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16. Fluorescent HGE was prepared from infected HL60 cultures incubated with the vital fluorescent dye CellTracker Green (Molecular Probes, Eugene, OR) (C. Nelson, M. Herron, J. L. Goodman, unpublished data). HGE bacteria were liberated by passage through a 25-gauge needle and washed twice in phosphate-buffered saline (PBS). Fluorescent HGE from 1 to 2 million infected HL60 was incubated for 30 min with 250,000 target cells at 22°C in 25 μl of PBS. Binding was stopped, and the cells and bacteria were fixed by addition of 0.25 ml of 1% paraformaldehyde in PBS. Cell surface binding of labeled HGE was assessed by FACS analysis of the mean cellular fluorescence of 5000 cells and expressed as the percentage of a control with no antibodies added, with subtraction of nonspecific background determined with a baseline control in which binding had been stopped by fixation immediately upon addition of HGE to target cells. This method was shown to provide results that linearly correlate with binding as determined by direct visualization by IFA.
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# The Prefrontal Cortex: Response Selection or Maintenance Within Working Memory?


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**It is controversial whether the dorsolateral prefrontal cortex is involved in the maintenance of items in working memory or in the selection of responses. We used event-related functional magnetic resonance imaging to study the performance of a spatial working memory task by humans. We distinguished the maintenance of spatial items from the selection of an item from memory to guide a response. Selection, but not maintenance, was associated with activation of prefrontal area 46 of the dorsal lateral prefrontal cortex. In contrast, maintenance was associated with activation of prefrontal area 8 and the intraparietal cortex. The results support a role for the dorsal prefrontal cortex in the selection of representations. This accounts for the fact that this area is activated both when subjects select between items on working memory tasks and when they freely select between movements on tasks of willed action.**


It has been controversial whether the dorsal prefrontal cortex is involved in the maintenance of working memory (*1*) or in the selection of responses (*2*, *3*). The first hypothesis accounts for the fact that in monkeys, there are cells in the dorsal prefrontal cortex that continue to fire during the delay on a working memory task (*4*). There is also activity in this area when humans perform working memory tasks (*5*, *6*), though there is no agreement as to whether to emphasize its role in the maintenance of information (*4*) or in the manipulation or monitoring of that information (*7*). However, there is also activity in the prefrontal cortex when humans freely select between manual or verbal responses (*8*). It could be argued that on such "free selection" tasks, the participants maintain a record of their responses on previous trials and that the activity can therefore still be related to working memory. But it has recently been shown that transcranial magnetic stimulation over the dorsal prefrontal cortex interferes with free selection of finger response even when there is no memory load (*9*). We have tried to reconcile these facts by using functional magnetic resonance imaging (fMRI) to measure activity in the dorsal prefrontal cortex during an experiment on working memory.

We used event-related fMRI to distinguish delay-related activity during the maintenance of items in memory ("set activity") from the transient activity related to selection of a single item from memory in that same trial. During working memory trials, the study participants remembered three spatial locations for up to 18 s (Fig. 1). They then selected the location of just one of these items to guide a response using a joystick. During the delay, the participants maintained the items in memory without requiring manipulation, monitoring, or preparation of their responses. They could not select the appropriate remembered location until the end of the working memory delay, and the stimulus locations changed at random from trial to trial. The control trials included similar stimuli and motor responses, but the participants were not required to remember or select spatial locations. We deliberately avoided the use of verbal material because we wished to ensure that the participants maintained items in memory during the delay without articulatory rehearsal.

A general linear model was applied to the time course of activation of each voxel (*10*). The model included separate covariates for transient neuronal activations in response to stimulus presentation, motor response, the selection from memory at the end of memory trials, sustained activation during working


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memory, and the equivalent period between stimuli during control trials ("nonmemory" periods). The long and variable delays used minimized the potential correlation between sustained set activity and transient selection events. We can therefore identify voxels that were activated by maintenance or selection or both. In the memory trials, 218 out of 253 (86%) responses were made to the correct target location (within one dot's diameter of that location).

The regions of brain activation associated with sustained maintenance of items in spatial working memory differ from those associated with transient selection of an item from within memory (Fig. 2 and Table 1). Working memory maintenance (contrasted with equivalent nonmemory periods in control trials) was associated with bilateral activation of prefrontal area 8 (Figs. 2 and 3B) and the intraparietal cortex (Figs. 2 and 3A) but not in prefrontal area 46. At a lower threshold [$t$ statistic ($t$) $> 3.10$, $P < 0.001$, uncorrected for multiple comparisons], there was an activation peak more anteriorly: it lay either within the anterior part of area 8 or in the area defined by Petrides and Pandya as 9/46 (coordinates 32, 24, 50; $t = 3.30$) (*11*), but there was still no activation in area 46 proper (*11*, *12*). Figure 3, C and D, shows the time course of activity during working memory trials as best fitted by the data for each length of memory delay (*13*). In both area 8 and the intraparietal cortex, there were sustained increases in blood oxygen level–dependent (BOLD) signal throughout the course of the working memory delay, seen as a plateau, the length of which is in direct proportion to the duration of the maintenance delay. This figure also shows that there was no additional activity in these areas that was associated with the selection of the response.

The selection of the target location from memory was associated with activations of the right prefrontal area 46 proper ($y = 38$) (Figs. 2 and 4B) and of a more posterior region lying either in area 8 or in the region identified by Petrides and Pandya as 9/46 ($y = 18$) (*11*). There was additional activation of the right ventral and orbital frontal cortex, and bilateral activation of the intraparietal cortex and the medial parietal cortex (Figs. 2 and 4A). The parietal activations for selection were more posterior and medial to those identified for maintenance of working memory. Figure 4, C and D, shows the time course of activity during working memory trials as best fitted by the model. In both area 46 and the medial parietal cortex, there was a transient increase in BOLD signal after the selection of the target location at the end of the working memory delay. There was no sustained activity in these areas associated with the maintenance of the locations during the working memory delay.

The activations associated with presentation of visual stimuli lay in visual and parietal areas (Table 2). The activations associated with movement of the joystick lay within the motor system, not including the prefrontal cortex; there were also activations in the prestriate cortex, perhaps associated with perception of the movement of the cursor.

Our results clearly show separate frontoparietal networks of activation associated with maintenance and the selection of items within the same working memory trial. Thus, although the attentional, mnemonic, and motor response components of tasks such as the delayed response task may all be considered to constitute "working memory" (*4*), they do not necessarily share the same neuroanatomical basis in humans. For our human participants, there was activation around the superior frontal sulcus (area 8) ($y = 8$) but not area 46 during maintenance of spatial working. There have been previous reports of activation posteriorly in or medial to the superior frontal sulcus during spatial memory anterior to the frontal eye fields (*14*–*16*). In our study, the activation in area 8 lay anterior to

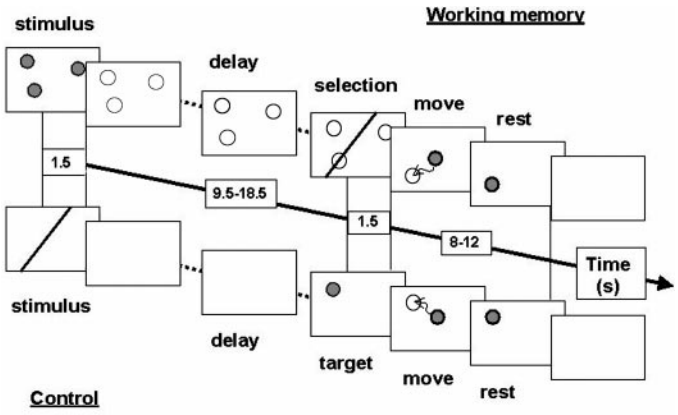


**Fig. 1.** A schematic representation of the spatial working memory and control trials. For a memory trial, the participants saw three red dots presented simultaneously for 1.5 s on a screen in front of them, in random locations (solid circles). There followed a delay of 9.5 to 18.5 s (in steps of 1 s, randomly ordered), during which the participants remembered the exact location of the dots (indicated here by white circles not actually presented to participants). A line then appeared for 1.5 s across the screen, running through the location of just one of the previous red dots. This indicated which of the remembered dots now became the target for response, without specifying the location directly. The line was then replaced by a central cursor identical in appearance to the red dots. The participants moved the cursor to the remembered target location using a joystick. After the response, the trial ended and was followed by a rest period of 8 to 12 s. For control trials, the visual and motor components of the task were similar to those of the memory trials, but the stimuli were presented in reverse order so that there were no spatial cues to remember during the prolonged delay (the "nonmemory" period in analyses). The target for the cursor response in the control trials was the location of the single red dot, with no intervening delay. Six healthy volunteers (age 24 to 34; five male, one female) gave written consent. The functional images were acquired by T2*-weighted echo planar MRI at 2 T; repeat time, 4500 ms; echo time, 40 ms; over 40 min of continuous whole-brain imaging (64 by 64 by 48 voxels at 3 mm isotropic resolution). Statistical parametric mapping software (SPM99) was used for image processing and analysis. The images were realigned to the mean image by rigid body transformation and were sinc interpolated in time to correct phase advance during volume acquisition (*32*). These realigned images were transformed to normal anatomic space (*33*) by nonlinear transformations (*34*). The data were spatially smoothed with a Gaussian kernel full width at half maximum of 6 mm. High-resolution structural T1-weighted MPRAGE images were also acquired on all participants to permit anatomical localization of activation foci.

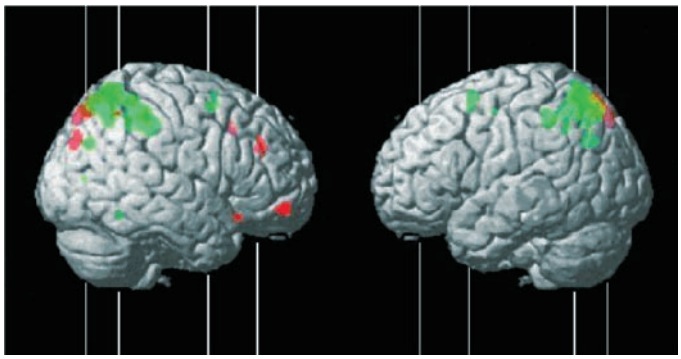

**Fig. 2.** The regions associated with maintenance of items in spatial working memory (green) and selection of an item from working memory (red) are projected together onto a surface-rendered representative brain in normal stereotactic space ($t > 4.91$, $P < 0.05$, corrected for multiple comparisons). There is clear regional specialization in the frontal lobe, with only selection being associated with activation of area 46. For clarity, regions associated with visual stimuli and motor responses are not shown but are listed in Table 2. The four white lines indicate the planes of coronal sections displayed in Figs. 3 and 4.

the anterior commissure ($y = 8$), whereas the frontal eye fields have been located at coordinates posterior to this line (*17*, *18*).

The results for frontal and parietal delay-related activations are consistent with the animal literature on spatial working memory. In monkeys, sustained neuronal activity has been reported in area 8a anterior to the arcuate sulcus (*19*, *20*), and in the same studies activity was found in the posterior third of the sulcus principalis, designated area 9/46 by Petrides and Pandya (*11*). The activity of many of these neurons can be shown to be associated with the retention of the sensory cues (*19*, *21*, *22*). Sustained parietal activation associated with the maintenance of spatial information has also been reported in the monkey intraparietal cortex (*20*, *23*).

It is, of course, possible that the negative result for maintenance in area 46 was due to insensitivity to underlying maintenance-related activation. In monkeys, delay-related activity has been reported in area 46 in the middle third of the sulcus principalis (*24*). However, in our study we specifically compared the activity during the spatial working memory interval with the equivalent period without working memory in control trials. Kojima and Goldman-Rakic made a similar comparison in a working memory paradigm in monkeys (*25*). They reported that for over 80% of cells in the sulcus principalis that had delay-related activity, this activity was at least as great for trials without working memory as for trials with working memory. Furthermore, in our study the participants were not able to prepare their response, yet in the study on monkeys the activity of the remainder of cells could have represented preparatory activity.

We could test whether there was a lack of sensitivity in our methods by reducing the statistical threshold to $t = 3.10$ ($P < 0.001$, uncorrected for multiple comparisons) or by searching within specified regions of interest. These were defined functionally, using the region of activation associated with selection; or anatomically, using a sphere of radius 10 mm around the peak of selection-related activation. Not one of these three approaches exposed any maintenance-related activation in area 46. Further, any such activity in these voxels would be revealed in the time course of activation shown in Fig. 4. These plots show the time course of activation derived from the condition-specific covariates (including maintenance) and their respective parameter estimates. Maintenance-related activity would be seen as a rise in activity above baseline during the delay period, even if it did not reach statistical significance (compare Fig. 3). It is true that specific delay-related activity in area 46 has been reported previously in fMRI studies. However, it is necessary to exclude from the comparison studies in which the participants could prepare their response or where they could manipulate the information in memory during the delay, as on the *n*-back task (*6*). Courtney *et al.* (*15*) instructed participants to actively rehearse faces to themselves and found that that in half of their participants the activity of area 46 correlated with a period of rehearsal for 8 s. One difference is that in our study the participants were not instructed to actively rehearse the items. Recently, Postle and D'Esposito (*14*) studied consecutive object and spatial working memory and reported statistically significant delay-related activity. However, the specificity of this delay-related activity is uncertain, because the short memory delay used would have induced a high degree of colinearity between the covariates for stimuli, memory, and probe events in their general linear model. This was avoided in our design, enabling us to conclude that selection dominates the activity of these voxels in area 46 and that in simple spatial working memory tasks the contribution of maintenance must be small, as has also been claimed by others (*26*).

Our design enabled us to distinguish the selection of a remembered location in memory from the maintenance of several locations in memory. In contrast to the findings for maintenance, we found transient activation in dorsolateral prefrontal cortex area 46 when the participants selected the appropriate location from memory. The activation at $y = 38$ clearly lies in area 46 proper (*11*, *12*), whereas a second dorsolateral prefrontal activation at $y = 18$ is more likely to lie in the posterior frontal area 9/46 (*11*).

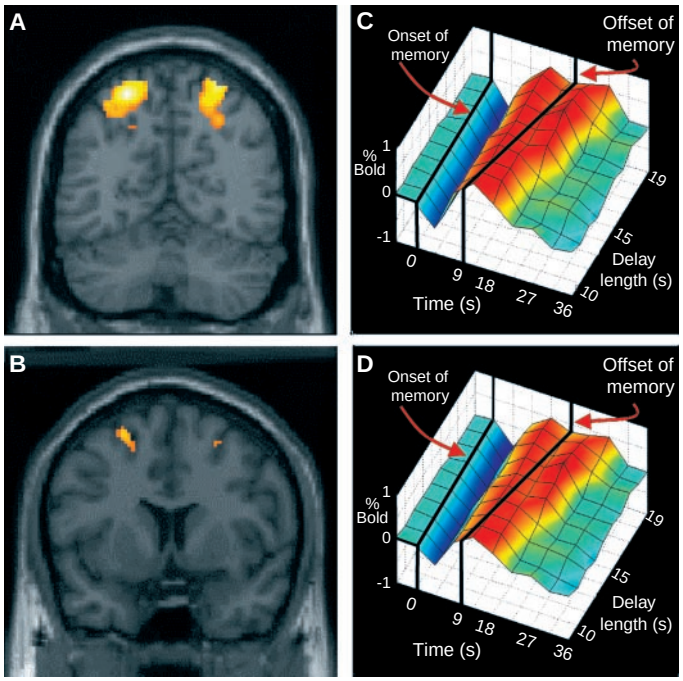


**Fig. 3.** The statistical parametric maps ($t > 4.91$) for the contrast of activation in working memory delay versus nonmemory delay in control trials, shown on coronal slices through (**A**) area 8 (24, 4, 54) and (**B**) the intraparietal cortex (26, −60, 64). The fitted data from the activation peaks in (**C**) area 8 and (**D**) the intraparietal cortex have been temporally realigned to the onset of working memory trials and are shown as changes in BOLD signal (*z* axis) over time (*x* axis) for each delay length of working memory (*y* axis). The thick black lines indicate the onset and offset of the working memory delays, and the color scale indicates the relative change in BOLD signal from the start of each trial. The plots demonstrate the sustained activity over the length of the working memory delay.

**Table 1.** Areas of significant activation associated with maintenance and response selection.

| Region | Laterality | Talairach coordinate | *t* statistic |
|---|---|---|---|
| *Working memory maintenance (versus nonmemory interval in control trials)* | | | |
| Superior frontal sulcus (area 8) | Right | 24, 4, 54 | 5.81 |
| | Left | −22, 8, 60 | 6.42 |
| Intraparietal cortex | Right | 26, −60, 64 | 9.98 |
| | | 44, −34, 42 | 7.61 |
| | Left | −22, −62, 60 | 10.33 |
| *Selection from memory* | | | |
| Dorsal lateral PFC (46) | Right | 42, 38, 28 | 5.29 |
| (9/46) | Right | 30, 18, 40 | 5.22 |
| Orbitofrontal PFC | Right | 40, 54, −12 | 6.22 |
| Ventral PFC | Right | 36, 22, −16 | 5.41 |
| Medial parietal cortex | Right | 10, −80, 48 | 5.94 |
| | Left | −14, −76, 54 | 5.62 |
| Intraparietal cortex | Right | 38, −82, 32 | 5.81 |

Previous imaging studies have reported activation in area 46 during working memory tasks that also required monitoring or manipulation (*5*, *6*, *27*, *28*). However, these tasks are complex. For example, on the *n*-back task (*6*) the participants must remember a series of spatial locations and their temporal order, update the list of recent items, and select responses according to the *n*-back rule.

We therefore suggest that the reason for the common activation of prefrontal area 46 in working memory tasks and free selection tasks is that both involve the selection of the target of the response. They are both examples of the general process of selecting representations to guide actions when there is no external prompt. Functional imaging studies have demonstrated greater activation of prefrontal area 46 when actions were freely selected rather than externally specified, whether the actions were finger movements, drawing, joystick control, or mouth movements (*8*).

A more general role has been suggested for the prefrontal cortex in the selection of representations by top-down attentional mechanisms (*3*, *29*). We propose that the critical feature of the tasks activating area 46 is the selection of items within memory. The crucial distinction is between tasks that require participants to report the contents of memory as presented and tasks that require participants to select between items in memory (*5*, *6*). This approach can explain the activation of dorsal prefrontal area 46 in specific working memory tasks and in tasks of free selection without working memory, because both involve the selection of representations. On the search task, the participants must select items in turn, rejecting ones previously chosen. On tasks requiring reordering, the participants must sequentially select items for report: This involves selecting out the item tagged as last, then the last but one, and so on. On the *n*-back task, the participants must not only remember the temporal order of items but also select recent items in memory in preference to earlier ones (*6*). Levy and Goldman-Rakic report that lesions of area 46 impaired delayed response without manipulation as well as analogues of the self-ordered search tasks (*30*). However, even on their delayed response task there is interference between trials (*31*): The monkey must select the last location rather than the one presented on the previous trial.

In the present study, an accurate response demanded selection of the particular location in memory. The participants needed to voluntarily focus awareness on the item in memory, a process termed "attentional selection" by Miller (*29*). Further research is needed to distinguish whether this involves the enhancement of selected items or the inhibition of nonselected items, or both. Goldman-Rakic (*1*) argued that prefrontal area 46 was essential for the guidance of behavior by internal representations in working memory. Our results support this, but we can be more specific. The critical operation is the selection of these representations as the target for the response and not their maintenance.

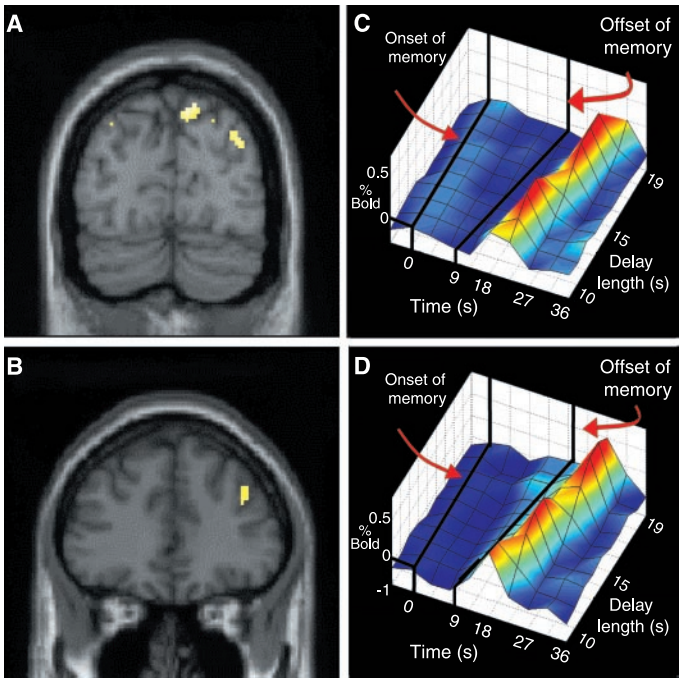


**Fig. 4.** The statistical parametric maps ($t >$ 4.91) for the main effect of selection from memory, shown on coronal slices through (**A**) prefrontal area 46 (42, 38, 28) and ( **B**) the parietal cortex (38, −82, 32). The fitted data from the activation peaks in (**C**) prefrontal area 46 and (**D**) the parietal cortex have been temporally realigned to the onset of working memory trials and are shown as changes in BOLD signal (*z* axis) over time (*x* axis) for each delay length of working memory (*y* axis). The thick black lines indicate the onset and offset of the working memory delays, and the color scale indicates the relative change in BOLD signal from the start of each trial. In both areas, there is no activation during the working memory interval, but there is a peak of activation after selection of the item at then end of the memory period.

**Table 2.** Areas of significant activation associated with visual presentation and use of the joystick.

| Region | Laterality | Talairach coordinate | *t* statistic |
|---|---|---|---|
| | *Visual presentation* | | |
| Striate cortex | Left | −8, −96, 0 | 8.46 |
| | Right | 10, −98, 12 | 7.82 |
| Parietal cortex | Left | −12, −58, 70 | 10.32 |
| | Right | 22, −60, 68 | 7.86 |
| | *Cursor positioning* | | |
| Motor cortex | Left | −24, −24, 74 | 21.54 |
| SII | Left | −52, −22, 18 | 12.55 |
| | Right | 66, −22, 18 | 13.63 |
| Prestriate cortex | Left | −26, −92, 0 | 10.16 |
| Prestriate cortex | Right | 34, −84, −10 | 14.97 |
| Insula | Left | −38, −2, 10 | 14.84 |
| | Right | 42, 2, 10 | 10.90 |
| Cerebellum | Left | −14, −72, −46 | 7.45 |
| | Left | −30, −54, −22 | 9.73 |
| | Right | 14, −64, −50 | 11.58 |
| | Right | 24, −50, −24 | 17.56 |
| Putamen | Left | −26, 0, 0 | 8.68 |
| | Right | 24, 0, 0 | 5.53 |
| Thalamus | Left | −12, −18, 6 | 9.53 |
| | Right | 10, −16, 6 | 6.79 |
| Cingulate motor area | | 0, 0, 52 | 14.54 |

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*et al.*, *Eur. J. Neurosci.* **11,** 567 (1999); E. E. Smith, J. Jonides, R. A. Koeppe, *Cereb. Cortex* **6,** 11 (1996)]. However, activation has been consistently reported in this area when participants are asked to monitor or manipulate items in memory in tasks such as the self-ordered task [M. Petrides, B. Alivisatos, E. Meyer, A. C. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **90,** 873 (1993)] or search task [A. M. Owen, A. C. Evans, M. Petrides, *Cereb. Cortex* **6**, 31 (1996)].

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# Generalized Potential of Adult Neural Stem Cells

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**The differentiation potential of stem cells in tissues of the adult has been thought to be limited to cell lineages present in the organ from which they were derived, but there is evidence that some stem cells may have a broader differentiation repertoire. We show here that neural stem cells from the adult mouse brain can contribute to the formation of chimeric chick and mouse embryos and give rise to cells of all germ layers. This demonstrates that an adult neural stem cell has a very broad developmental capacity and may potentially be used to generate a variety of cell types for transplantation in different diseases.**

Multicellular organisms are formed from a single totipotent stem cell. As this cell and its progeny undergo cell divisions, the potential of the cells becomes restricted, and they specialize to generate cells of a certain lineage. In several tissues a stem cell population is maintained in the adult organ, and it may generate new cells continuously or in response to injury. The adult brain and spinal cord retain neural stem cells that can generate neurons, astrocytes, and oligodendrocytes (*1*–*5*). Two stem cell populations have recently been identified in the adult central nervous system—ependymal cells (*6*) and subventricular zone astrocytes (*7*)—although it is not yet clear whether these two cell types represent independent populations or whether they share a lineage relationship (*3*, *8*). These cells can be cultured as clonal cell aggregates referred to as neurospheres (*9*).

Most of the available data indicate that progeny produced by nervous system stem cells is limited to neural cell fates (*1*–*5*). It is, however, possible that the cellular fates generated by adult neural stem cells are restricted because of the limitations imposed on them by the particular environment in which they have been evaluated. In line with this, neural stem cells isolated from the adult forebrain were recently shown to be capable of repopulating the hematopoietic system and produce blood cells in irradiated adult mice (*10*). However, because this method of addressing the potency of neural stem cells fell within the limits of the hematopoietic system, their repertoire of progeny was still restricted. We have expanded the question of the differentiation potential of adult neural stem cells by exposing them to different inductive environments.

Embryonic stem (ES) cells are totipotent and can be induced to differentiate into a variety of cell types when cultured as embryoid bodies (*11*). We reasoned that inductive signals for differentiation to diverse lineages must be present in these cultures. To evaluate the capacity of inductive signals from ES cells to guide the differentiation of neural stem cells, we cultured adult neural stem cells together with embryoid bodies. The neural stem cells, derived from ROSA26 mice (*12*), express β-galactosidase (β-Gal), enabling identification of their progeny by X-Gal histochemistry or with antibodies against β-Gal (*13*). Moreover, ROSA26 cells express the neomycin resistance gene, which allowed us to later eliminate the G418-sensitive ES cells from the cocultures and specifically study the remaining resistant neural stem cell–derived

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