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**Cross-modal and cross-temporal association in neurons of frontal cortex**

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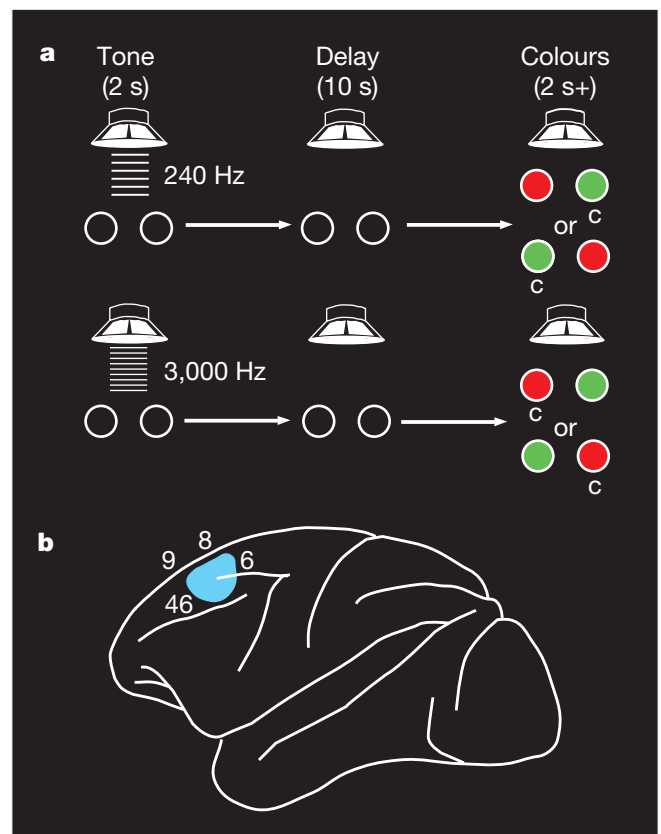
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The prefrontal cortex is essential for the temporal integration of sensory information in behavioural and linguistic sequences<sup>1,2</sup>. Such information is commonly encoded in more than one sense modality, notably sight and sound. Connections from sensory cortices to the prefrontal cortex support its integrative function<sup>3–5</sup>. Here we present the first evidence that prefrontal cortex cells associate visual and auditory stimuli across time. We gave monkeys the task of remembering a tone of a certain pitch for 10 s and then choosing the colour associated with it. In this task, prefrontal cortex cells responded selectively to tones, and most of them also responded to colours according to the task rule. Thus, their reaction to a tone was correlated with their subsequent reaction to the associated colour. This correlation faltered in trials ending in behavioural error. We conclude that prefrontal cortex neurons are part of integrative networks that represent behaviourally meaningful cross-modal associations. The orderly and timely activation of neurons in such networks is crucial for the temporal transfer of information in the structuring of behaviour, reasoning and language.

The cardinal function of the association cortex of the frontal lobes, or prefrontal cortex (PFC), is the temporal organization of behaviour (see ref. 2 for a review of the evidence). Prefrontal neurons support this function, in part, by their well documented contribution to short-term memory<sup>6–10</sup>. Some neurons are attuned to one or more attributes of visual stimuli in memory, such as spatial location or colour<sup>11,12</sup>. Others are attuned to the associations

of sensory stimuli with motor acts<sup>13</sup> or with reward<sup>14</sup>. Until now, however, there has been no evidence that PFC cells represent the association of sensory items of more than one sensory modality, or that they integrate these items across time. The purpose of our research was to obtain such evidence and establish a role of the PFC in cross-modal and cross-temporal integration. In contrast to previous studies, which have focused on associations between visual stimuli and motor acts, this study demonstrates that prefrontal cells integrate sensory stimuli of different modality that do not share physical dimensions. We show that PFC cells are not only accessible to auditory and visual stimuli, but also treat these stimuli as paired associates in accord with their behavioural role. The paired association by PFC cells takes place across modalities, across time and towards a goal.

The experiment was conducted on two rhesus monkeys trained to perform an audio-visual memory task (Fig. 1a). A trial in the task consisted of the following sequence: (1) a 2-s tone, high-pitch or low-pitch; (2) a 10-s delay; (3) the simultaneous display of two colours, red and green; (4) the manual selection of one colour depending on the tone (red if high tone, green if low tone); and (5) reward if the tone–colour match was correct. During the task, neuronal action potentials (spikes) were recorded extracellularly with microelectrodes from a wide region of dorsolateral frontal cortex bilaterally, including portions of areas 6, 8, 9 and 46 (Fig. 1b).



**Figure 1** Sound–colour matching task. **a**, Monkey faces panel with two translucent buttons. A trial begins with 240-Hz (low) or 3,000-Hz (high) tone of 2-s duration. Ten-second delay follows, at the end of which buttons are simultaneously lit, red and green. Animal must touch red button if tone was high, green if low. Correct choice (c) is rewarded. Tone and colour position change at random; about 30 s elapse between trials. During inter- and intratrial (delay) intervals, animal rests operant hand on a fixed pedal. Tone is delivered through overhead speaker, with an intensity of 45 dB above background. Each button subtends 8°. Colours are isoluminous (13.5 cd m<sup>-2</sup> ± 0.1 log unit); dominant λ: 620 nm for red, 530 nm for green. **b**, Brain diagram. Numbers indicate frontal cytoarchitectonic areas; blue depicts region containing most of the tone- and colour-differential units recorded.

**Table 1** Behavioural coherence of differential firing across trial periods\*

	Tone/delay (Δ <sub>1</sub> versus Δ <sub>2</sub> )	Colours/delay (Δ <sub>3</sub> versus Δ <sub>2</sub> )	Colours/tone (Δ <sub>3</sub> versus Δ <sub>1</sub> )	Chosen-colour/tone (Δ <sub>4</sub> versus Δ <sub>1</sub> )
Coherent	68	66	73	69
Non-coherent	26	28	21	25
Total units	94	94	94	94
	χ <sup>2</sup> = 18.76 P < 0.001	χ <sup>2</sup> = 15.36 P < 0.001	χ <sup>2</sup> = 28.76 P < 0.001	χ <sup>2</sup> = 20.59 P < 0.001

\* Coherence signifies the same sign of firing difference (delta) in two trial periods paired for comparison.

The firing frequency of isolated neurons was scrutinized for changes occurring in temporal relation to events in the task. In particular, the analysis focused on the cellular response to each tone and each colour. One objective of this analysis was to examine whether the selective reaction of a given cell to a tone was reflected subsequently, at the time of visual choice, by a comparable reaction to the behaviourally associated colour. This relationship would constitute evidence that the cell belongs to the cortical network representing the two associated stimuli in long-term memory (paired cross-modal association).

Most units in the sample of 325 showed changes of firing frequency in temporal relation to one or more task events or periods, although most of these changes were nonspecific for tone or colour. In 94 cells, however, firing at the tone or in the subsequent delay, or both, differed significantly depending on the tone that the monkey had to memorize for correct tone–colour match; the two tones induced a different degree of excitation or inhibition in the cells. Seventy-two of the 94 cells, called hereafter ‘tone-selective cells’, responded with increased firing to one tone and with a lesser excitation, or an inhibition, to the other. (The other 22 differential cells responded to the two tones with different degrees of inhibition.) Whereas in some cells the differential or selective reaction to a tone was limited to the tone period, in others it persisted during part or the entirety of the delay or memory period; and in some cells, tone-selective firing disappeared with the end of the tone only to reappear later in the delay, in anticipation of the colour. Figure 2 illustrates three low-tone selective cells with varying degree and duration of tone-differential firing in the delay period.

Regardless of discharge pattern during the delay, most of the 94 tone-differential units also responded differentially to the two simultaneously presented colours, depending on which of the two was the correct choice. Thus, their responses to colour presentation depended on the tone that had initiated the trial. These colour responses agreed, in most cells, with the behavioural associations of tones and colours. A cell selectively excited by high tone would be more excited at colour presentation in high-tone trials, when red was the correct choice, than in low-tone trials, when green was the correct choice; and vice versa for a low-tone selective cell (Figs 2 and 3). In summary, most differential units showed the same relation of firing to high and low tones as they did to red and green, respectively.

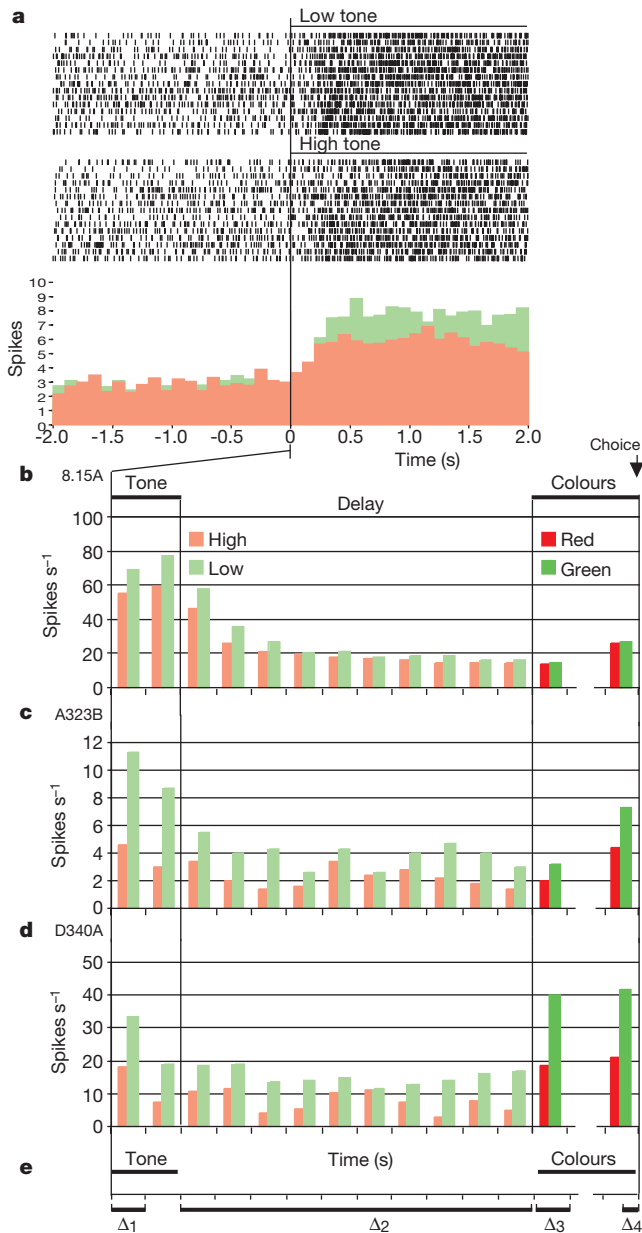
All 94 cells that differentiated the tones—and most of them also the colours—were located in areas 6, 8 or 9/46. Their estimated distribution by area<sup>15</sup> was as follows: 33 cells in area 6; 45 cells in area 8 (mainly 8b, upper prefrontal convexity); and 16 cells in area 9/46. By pooling the stereometric data from both hemispheres and monkeys, most differential units were estimated to lie within a frontal region (Fig. 1, blue area) that straddled and included parts of those three areas. The 231 non-differential units, whether task-related or not, were scattered more or less uniformly throughout the entire dorsolateral frontal region from which cell records were obtained.

Above we have shown in single cells the behavioural–associative–coherence of firing differences (deltas) between high-tone–red and low-tone–green trials across trial periods (tone, delay and colour). Not all cells, however, show coherence across all three periods; some do it only between tone and delay, others between delay and colour, and others between tone and colour. Furthermore, some cells show non-coherent deltas between periods (that is, deltas of opposite sign). The aggregate of the 94 cells, however, shows a highly significant predominance of coherence over non-coherence across all pairs of trial periods (Table 1).

The average deltas between tones and between colours were significantly correlated across trial periods not only in terms of sign but also in terms of magnitude (Fig. 4a). In trials terminating with incorrect choice, the four correlations of average differential firing between trial periods diminished or became negative (Fig. 4b).

By comparing mean spike-frequency responses to the two colours in the chosen-colour period ( $\Delta_4$ ), in high-tone selective cells and in low-tone selective cells, a significant bias for the corresponding colour could be observed in both groups of cells (Fig. 4c).

In conclusion, neurons of the frontal cortex integrate two sense modalities across time in support of behaviour. Because these

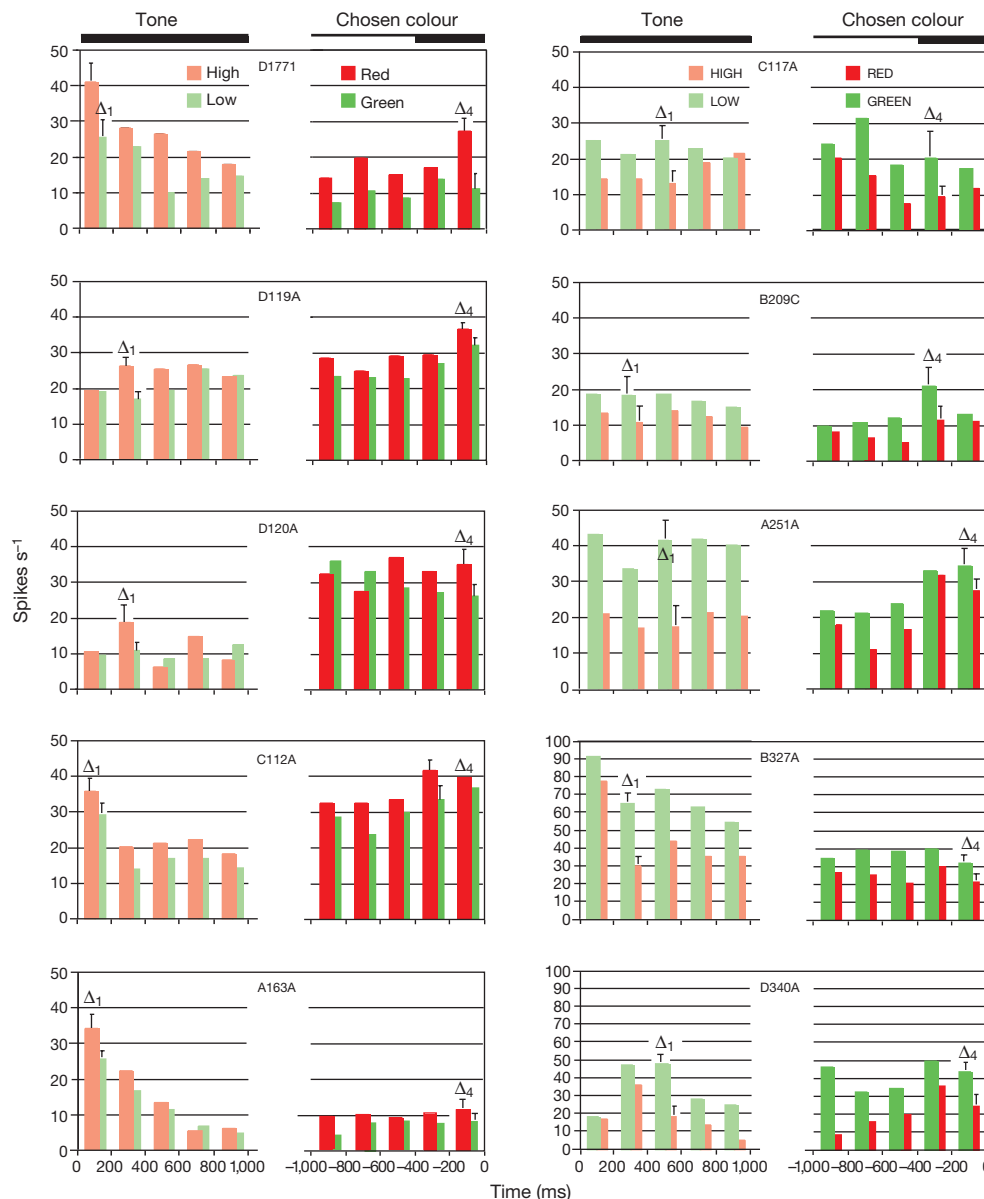


**Figure 2** Whole-trial activity of three low-tone selective cells. **a**, Spike rasters and average frequency histograms in baseline and tone period from cell in area 8. Light green indicates low-tone trials; pink indicates high-tone trials. **b**, Frequency histograms through high- and low-tone trials, 1-s time bins in the same cell. (Break of time line indicates variance in reaction time, generally 2.0–3.5 s.) The last bin of the histogram indicates mean firing in 1 s immediately preceding colour choice. **c**, Differential, low-tone selective activity throughout the delay from cell in area 6, bordering 8. As the animal selects a colour (trial's last second), discharge is higher in low-tone trials (green) than in high-tone trials (red). **d**, Increase in low-tone selective activity toward end of delay, anticipating green, from cell near that in **c**. Selectivity is maximal in colour period, when differential firing to colours matches differential firing to tones at trial's start. **e**, Schematic trial sequence indicating four periods in which firing-frequency differences (deltas) were measured for analysis of tone- and colour-dependent firing:  $\Delta_1$ , tone;  $\Delta_2$ , delay;  $\Delta_3$ , colours;  $\Delta_4$ , chosen colour.

neurons respond in correlated fashion to behaviourally associated stimuli of separate sensory origin, and because they are found over a broad frontal region, we infer tentatively that they belong to wide cortical networks representing cross-modal associations in permanent storage. Our results deviate from the model of the PFC which posits separate areas for object and spatial representations. The differential cells described here appear to represent cross-modal associations, not specific stimulus properties; furthermore, these cells are 'what' cells and are found in dorsolateral PFC, which has been assumed to represent the 'where' properties of external stimuli<sup>16</sup>.

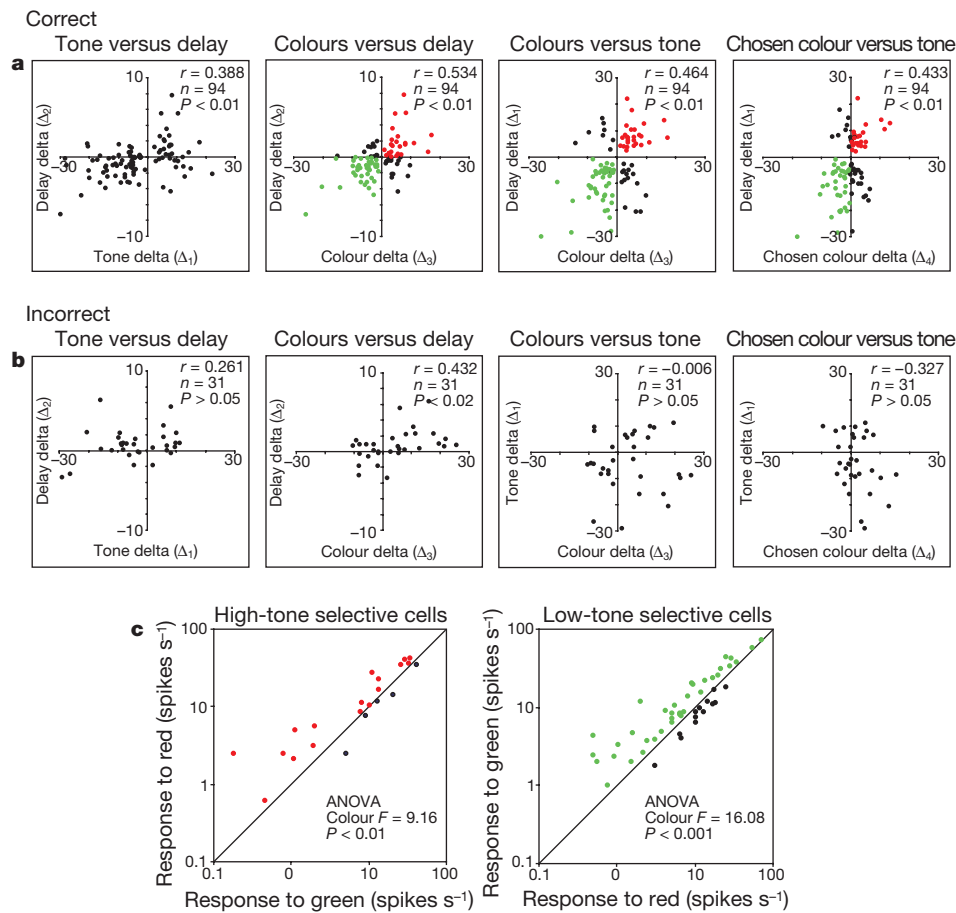
It cannot be concluded, however, that these cells perform only a sensory function—auditory, visual or cross-modal. Their associative properties probably extend to other attributes of the monkey's task that here were not controlled or measured (for example, hand and ocular movements). The representation of non-sensory attributes may be as much a part of the presumptive mnemonic network

to which the cells belong as the sounds and colours of the task. Non-differential reactions of many cells to sounds and colours, as well as the excitatory reactions of differential cells to both tones or both colours below the delta, probably reflect neuronal response to associated sensory qualities that are common to all trials (for example, loudness, brightness, location). Thus, the cells' cross-modal and cross-temporal associations that we have observed would simply contribute, with many others, to the structuring of behaviour by a vast sensory-motor network that extends well beyond our region of interest. Our cells seem to take part in at least three aspects of temporal integration: (1) activation of that network by the tone, which is one of the sensory components of the audio-visual association in permanent storage; (2) sustained low-level activation of the network in the 'working memory' of that association; and (3) reactivation of the network before and during presentation of the associated colour. Different cells appear to participate to a different degree in these three processes. The



**Figure 3** Firing-frequency histograms from ten prefrontal units showing behavioural concordance of firing at tones (first second of tone) and colours (last second of colour). Left, units responding preferentially to high tone and red correct; right, units preferring low tone and green correct. Estimated unit locations: left, from the top, area 9/46, 8b, 8b, 8b

and 8b; right, from the top, area 8b, 9/46, 8b, border 8b/6, and border 8b/6. Note that in all ten cells the firing difference between colours ( $\Delta_4$  and adjacent bins) matches in direction the difference between tones some 14 s earlier, at the start of the trial ( $\Delta_1$  and adjacent bins).



**Figure 4** Behaviourally concordant correlations between cell responses to tones and colours. **a**, Correlations between firing deltas across trial periods in 94 tone-differential cells, correct-response trials. **b**, Correlations between deltas in incorrect-response trials.

**c**, Scatter plots of response to colours by the 72 tone-selective cells (23 high-tone and 49 low-tone). Here, as in **a**, cells with high-tone-red bias are coded red and those with low-tone-green bias are coded green.

continuity of discriminant firing in the cellular aggregate through a trial denotes the selective and orderly activation of the network in the temporal transfer of cross-modal information, from the tone to the monkey's hand.

Beyond sensory cortices, complex sensory features are integrated in associative areas of temporal and parietal cortex<sup>17–20</sup>. Some of these areas are involved in intermodal association<sup>21–24</sup>. The integration of sensory stimuli with motor acts engages these areas in dynamic interaction with frontal cortex<sup>25–27</sup>, especially the PFC when the integration is across time, as our data indicate. The frontal region that we have explored is one of the highest stages of sensory-motor integration. It receives afferent connections from multiple areas of sensory association<sup>3–5,28</sup>. Our data indicate that a subregion within it (Fig. 1b, blue area) is especially rich in audiovisual associative cells. Accordingly, lesions of it produce deficits in conditional auditory and visual discrimination<sup>29</sup>. That frontal region is also strategically situated for the organization of action in time. The available evidence<sup>2</sup> supports a critical role of dorso-lateral prefrontal and premotor cortices in motor set and motor sequences, and thus, more broadly, in the temporal organization of behaviour. This role extends to the organization of language, which depends on the temporal integration of stimuli (words) encoded in the two sense modalities of our cross-modal task. □

**Methods**

**Behaviour**

The animals were trained in the audiovisual task to a criterion of a correct performance of 75% or higher. Training was laborious and took several months, mainly because of the

difficulty that monkeys have in learning auditory discriminations. Even after reaching criterion, performance remained variable throughout the experiment. No attempt was made to restrict or control the animals' ocular motility. As verified by a previous study<sup>30</sup>, monkeys performing colour choices, as they do here at the end of each trial, foveate the colour they are about to chose by hand. The experiment was conducted by strict adherence to animal use guidelines of the UCLA School of Medicine, the National Institutes of Health and the Society for Neuroscience.

**Recording**

The database for the present study consists of 325 units. Each cell was selected according to the following criteria: (1) clear separation of its action potentials from those of other cells; (2) stability of intertrial baseline firing; (3) records from a sufficient number of trials with each of the test stimuli; and (4) monkey's performance was at least 55% correct. The number of trials analysed varied from cell to cell depending on cell stability, firing frequency and the monkey's performance. On average, the 325 cells that met the criteria were recorded through a minimum of 10 correct-response trials with each tone. The median level of performance during recording was 76.6% correct. Recording sites were estimated by histology in one animal, and by stereotaxia and cortical landmarks in the other.

**Data analysis**

In a first stage of analysis, trial-related changes of firing frequency were computed for the 325 units in terms of deviations from baseline firing, that is, from the average firing of each cell in the period (10 s or longer) immediately preceding the trial. Spike discharge in the course of the trial was divided in time bins of 0.2, 0.5, 1.0, 5.0 or 10 s (last two bin-durations applied only to delay period). Average per bin difference from baseline, in spikes  $s^{-1}$ , was calculated across all correct-response trials with each tone. Average differences between blocks of trials with different tone were submitted to a Student's *t*-test for correlated means, with intertrial variance in the error term (significance of differences ascribed to  $P < 0.05$ ).

The analysis of deviations from baseline firing led to the identification of the 94 units on which the cross-modal and cross-temporal findings of this study are based. They were

selected by the following criteria: (1) stable intertrial baseline, without statistically significant differences in average firing rate between pre-low-tone and pre-high-tone baselines (the overall average baseline firing for the 94 cells was 10.03 spike s<sup>-1</sup>; and (2) statistically significant differences in firing between the two tones, at tone presentation or in the subsequent delay. These cells were submitted to the correlational analysis of average tone- and colour-related differences (deltas) between trial periods. Four such periods were the object of analysis: (1) first 1 s of tone; (2) 10-s delay; (3) first 1 s of colour presentation; and (4) 400-ms period immediately preceding manual choice of colour. Tone and colour periods (1, 3 and 4) were divided in 200-ms bins, and the entire delay period was treated as a 10-s bin. Four tone- or colour-related differences or deltas (Fig. 2e) were determined between high-tone (red choice) trials and low-tone (green choice) trials:  $\Delta_1$  (tone), largest 1-bin difference between responses to the two tones;  $\Delta_2$  (delay), tone-related difference in delay firing;  $\Delta_3$  (colours), largest 1-bin difference in response to the two simultaneous colours; and  $\Delta_4$  (chosen colour), largest 1-bin difference in pre-choice firing. Table 1 tabulates cells by correlation of delta sign across trial periods. Figure 4a, b shows delta correlations by magnitude. Expected correlations by chance were calculated by shuffling 1,000 times the delta values from both animals (94 differential units) in correct-response trials: the mean coefficient ( $r$ ) thus obtained for all four interperiod correlations ( $\Delta_1$  versus  $\Delta_2$ ,  $\Delta_3$  versus  $\Delta_2$ ,  $\Delta_3$  versus  $\Delta_1$ , and  $\Delta_4$  versus  $\Delta_1$ ) ranged between minus 0.002 and plus 0.003 (s.d. 0.103–0.105).

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**Phenotypic suppression of *empty spiracles* is prevented by *buttonhead***

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 Unlike the trunk segments, the anterior head segments of *Drosophila* are formed in the absence of pair-rule<sup>1,2</sup> and HOX-cluster gene<sup>3</sup> expression, by the activities of the gap-like genes *orthodenticle* (*otd*), *empty spiracles* (*ems*) and *buttonhead* (*btd*)<sup>4,5</sup>. The products of these genes are transcription factors<sup>6,7</sup>, but only EMS has a HOX-like homeodomain<sup>8,9</sup>. Indeed, *ems* can confer identity to trunk segments<sup>10</sup> when other HOX-cluster gene activities are absent<sup>3,11</sup>. In trunk segments of wild-type embryos, however, *ems* activity is prevented by phenotypic suppression<sup>10</sup>, in which more posterior HOX-cluster genes inactivate the more anterior without affecting transcription or translation<sup>12</sup>. *ems* is suppressed by all other Hox-cluster genes and so is placed at the bottom of their hierarchy<sup>10</sup>. Here we show that misexpression of EMS in the head transforms segment identity in a *btd*-dependent manner, that misexpression of BTD in the trunk causes *ems*-dependent structures to develop, and that EMS and BTD interact *in vitro*. The data indicate that this interaction may allow *ems* to escape from the bottom of the HOX-cluster gene hierarchy and cause a dominant switch of homeotic prevalence in the anterior–posterior direction.

Combined activities of *otd*, *ems* and *btd*<sup>5</sup> generate and specify *Drosophila* head segments (Fig. 1a–d) in the absence of pair-rule and homeotic gene activities<sup>4</sup> (Fig. 1e). *btd* alone is required for development of the mandibular segment, *btd* plus *ems* for the intercalary segment, and *btd*, *ems* plus *otd* for the antennal segment (Fig. 1e). Misexpression of *btd* or *otd* in the prospective head region failed to cause homeotic transformations<sup>13,14</sup> showing that neither of the two genes carries the proposed homeotic function in head segmentation<sup>5</sup>. To explore the untested homeotic role of *ems* and to address a possible cooperation with *btd*, we misexpressed the *ems* protein (EMS) in the *btd* domain of otherwise wild-type embryos. EMS expression was achieved by an *ems* complementary DNA transgene under control of the *btd* cis-acting promoter region<sup>13</sup>.

EMS expression in the *btd* domain of wild-type embryos caused a second intercalary-like *engrailed* expression domain in place of the mandibular segment (Fig. 2a and d). Furthermore, these embryos developed a duplicate set of intercalary cuticle elements in place of mandibular structures (compare Fig. 2b, c with e, f). We observed the same results in response to EMS expression in the anterior third of blastoderm embryos mediated by a Gal4/UAS system<sup>15</sup>. However, misexpression of OTD in the *btd* domain had no effect on head

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