Coding the Locations of Objects in the Dark

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2.70,2.74 (α), 35.58,35.67/1.85,1.89 (β), and 61.78,61.84/3.60 (γ).
25. S. Quideau and J. Ralph, *J. Agric. Food Chem.* **40**, 1108 (1992); F. H. Ludley and J. Ralph, *ibid.* **44**, 2942 (1996).
26. J. Ralph, R. F. Helm, S. Quideau, R. D. Hatfield, *J. Chem. Soc. Perkin Trans.* **1**, 2961 (1992). The synthetic lignin was prepared by slow addition of solutions of $[O^{12}C^{2}H_{3}]$-coniferyl alcohol and hydrogen peroxide to a buffered solution of horseradish peroxidase. The isotopic labeling in the coniferyl alcohol was done to reduce the impact on NMR spectra of the normally intense methoxyl peaks.
27. R. A. Savidge, *Phytochemistry* **26**, 93 (1987).
28. S. Kamisaka, N. Sakurai, K. Shibata, *Plant Cell Physiol.* **24**, 369 (1983).
29. We made these determinations by analyzing monomers released after cleaving all α- and β-ethers in the lignin by the DFRC method (derivatization followed by reductive cleavage) (F. Lu and J. Ralph, *J. Agric. Food Chem.*, in press), which gives data analogous to analytical thioacidolysis [C. Lapierre, in *Forage Cell Wall Structure and Digestibility*, H. G. Jung, D. R. Buxton, R. D. Hatfield, J. Ralph, Eds. (American Society of Agronomy, Madison, WI, 1993), pp. 133–166; C. Rolando, B. Monties, C. Lapierre, in *Methods in Lignin Chemistry*, C. W. Dence and S. Y. Lin, Eds. (Springer-Verlag, Berlin, Heidelberg, 1992), pp. 334–349]. Dihydroconiferyl peracetate (*m/z* 266) was abundant. There was no difference between the mutant and the normal pine for the small amounts of dihydro-*p*-coumaryl acetates (*m/z* 236).
30. L. B. Davin *et al.*, *Science* **275**, 362 (1997).
31. J. Ralph *et al.*, in *Lignin and Lignin Biosynthesis*, N. G. Lewis and S. Sarkanen, Eds. (American Chemical Society, Washington, DC, in press); J. Ralph, J. H. Grabber, R. D. Hatfield, *Carbohydr. Res.* **275**, 167 (1995).
32. C. Lapierre, M. T. Tollier, B. Monties, *C. R. Acad. Sci. Ser. 3* **307**, 723 (1988).
33. B. Monties, *Ann. Proc. Phytochem. Soc.* **5**, 161 (1995).
34. We are grateful to F. Lu for providing analytical data obtained by DFRC, which verified the NMR findings and provided data on the high *p*-coumaryl alcohol component; to Y. Zhang for preparing the synthetic lignin; to S. Ralph, L. Landucci, and F. Ludley for help in lignin preparation steps and model work on the coniferaldehyde components; and to J. Grabber for valuable input. NMR studies at 750 MHz for supporting data were carried out at the National Magnetic Resonance Facility at Madison, WI. Samples were provided from a Westvaco planting containing selfs of 7-56 by L. Pearson (Westvaco, Summerville, SC) and G. Askew (Baruch Experimental Forest, Clemson University, Georgetown, SC). We are grateful for partial funding from the U.S. Department of Agriculture–National Research Initiatives, Plant Growth and Development section (grants 94-02764 and 96-02587), and for grants from NIH (GM45344-07), U.S. Department of Energy (DE-FG05-92ER20085), and the NCSU Forest Biotechnology Industrial Research Consortium.

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Coding the Locations of Objects in the Dark

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The ventral premotor cortex in primates is thought to be involved in sensory-motor integration. Many of its neurons respond to visual stimuli in the space near the arms or face. In this study on the ventral premotor cortex of monkeys, an object was presented within the visual receptive fields of individual neurons, then the lights were turned off and the object was silently removed. A subset of the neurons continued to respond in the dark as if the object were still present and visible. Such cells exhibit "object permanence," encoding the presence of an object that is no longer visible. These cells may underlie the ability to reach toward or avoid objects that are no longer directly visible.

A scientist sitting in her office reaches for a book on the shelf. She knows where the book is located and does not need to look in order to guide her hand. Later, while driving home, she adjusts the car radio while her eyes are fixed on the road. That night, in darkness, she reaches toward a box of tissues on the bedside table. How does the brain keep track of the locations of objects that are no longer in sight, and how does it guide movements toward or away from those objects? Piaget (*1*) was the first to emphasize the importance of object permanence, that is, the knowledge that an object is still present even though it is no longer visible. More recently, researchers have emphasized the more specific problem of how movements toward these unseen objects are guided (*2*). Here we describe visually responsive neurons in the ventral premotor cortex (PMv) of the monkey brain that appear to solve the problem of object permanence. These neurons keep track of the locations of objects near the monkey's body, even after the lights are turned off and the monkey is in darkness.

PMv, the area of cortex just posterior to the lower limb of the arcuate sulcus, is thought to be involved in the sensory guidance of movement (*3*). Its neurons respond to tactile and visual stimuli and also during movements of the head and the arms (*4*). About 40% of the neurons in PMv have both a tactile and a visual receptive field (RF). For these bimodal cells, the visual RF extends from the approximate region of the tactile RF into the immediately adjacent space (Fig. 1). For most cells with a tactile RF on the arm, when the arm moves, the visual RF moves with it, and for most cells with a tactile RF on the face, when the head is rotated, the visual RF moves with it (*5*). In contrast, when the eyes move, the visual RFs do not move but remain anchored to the body surface (*5*, *6*). These visual RFs, therefore, encode the locations of nearby stimuli relative to different parts of the body. One suggestion is that the bimodal neurons help to guide movements of the head and arms toward or away from nearby stimuli (*7*).

We tested whether the bimodal neurons in PMv encode the locations of nearby stimuli that are no longer visible. Responses of single neurons in PMv were studied in two tame male *Macaca fascicularis* (4.6 and 5.0 kg). For details of the experimental procedures, see (*5*). Daily recording sessions were conducted on each monkey while the animal was seated in a primate chair with the head fixed. A hydraulic microdrive was used to lower an electrode into PMv. Once a neuron was isolated, it was tested for somatosensory and visual responsiveness. Somatosensory RFs were plotted by manipulating the joints and stroking the skin, and visual RFs were plotted with objects presented on a wand. In addition, we made the unexpected observation that neurons with a tactile RF extending onto the back of the head often responded to auditory stimuli; therefore, we also routinely tested for auditory responsiveness.

Of 153 isolated single neurons, 6 (4%) responded only to visual stimuli, 34 (22%) responded only to somatosensory stimuli, 55 (36%) were bimodal, responding to visual and tactile stimuli, and 11 (7%) were tri-

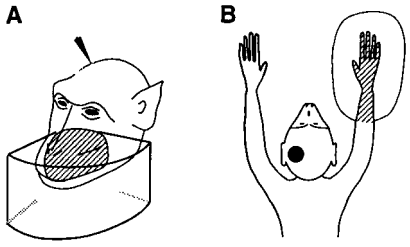

Fig. 1. Receptive fields of two bimodal, visual-tactile neurons in PMv. (**A**) The tactile RF (shaded) is on the snout, mostly contralateral to the recording electrode (indicated by the arrowhead) but extending partially onto the ipsilateral side of the face. The visual RF (boxed) is contralateral and confined to a region of space within ~10 cm of the tactile RF. (**B**) The tactile RF for this neuron is on the hand and forearm contralateral to the recording electrode (indicated by the black dot), and the visual RF (outlined) surrounds the tactile RF.

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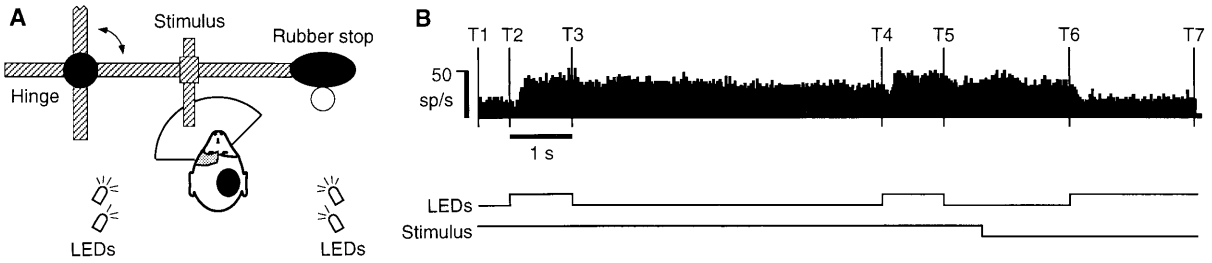

Fig. 2. (**A**) Experimental apparatus for testing object permanence. The visual stimulus, a plastic dowel 2.5 cm in diameter, was adjusted for each neuron until it extended into the center of that neuron's visual RF. The stimulus was mounted on an oiled plastic hinge and could be silently swiveled out of the visual RF or back into it again. A rubber stop prevented the stimulus from making noise when it reached its final position near the monkey's face. The experimenter sat behind a black drape and presented the stimulus manually. Four LEDs were used to illuminate the space near the monkey in the otherwise dark room. The black dot on the head shows the hemisphere recorded from. The stippling on the left eyebrow and the outlined area near the face show the tactile and visual RFs, respectively, of the neuron whose responses are shown in (B). (**B**) Responses of a neuron, averaged over 50 trials, as a function of onset and offset of the LEDs and presence or absence of the stimulus near the face (sp/s, spikes per second). The stimulus was first positioned near the face during an 8-s intertrial interval in darkness (not shown).

modal, responding to visual, tactile, and auditory stimuli. All 11 trimodal cells had a tactile RF that extended from the front of the face onto the back of the head. Forty-seven cells (31%) were unresponsive under our experimental conditions.

Each of the 72 visually responsive neurons (61 from monkey 1, 11 from monkey 2) was tested with the apparatus shown in Fig. 2A. The visual stimulus was silently presented in the dark, then the space near the monkey was illuminated with four "super-bright" light-emitting diodes (LEDs) (total of 20,000 millicandela), used because of their fast rise time. The surfaces of the LEDs were abraded to diffuse the light. The onset of the LEDs revealed the stationary stimulus within the visual RF of the neuron. Thirty of the visual neurons did not respond under these conditions. These cells gave a vigorous response only to a visual stimulus in motion within the RF (8). The remaining 42 neurons were tested for their responses to the stationary visual stimulus while it was visible within the RF and after the LEDs were turned off. During these tests the monkey sat awake in the primate chair and did not perform any task (9).

The responses of an example neuron with a tactile RF on the face and a visual RF within the space near the face are shown in Fig. 2B. During the 8-s intertrial interval (not included in the figure), the monkey was in darkness and the stimulus was moved into the area of the visual RF. The neuron did not respond to the stimulus, presumably because it was inaudible and invisible. The interval from T1 to T2 shows the relatively low baseline firing rate of the neuron at the beginning of the trial when the monkey was still in darkness. At T2, the LEDs were illuminated and the monkey could see the stimulus located in the neuron's visual RF. The cell began to respond with a 200-ms latency. At T3, 1 s later, the LEDs were extinguished and the monkey could no longer see the stimulus; however, the neuron continued to fire at an elevated rate. By T4, after 5 s of darkness, the firing rate was still more than twice the baseline rate. The LEDs were illuminated again at T4, revealing the presence of the stimulus, and the neuron's firing increased in response. At T5, the LEDs were extinguished. Within 1 s of the offset of the LEDs, the stimulus was removed from the area of the visual RF. The neuron continued to respond as if the object were present in the RF, presumably because the stimulus was moved silently and in darkness and therefore the monkey did not know that it had been retracted. At T6, the LEDs were illuminated, revealing that the stimulus was no longer present. The neuron's firing rate abruptly dropped to its baseline level.

These results show that the neuron responded to the presence of an object near

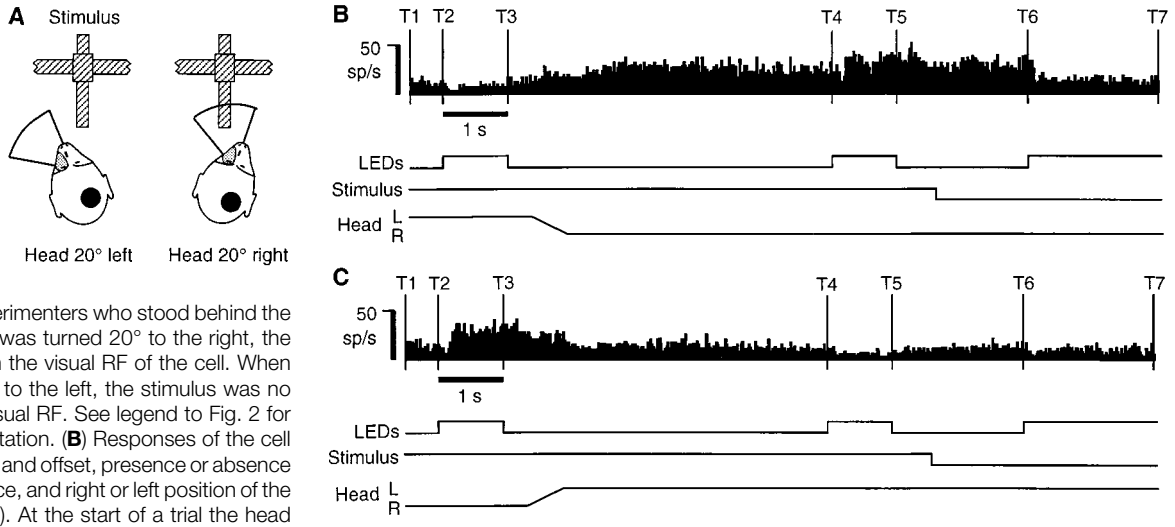

Fig. 3. Responses of a second example neuron. This cell had a small tactile RF on the left snout and a small associated visual RF. (**A**) Passive rotation of the head caused the visual RF of this neuron to move into and out of alignment with the stimulus. The bolt that held the head was rotated by one of the experimenters who stood behind the monkey. When the head was turned 20° to the right, the stimulus was aligned with the visual RF of the cell. When the head was turned 20° to the left, the stimulus was no longer aligned with the visual RF. See legend to Fig. 2 for details of stimulus presentation. (**B**) Responses of the cell as a function of LED onset and offset, presence or absence of the stimulus near the face, and right or left position of the head (average of 35 trials). At the start of a trial the head was 20° to the left. Within 1.5 s after T3, the head was moved 20° to the right. (**C**) Same as (B) except at the start of a trial the head was 20° to the right. Within 1.5 s after T3, the head was moved 20° to the left. Trials in the conditions described in (B) and (C) were interleaved in an alternating fashion.

the face. When the monkey saw that the object was present, the neuron began to respond. When the monkey saw that the object was absent, the neuron ceased to respond. During the periods of darkness between, the firing of the neuron reflected the stimulus configuration that the monkey had most recently seen. This pattern of response cannot be explained by a dim or partial view of the object in the dark. Instead, it can only be explained by the monkey's memory of the presence or absence of the stimulus. That is, the cell showed object permanence.

Of the 42 neurons tested, 15 (36%) showed object permanence: They began to respond at T2, their activity remained significantly above baseline until T6, and then they returned to baseline after T6 (*10*). In particular, the activity during the 5-s dark period, between T3 and T4, was well over baseline, with $P < 0.005$ in every case and $P < 0.0001$ in 11 of the 15 cases. Twenty-two neurons (52%) responded significantly to the sight of the stimulus at T2 and T4 but returned to their baseline firing rate when the monkey was in darkness. Five neurons (12%) did not respond to the stimulus but instead responded significantly to the onset of the LEDs at times T2, T4, and T6.

As described above, for almost all bimodal neurons in PMv with a tactile RF on the face, the visual RF is anchored to the head and moves as the head is moved (*5*). The responses of a neuron that was tested by moving the head is shown in Fig. 3. The cell had a tactile RF on the left side of the face and an excitatory visual RF near the tactile RF. The cell also gave a weak, transient inhibitory response to visual stimuli near the opposite side of the face. As shown in Fig. 3A, when the head was positioned 20° to the left, the excitatory visual RF was out of register with the stimulus. When the head was positioned 20° to the right, the visual RF was aligned with the stimulus. Figure 3B shows the result when the head was 20° to the left at the start of the trial. The interval from T1 to T2 shows the baseline firing rate of the neuron in darkness. At T2, the LEDs were illuminated, revealing the stimulus near the ipsilateral side of the face, outside the excitatory visual RF. The neuron's firing rate was transiently inhibited and then began to recover. By T3, the firing rate had returned to baseline. At T3 the LEDs were extinguished. Within the next 1.5 s the head was turned 20° to the right. In this new position, the excitatory RF of the neuron should be in alignment with the stimulus. Note that during this time interval the monkey was in darkness and did not see the stimulus inside the visual RF. Instead, the visual RF overlapped the remembered location of the stimulus. The neuron responded vigorously and continued to respond throughout the remaining 3.5 s of darkness. At T4 the LEDs were lit again, revealing the stimulus near the face and inside the visual RF. At T5 the LEDs were extinguished. Within 1 s after T5 the stimulus was silently removed. The cell continued to respond as if an object were present in the RF, presumably because the monkey did not know that the stimulus had been removed. At T6 the LEDs were lit again, revealing that the stimulus was no longer in the visual RF. The neuron's firing rate abruptly returned to baseline.

The result on interleaved trials is shown in Fig. 3C. The head was 20° to the right at the start of the trial. At T2 the LEDs were illuminated, revealing the stimulus inside the excitatory visual RF, and the neuron responded. At T3, the LEDs were extinguished, and the neuron continued to respond in the dark. Within the next 1.5 s, the head was turned 20° to the left. In this position, the excitatory RF of the neuron was no longer in register with the position where the monkey last saw the stimulus, and the firing rate of the neuron returned to baseline.

This neuron, therefore, not only encoded the presence or absence of a stimulus that was no longer visible, but also encoded the position of the stimulus relative to the head. As the head was rotated in one direction, the visual RF shifted into alignment with the remembered location of the stimulus, and the neuron began to respond. As the head was rotated in the opposite direction, the visual RF shifted out of alignment with the remembered location of the stimulus, and the neuron stopped responding. Of the 15 neurons that showed object permanence, only two had visual RFs that were sufficiently small to test by turning the head. The second neuron showed the same pattern of response as illustrated in Fig. 3.

These results show that a subset of the bimodal neurons in PMv is able to keep track of the locations of stimuli near the monkey's body, even after the lights are extinguished and the monkey is in darkness. As the monkey's head turns, cells with visual RFs that are anchored to the head become active or fall quiet as their RFs pass over the remembered location of the stimulus. Because these neurons are found in a premotor area that has a high incidence of movement-related responses (*3*, *5*), we suggest that they may play a role in the guidance of movement toward objects that are no longer visible, such as objects that are occluded, that are behind the animal, or that are no longer foveated. Neurons with tactile RFs on the face and visual RFs anchored to the head would be able to code locations of objects relative to the head and therefore would be useful for guiding movements of the head toward or away from those objects. Neurons with tactile RFs on the arm and visual RFs anchored to the arm might be more useful for guiding movements of the arm.

REFERENCES AND NOTES

1. J. Piaget, *The Origins of Intelligence in Children* (International Universities Press, New York, 1952).
2. L. Proteau and D. Elliott, Eds., *Vision and Motor Control* (Elsevier, New York, 1992).
3. G. Rizzolatti *et al.*, *Exp. Brain Res.* **71**, 491 (1988); S. P. Wise, *Annu. Rev. Neurosci.* **8**, 1 (1985).
4. M. Gentilucci *et al.*, *Exp. Brain Res.* **71**, 475 (1988); M. S. A. Graziano and C. G. Gross, in *The Cognitive Neurosciences,* M. S. Gazzaniga, Ed. (MIT Press, Cambridge, MA, 1995), pp. 1021–1034; G. Rizzolatti, C. Scandolara, M. Matelli, M. Gentilucci, *Behav. Brain Res.* **2**, 147 (1981).
5. M. S. A. Graziano, X. T. Hu, C. G. Gross, *J. Neurophysiol.,* in press; M. S. A. Graziano, G. S. Yap, C. G. Gross, *Science* **266**, 1054 (1994).
6. M. Gentilucci *et al.*, *Exp. Brain Res.* **50**, 464 (1983); L. Fogassi *et al.*, *J. Neurophysiol.* **76**, 141 (1996).
7. C. G. Gross and M. S. A. Graziano, *Neuroscientist* **1**, 43 (1995).
8. Most bimodal PMv neurons are sensitive to the speed and direction of the visual stimulus, and many will respond only to moving stimuli (*4*–*6*).
9. Because most visual RFs in PMv are anchored to the body and do not move when the eyes move, it is not necessary to control the position of the eyes in order to present a stimulus within the visual RF (*5*). A fixation task would have been inappropriate here anyway, for two reasons. First, the fixation light would have illuminated the room when the experiment required darkness. Second, each trial was 12 s long, and it is difficult to train monkeys to fixate reliably for that period.
10. Each neuron was analyzed as follows: The mean baseline activity (spikes per second) was calculated with the interval T1-T2, and then subtracted from the activity in intervals T2-T3, T3-T4, T4-T5, T5-T6, and T6-T7, resulting in five data samples expressed in spikes per second above baseline. A *t* test was then performed on each of these samples to determine whether it was significantly above zero (criterion $P < 0.05$). In this way, although five *t* tests were performed on each neuron, the tests were orthogonal and therefore no statistical correction was needed. A neuron was considered to have object permanence if it responded significantly above baseline in intervals T2-T3, T3-T4, T4-T5, and T5-T6, but not in interval T6-T7. The following population statistics describe the 15 neurons that showed object permanence. The activity in T2-T3 was a mean of 125% above baseline, with a range of 14 to 484%; activity in T3-T4: mean 84% above baseline, range 12 to 423%; activity in T4-T5: mean 160% above baseline, range 20 to 400%; activity in T5-T6: mean 101% above baseline, range 19 to 469%; and activity in T6-T7: mean 6% below baseline, range 42% below baseline to 28% above baseline.

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