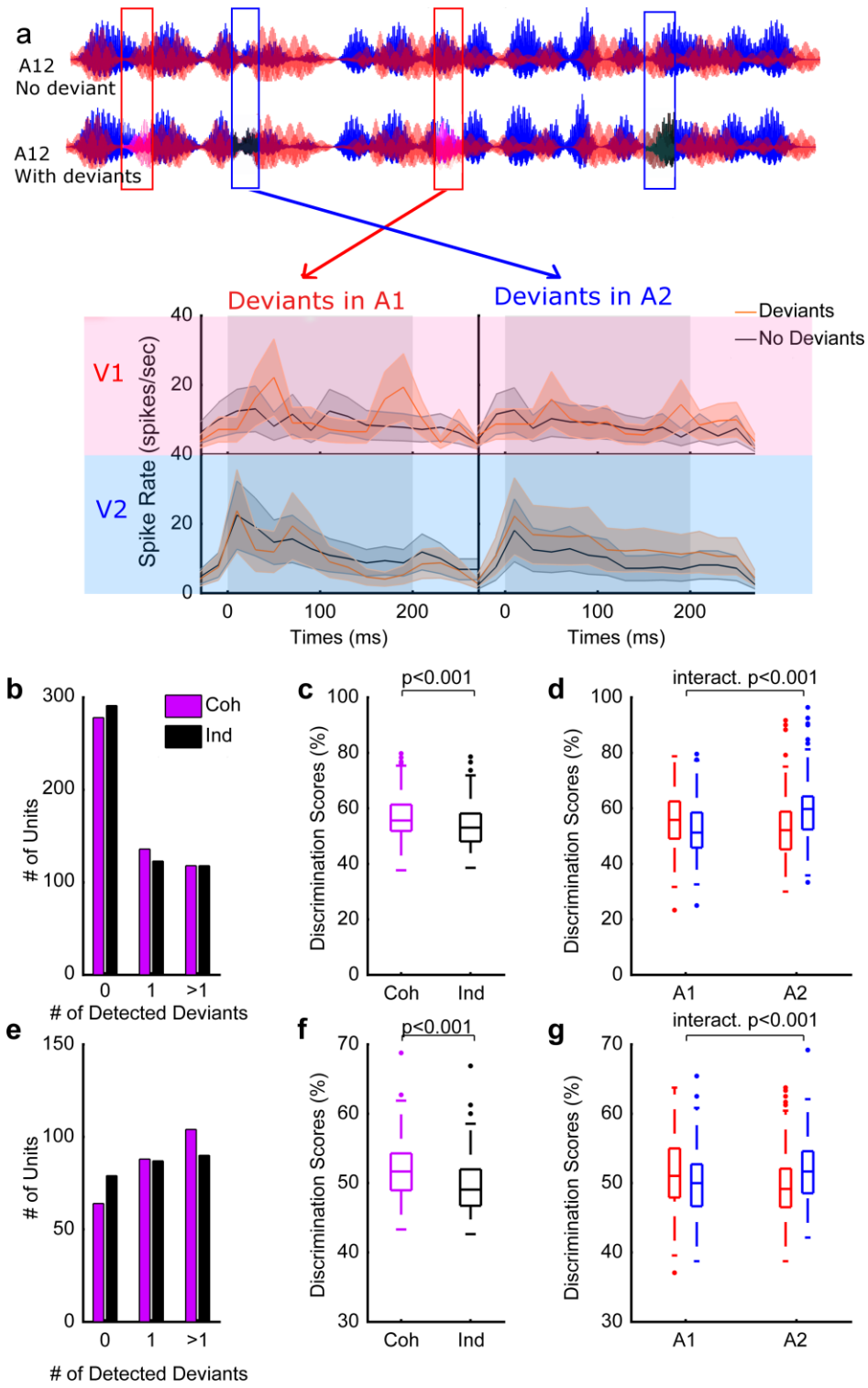
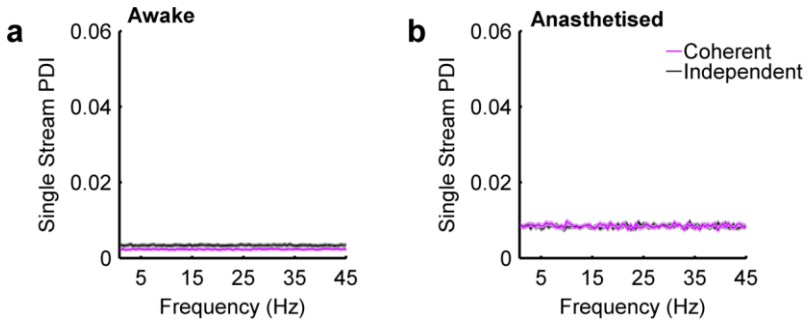


Figure 8: Temporally coherent changes in visual luminance and auditory intensity enhance the coding of a non-binding auditory feature.

a Example unit (from the awake dataset) showing the influence of visual temporal coherence on spiking responses to dual stream stimuli with or without deviants embedded. Shaded rectangles

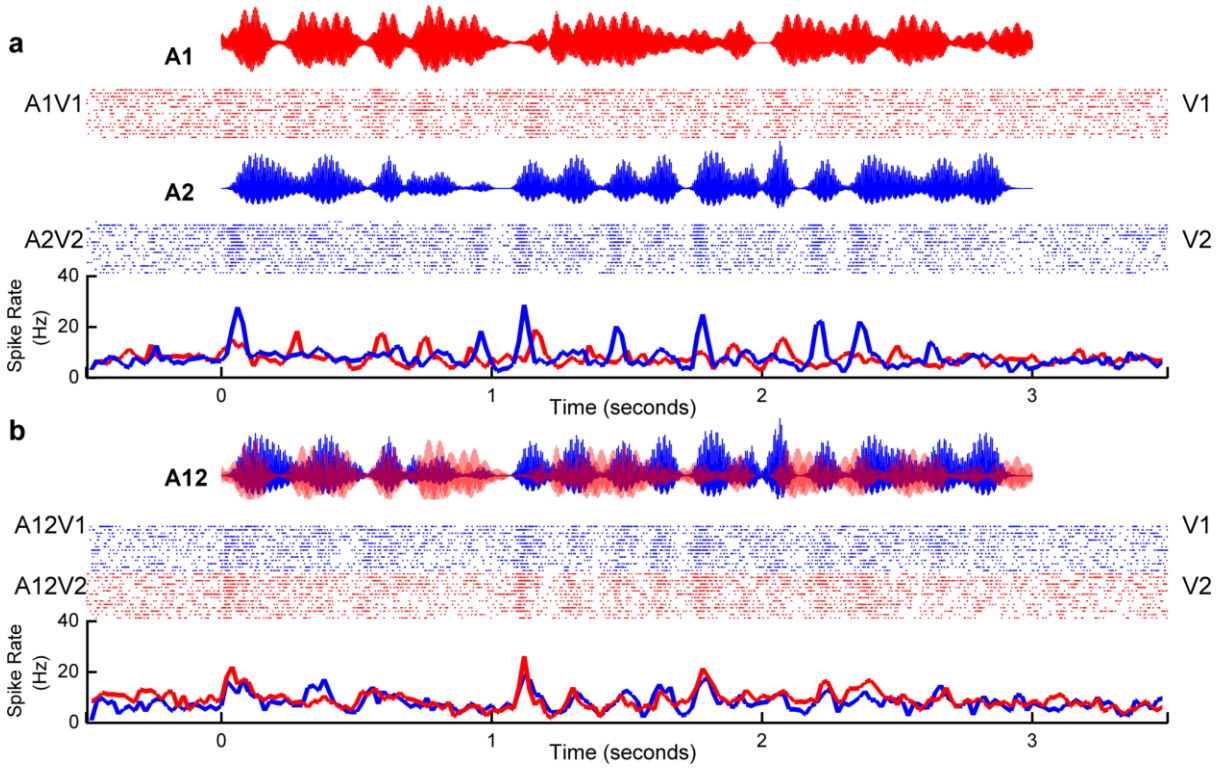
indicate the 200 ms window over which the timbre deviant occurred and over which analysis was conducted. **b-d** timbre deviant discrimination in the awake dataset. Two deviants were included in each auditory stream giving a possible maximum of 4per unit **b,** Histogram showing the number of deviants (out of 4) that could be discriminated from spiking responses **c,** Box plots showing the average timbre deviant discrimination scores in the single stream condition across different visual conditions (Coh: coherent, ind: independent). The boxes show the upper and lower quartile values, and the horizontal lines at their "waist" indicate the median. **d,** Discrimination scores for timbre deviant detection in dual stream stimuli. Discrimination scores are plotted according to the auditory stream in which the deviant occurred and the visual stream that accompanied the sound mixture. **e-g** show the same as **b-d** but for the anesthetised dataset.

Supplementary Figures



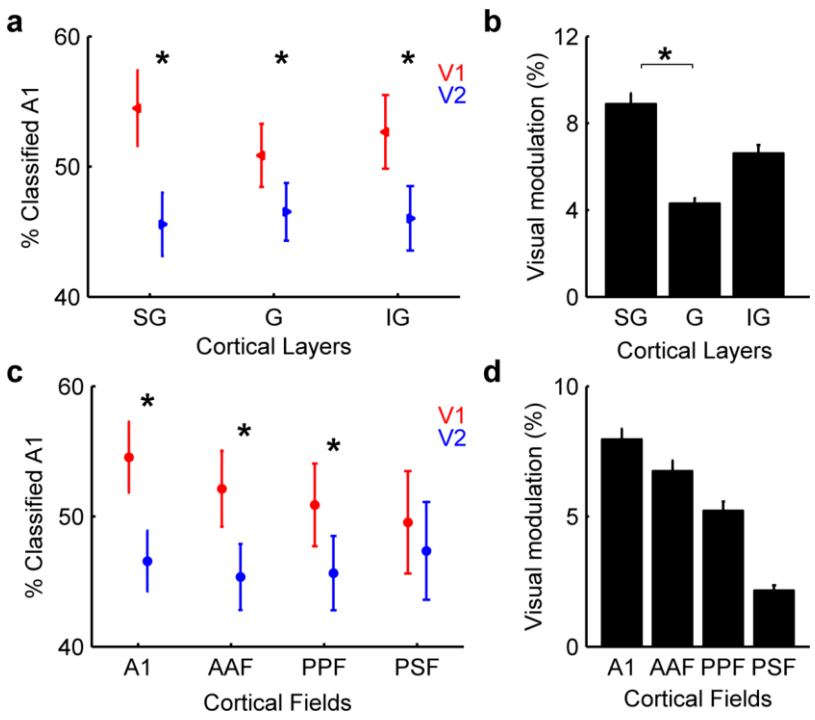
Supplementary figure 1: Dynamic visual stimuli do not elicit reliable changes in LFP power.

a, b: Mean (±SEM) single stream phase dissimilarity index values for coherent (A1V1 and A2V2) and independent (A1V2 and A2V1) stimuli at all recording sites at which there was a driven spiking response for awake (a) and anaesthetised (b) animals.



Supplementary figure 2: Visual stimuli can determine which sound stream auditory cortical neurons follow in a mixture in auditory-discriminating units

467 The spiking responses of an example unit are shown to coherent single stream (**a**) and dual stream
468 stimuli (**b**). This example unit was an auditory discriminating unit recorded from an awake animal.
469 When the visual stimulus was V1, 67% of trials were classified as A1V1, when the visual stimulus was
470 V2 only 29% of trials were classified as A1V1.

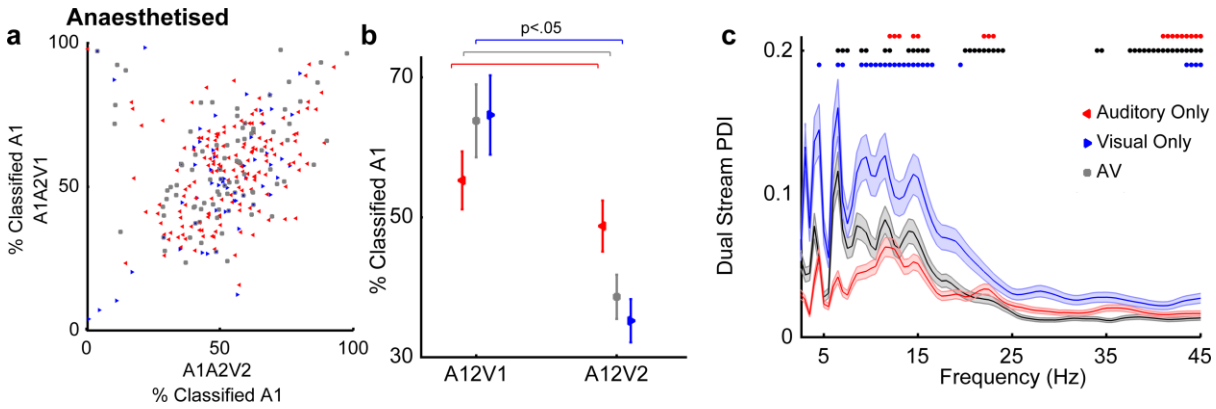


471

472 **Supplementary figure 3: Preference to the auditory stream with which the visual stimulus was**
473 **coherent observed across all cortical layers and in three tonotopic fields.**

474 In the anesthetised animal, we determined tonotopic gradients from responses to pure tone stimuli
475 to accurately confirm the cortical field in which any given recording was made. Recordings were made
476 with linear shank electrodes, facilitating current source density analysis to identify the cortical layers.
477 **a** Proportion of responses classified as A1 when the visual stimulus was V1 or V2 are shown for
478 supragranular layers (SG, t_{700}=5.686, p<0.001), granular layer (G, t_{878}=3.481 p<0.001) and intragranular
479 layer (IG, t_{690}=4.418, p<0.001). Data shown as mean (± SEM) across units. A two-way ANOVA across
480 visual condition and cortical layers showed only a significant effect of visual condition (F (1, 2273)
481 =64.288, p<0.001) but not of layers (F (1, 2273) =0.91, p =0.404) and no interaction was observed (F
482 (1, 2273) =2.679, p=0.068). Significant post-hoc comparisons (p<0.05) are shown with an asterisks. **b,**

483 In order to compare the magnitude of the effect of the visual stimulus across cortical layers we
484 calculated visual modulation as the decoding score when the visual stimulus was V1 – the decoder
485 score when the visual stimulus was V2. Plotted are the mean (± SEM) visual modulation across
486 different layers. A one-way ANOVA across cortical layers showed a significant effect of cortical layer
487 (F (2, 1134) =3.15, p =0.043). Post hoc comparison revealed that visual modulation in SG is greater
488 than G. **c**, as for a but for different cortical fields (A1, t_{798} =5.435, p<0.001; AAF, t_{636} =4.302, p<0.001;
489 PPF, t_{510} =3.609, p<0.001; PSF, t_{317} =0.932, p=0.352). A two way ANOVA across visual condition and
490 cortical field showed only a significant effect of visual condition (Visual condition: F (1, 2267) =40.301,
491 p<0.001; fields: F (1, 2267) =1.937, p=0.121; visual condition x fields: F (1, 2267) =1.804, p=0.144).
492 Significant post-hoc comparisons (p<0.05) are shown with an asterisks. In order to compare the
493 magnitude of the effects across cortical fields we again calculated a visual modulation value. **b,** Mean
494 (± SEM) visual modulation across different fields. A one-way ANOVA across cortical layers showed a
495 no significant effect of cortical field (F (3, 1130) =2.19, p =0.0877).



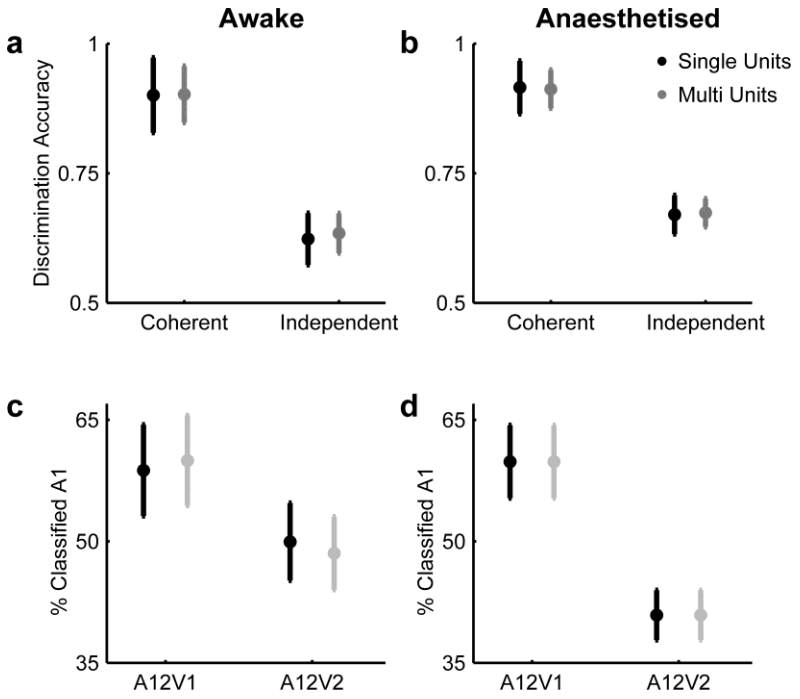
496

497 **Supplementary figure 4: Auditory, visual and auditory-visual units are influenced by visual stimuli.**

498 In anesthetised animals we also recorded responses to 100 ms noise bursts and/or LED flashes in order
499 to apply conventional classification of units as either auditory, visual or auditory-visual. Unit
500 classification was based on a two way ANOVA of spike counts calculated over a 200ms window
501 following stimulus onset with auditory and visual stimuli as factors. Units in which both auditory and
502 visual stimuli significantly modulated spiking or in which there was a significant interaction between

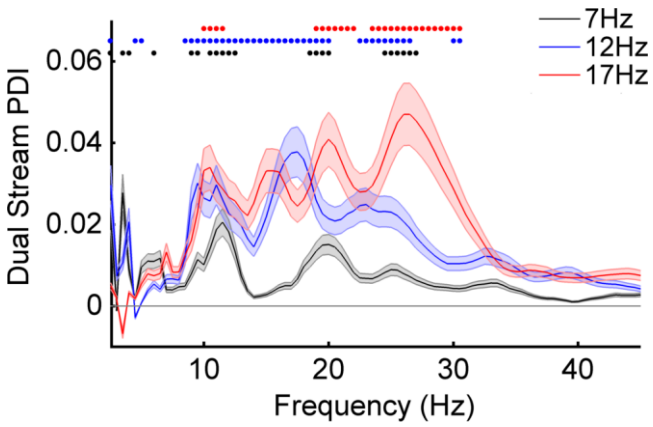
503 stimuli were classified as auditory-visual (AV). This analysis yielded recording sites at which units were
504 classified as auditory only (A; n=177 units), visual only (V, n=130) and AV (grey, n=150). This allowed
505 us to explore the visual modulation effects according to independently determined functional
506 definitions of units as auditory, visual or auditory visual.

507 **a**, The proportion of responses classified as A1 when visual stimuli as V1 or V2 is plotted with each
508 unit color coded according classification as A, V or AV. **b**, Mean (± SEM) values across the population.
509 Two way ANOVA revealed a significant effect of visual condition ($F(1, 1212) = 173.463$, $p<0.001$) and
510 unit type ($F(2, 1212) = 1780.803$ $p<0.001$), and interaction between factors ($F(2, 1212) =88.66$,
511 $p<0.001$) on the proportion of responses classified as A1. While the effect was strongest in AV and V
512 units, pairwise post-hoc comparisons revealed a significant effect of visual stimulus in all subgroups
513 ($p<0.05$). **c**, Mean (± SEM) dual stream phase dissimilarity index (PDI) values for recording sites
514 categorised according to the spiking responses recorded there. Symbols indicate where the dual
515 stream PDI index was significant (pairwise t-test, $p<0.001$ with correction). While the phase effects
516 are greatest at the sites where visual activity was recorded, significant dual stream PDI values were
517 observed in all three unit types. In all three cases significant phase coherence was seen at 12Hz,
518 13.5Hz-14.5Hz and 42.5-44.5Hz. Modulation at 10-12 Hz was only observed at sites in which AV and V
519 responses were recorded.



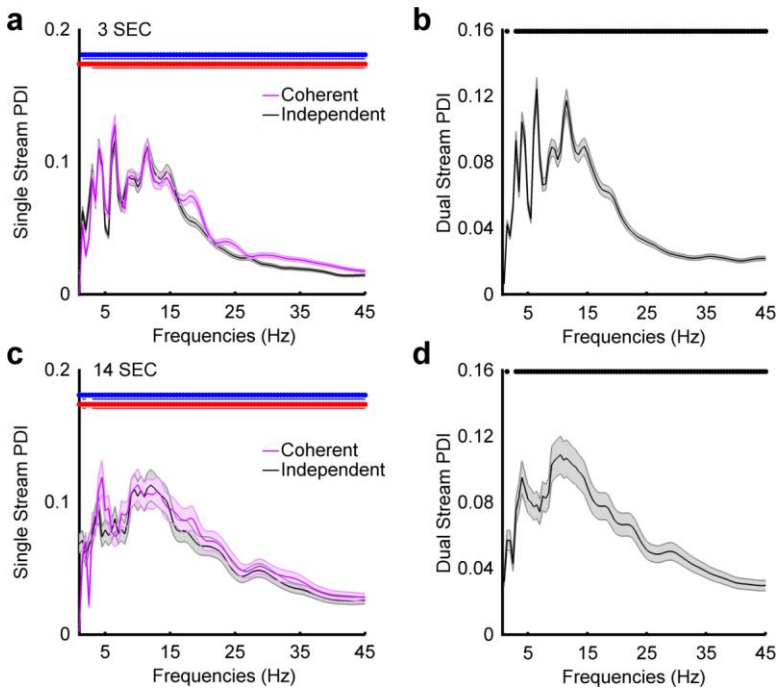
Supplementary figure 5: The effects of temporal coherence on single stream decoding and of visual identity on dual stream decoding are evident in both single and multi-units.

a,b Discrimination accuracy for single stream decoding is indistinguishable for single units and multi-unit activity. Two way ANOVA revealed a significant effect of visual condition and no effect of unit type in discrimination accuracy values in awake recording (a, Visual condition: $F(1,851) = 14.6607$ $p<0.01$, Unit Type: $F(1,851)= 1.02$, $p=0.312$; interaction: $F(1,851)=0.005$, $p=0.449$) and anaesthetised recording. (b, Visual condition: $F(1,1847) = 24.514$, $p<0.01$, Unit Type: $F(1,1847)= 0$, $p=0.986$; interaction: $F(1,1847)=0.66$, $p=0.418$) **c, d**. Impact of visual stimulus on dual stream decoding in single units and multi units. A two way ANOVA revealed only significant effect of visual condition and no effect of unit type in discrimination accuracy values in awake recording (c, Visual condition: $F(1,449) = 19.41$ $p<0.01$, Unit Type: $F(1,851)= 1.07$, $p=0.414$; interaction: $F(1,851)=0.33$, $p=0.568$) and anaesthetised recording. (d, Visual condition: $F(1,1847) = 15.41$, $p<0.01$, Unit Type: $F(1,1847)= 1.1$, $p=0.506$;interaction: $F(1,1847)=0.66$, $p=0.418$).



Supplementary figure 6: Dual stream PDI values in the alpha range are independent of amplitude modulation rate.

Stimuli were generated with three different amplitude modulation rates (<7Hz, as in the main experiment, <12Hz and <17Hz) and the responses to these were recorded in 92 units. Symbols indicate where the dual stream phase selectivity index was significant (pairwise t-test, $p<0.05$ with correction). In all three cases significant phase coherence is seen between 10Hz-11.5Hz, 19Hz-20Hz and 24-26 Hz.



Supplementary figure 7: Stimulus duration does not influence the frequency range across which significant single stream PDI values and dual stream PDI values are observed.

We observed that phase coherence values were higher for longer duration stimuli and therefore in order to directly compare anesthetised and awake datasets, all analysis in the anesthetised dataset was restricted to the first three seconds of stimuli. At longer stimulus durations the ITPC profile and resulting PDI varied more smoothly with frequency (a, c); However at both durations phase values were significantly different from zero at all frequencies. The pattern of significant phase selectivity values was also preserved across stimulus durations. (**b, d**). Frequency points at which the single stream PDI value and dual stream PDI values were similar in 3 second length (**a, b**) and 14 second length (**c, d**) Blue, red and black symbols indicate where the PDI was significant (pairwise t-test, α = 0.0012 with correction).

554 ## Online Methods

555 Animal preparation: The experiments were approved by the Committee on Animal Care and Ethical
556 Review of University College London and The Royal Veterinary College, and performed under license
557 from the UK Home Office and in accordance with the Animals Scientific Procedures Act 1986. Neural
558 responses were recorded in a total of 11 awake pigmented adult female ferrets (Mustela putorius
559 furo; 1-5 years old). Data from 9 of these animals was used for the main experiment (532 units), data
560 from 8 other animals (6/9 and two additional ferrets, 89 units) was collected for additional control
561 analysis (Figures 6e, supplemental figure 6). Animals were chronically implanted with recording
562 electrodes and passively listening/watching stimuli while holding their head at a water spout and
563 receiving continuous water reward. These animals were trained in various listening tasks for other
564 studies. An additional 5 adult females were used to record responses under anaesthesia.

565

566 Full methods for recording under anesthesia can be found in Bizley et al., [44]. Briefly, ferrets were
567 anesthetized with medetomidine (Domitor; 0.022mg/kg/h; Pfizer, Sandwich, UK) and ketamine
568 (Ketaset; 5mg/kg/h; Fort Dodge Animal Health, Southampton, UK). The animal was intubated and the
569 left radial vein was cannulated in order to provide a continuous infusion (5 ml/h) of a mixture of
570 medetomidine and ketamine in lactated ringers solution augmented with 5% glucose, atropine sulfate
571 (0.06 mg/kg/h; C-Vet Veterinary Products) and dexamethasone (0.5 mg/kg/h, Dexadreson; Intervet,
572 UK). The ferret was placed in a stereotaxic frame in order to implant a bar on the skull, enabling the
573 subsequent removal of the stereotaxic frame. The left temporal muscle was largely removed, and the
574 suprasylvian and pseudosylvian sulci were exposed by a craniotomy, revealing auditory cortex (Kelly
575 et al., 1986). The dura was removed over auditory cortex and the brain protected with 3% agar
576 solution. The eyes were protected with zero-refractive power contact lenses. The animal was then
577 transferred to a small table in a sound-attenuating chamber. Body temperature, end-tidal CO2, and
578 the electrocardiogram were monitored throughout the experiment. Experiments typically lasted

between 36 and 56 h. Neural activity was recorded with multisite silicon electrodes (Neuronexus Technologies) in a 1x 16, 2x 16 or 4x 8 (shank x number of sites) configuration.

Full surgical methods for recording implanting electrode arrays to facilitate recording from awake animals are available in Bizley et al. [45]. Briefly, animals were bilaterally implanted with WARP-16 drives (Neuralynx, Montana, USA) loaded with high impedance tungsten electrodes (FHC, Bowdoin, USA) under general anaesthesia (medetomidine and ketamine induction, as above, isoflurane maintenance 1-3%). Craniotomies were made over left and right auditory cortex, a small number of screws were inserted into the skull for anchoring and grounding the arrays, and the WARP-16 drive was anchored with dental acrylic and protected with a capped well. Animals were allowed to recover for a week before the electrodes were advanced into auditory cortex. Pre-operative, peri-operative and post-operative analgesia were provided to animals under veterinary advice.

Stimulus presentation: All stimuli were created using TDT System 3 hardware (Tucker-Davis Technologies, Alachua, FL) and controlled via MATLAB (Mathworks, USA). For recordings in awake animals, sounds were presented over two loud speakers (Visaton FRS 8). Water deprived ferrets were placed in a dimly lit testing box (69 x 42 x 52 cm length x width x height) and received water from a central reward spout located between the two speakers. Sound levels were calibrated using a Brüel and Kjær (Norcross, GA) sound level meter and free-field ½-inch microphone (4191). Auditory streams were presented at 65 dB SPL (Fig. 1a). Visual stimuli were delivered by illuminating the spout with a white LED which provided full field illumination (Precision Gold N76CC Luxmeter, 0 to 36.9 lux). The animals were not required to do anything other than maintain their heads in position at the spout where they were freely rewarded. Recording was terminated when animals were sated.

602 For anesthetised recordings, acoustic stimuli were presented using Panasonic headphones (Panasonic
603 RP-HV297, Bracknell, UK) at 65 dB SPL. Visual stimuli were presented with a white Light Emitting Diode
604 (LED) which was placed in a diffuser at a distance of roughly 10 cm from the contralateral eye so that
605 it illuminated virtually the whole contralateral visual field.

606

607 Stimuli and data acquisition: *Auditory stimuli* were artificial vowel sounds that were created in
608 Matlab (MathWorks, USA). In the behavioural experiment that motivated this study [5],stimuli were 14
609 seconds in duration. However, we adapted the stimulus duration in awake recordings to 3 seconds in
610 order to collect sufficient repetitions of all stimuli, and to ensure animals maintained their head
611 position facing forwards for the whole trial duration. In the anesthetised recording stimulus streams
612 were 14 seconds long, as in the human psychophysics [5] but we only analysed the first 3 seconds to
613 ensure datasets were directly comparable (Supplemental Figure 7).

614 Stimulus A1 was the vowel [u] (formant frequencies F1-4: 460, 1105, 2857, 4205 Hz, F0= 195Hz), A2
615 was [a] (F1-4: 936, 1551, 2975, 4263 Hz, F0= 175Hz). Streams were amplitude modulated with a noisy
616 lowpass (7 Hz cutoff) envelope. Unless specifically noted, the timbre of the auditory stream remained
617 fixed throughout the trial. However, we also recorded responses to auditory streams that included
618 brief timbre deviants. As in our previous behavioural study, deviants were 200ms epochs in which the
619 identity of the vowel was varied by smoothly changing the first and second formant frequencies to
620 and from those identifying another vowel. Stream A1 was morphed to/from [ɛ] (730, 2058, 2857,
621 4205 Hz) and A2 to/from [i] (437, 2761, 2975, 4263 Hz).

622 *Visual stimuli* were generated using an LED whose luminance was modulated with dynamics that
623 matched the amplitude modulation applied to A1 or A2. In single stream conditions a single auditory
624 and single visual stream were presented (e.g. A1V1, A1V2, A2V1, or A2V2) whereas in dual stream
625 conditions both auditory streams were presented simultaneously, accompanied by a single visual
626 stimulus (A12V1, A12V2, A12V1 A12V2) (Fig. 1e). Auditory streams were always presented from both

627 speakers so that spatial cues could not facilitate segregation, and stimulus order was varied pseudo-
628 randomly. In the anesthetised recordings each stimulus was presented 20 times. In the awake dataset,
629 where recording duration was determined by how long the ferret remained at the central location
630 (mean repetitions: 20, minimum: 14, maximum: 34).

631 During anaesthetised recordings, pure tone stimuli (150 Hz to 19 kHz in 1/3-octave steps, from 10 to
632 80 dB SPL in 10 dB, 100 ms in duration, 5 ms cosine ramped) were also presented. These allowed us
633 to characterize individual units and determine tonotopic gradients, so as to confirm the cortical field
634 in which any given recording was made. Additionally broadband noise bursts and diffuse light flashes
635 (100 ms duration, 70 dB SPL) were presented and used to classify a stimulus as auditory, visual or
636 auditory visual. LFPs were subjected to current source density analysis to identify sources and sinks as
637 described by Kaur et al. [46]

638

639 Data Analysis: Electrophysiological data were analysed offline. Spiking activity and local field potential
640 signals were extracted from the broadband voltage waveform by filtering at 0.3-5kHz and 1-150 Hz
641 respectively. Spikes were detected, extracted and then sorted with a spike-sorting algorithm
642 (WaveClus) (Quiroga et al., 2004).

643 We used a Euclidean distance based pattern classifier (Schnupp et al., 2006) with leave-one-out cross
644 validation to determine whether the neuronal responses to different stimuli could be discriminated.
645 Spiking responses to a given stimulus were binned into a series of spike counts from stimulus onset (0
646 s) to offset (3s) in 20 ms bins. The average across-repetition response to each stimulus (excluding the
647 to-be-classified response) were used as templates and the response to a single stimulus presentation
648 was classified by calculating the Euclidean distance between itself and the template sweeps and
649 assigning it to the closest template. To determine whether the classifier performed significantly better
650 than expected by chance, a 1000 iteration permutation test was performed where trials were drawn
651 (with replacement) from the observed data and randomly assigned to a stimulus that was then used

652 for template formation / decoding. A neural response was considered to be significantly informative
653 about stimulus identity if the observed value exceeded the 95th percentile of the randomly-drawn
654 distribution.

655 This approach allowed us to classify units according to their functional properties: auditory units
656 discriminated two auditory stimuli based on the amplitude modulation of sound (A1 versus A2)
657 regardless of visual dynamics, (Fig. 2a, b), visual units discriminated visual presentations based on
658 temporal envelope of visual stimuli (V1 versus V2) regardless of auditory presentation (Fig. 2c, d) and
659 AV units could do both. This approach was extended to classify dual stream responses by using the
660 average response to each of the temporally coherent single stream stimuli (A1V1 or A2V2) as
661 templates. Performance was (arbitrarily) expressed as the proportion of responses classified as being
662 from the A1, and compared for the two dual stream stimuli with different visual conditions (Figure 5).
663 To be considered in this analysis the response of a unit had to be informative about the single stream
664 stimuli (i.e. classified as either auditory and/or visual discriminating).

665

666 Phase/power dissimilarity analysis: Local field potential recordings were considered for all sites at
667 which there was a significant driven spiking response, irrespective of whether that response could
668 discriminate auditory or visual stream identity. For the single stream trials, we computed a single
669 Stream Phase Dissimilarity Index (PDI), which characterizes the consistency and uniqueness of the
670 temporal phase/power pattern of neural responses to continuous auditory stimuli (Luo and Poeppel,
671 2007). This analysis compares the phase (or power) consistency across repetitions of the same
672 stimulus with a baseline of phase-consistency across trials in which different stimuli were presented.

673 In the first stage of PDI analysis, we obtained a time-frequency representation of each response using
674 wavelet decomposition with complex 7-cycle Morlet wavelets in 0.5 steps between 2.5–45 Hz,
675 resulting in 86 frequency points. Next, we calculated the inter-trial phase-coherence value (ITPC;
676 Equ.1) at each time-frequency point, across all trials in which the same stimulus was presented. For

each frequency band, the ITPC time-course was averaged over the duration of the analysis window and across all repetitions to obtain the average *within-stimulus ITPC*.

$$ITPC_{t,f} = \left| \frac{\sum_{k=1}^{N} e^{i\theta_{k,t,f}}}{N} \right| \qquad \text{Equ.1}$$

In which N is equal to the number of trials, and θ is the phase of trial *k* at a given frequency (*f*) and time (*t*). The *across-stimuli ITPC* was estimated using the same approach but using shuffled data, such that the ITPC was computed across trials with the same auditory stimulus but randomly drawn visual stimuli. The single stream phase dissimilarity index (Single stream PDI) was computed as the difference between the ITPC value calculated for *within* trials and the ITPC values calculated *across* visual trials (Equ.2). y. The dissimilarity function for each frequency bin *i* was defined as;

$$Single\ Stream\ PDI_i = \frac{\sum_{j=1}^{N} ITPC_{ij}\ within_{vis}}{N} - \frac{\sum_{j=1}^{N} ITPC_{ij}\ ,across_{vis}}{N} \qquad \text{Equ.2}$$

Large positive PDI indicate that responses to individual stimuli have a highly consistent response on single trials. Single stream PDI values were calculated for each stimulus type and then averaged across stimuli to calculate values for temporally coherent and temporally independent auditory visual stimuli. Single stream PDI was positive if within stimulus ITPC was larger than across-stimulus ITPC (pairwise t-test, $p<0.05$ Bonferroni correction for 86 frequencies points) and was considered significant if a minimum of 2 adjacent bins exceeded the corrected threshold.

Dual stream phase dissimilarity index (dual stream PDI) values were calculated by extending this approach for dual stream stimuli with the goal of determining how the temporal envelope of the visual stimulus influences the neural response to a sound mixture. To this end, we calculated the *within-dual ITPC* from the A12V1 trials and A12V2 trials separately and *across-dual ITPC* by randomly selecting

trials from both stimuli (Equ.3). The within-dual and across-dual ITPCs were then averaged over time and subtracted to yield the dual stream PDI (Equ.3).

$$Dual\ Stream\ PDI_i = \frac{\sum_{j=1}^{N} ITPC_{ij}\ within_{dual}}{N} - \frac{\sum_{j=1}^{N} ITPC_{ij}\ across_{dual}}{N} \qquad \text{Equ.3}$$

Positive dual stream PDI values indicate that the time course of the neural responses was influenced by visual input, despite the identical acoustic input. We determined whether the dual stream PDI was greater if the *within_dual ITPC* was significantly larger than *across_dual ITPC* (pairwise t-test, $p<0.05$ Bonferroni correction, as above).

Timbre deviant analysis***:*** In order to determine how a visual stimulus influenced the ability to decode timbre deviants embedded within the auditory streams we used the cross-validated pattern classifier described above for analysing single stream stimuli to discriminate deviant from no-deviant trials. Responses were considered over the 200 ms time window that the deviant occurred (or the equivalent point in the no-deviant stimulus) binned with a 10 ms resolution. Significance was assessed by a 1000 iteration permutation test in which trials were randomly drawn with replacement from deviant and no-deviant responses. The discrimination score was calculated as the proportion of correctly classified trials.

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