391 (1995); J. J. Tomasek *et al.*, *J. Biol. Chem.* **14**, 7482 (1997).

3. P. Tremble, C. H. Damsky, Z. Werb, *J. Cell Biol.* **129**, 1707 (1995); P. Huhtala *et al.*, *ibid.*, p. 867.
4. A. Ridley, *Curr. Biol.* **6**, 1256 (1996); N. Tapon and A. Hall, *Curr. Opin. Cell Biol.* **9**, 86 (1997); P. T. Hawkins *et al.*, *Curr. Biol.* **5**, 393 (1995).
5. Z. Werb, P. M. Tremble, O. Behrendtsen, E. Crowley, C. H. Damsky, *J. Cell Biol.* **109**, 877 (1989).
6. R. G. Qiu, J. Chen, D. Kirn, F. McCormick, M. Symons, *Nature* **374**, 457 (1995).
7. R. G. Qiu, A. Abo, F. McCormick, M. Symons, *Mol. Cell Biol.* **17**, 3449 (1997); R. G. Qiu, J. Chen, F. McCormick, M. Symons, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 11781 (1995).
8. RSFs (*2*) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, Denver, CO). RSFs used between passages 4 through 15 were subcultured 24 hours before transfection or other experimental procedures. We used adenovirus-mediated transfection for transient transfection: RSFs (4×10^6) were harvested from subconfluent plates with trypsin (0.05%; Cell Culture Facility, University of California, San Francisco), were mixed at a 1:40 final dilution of a replication-defective mutant of human adenovirus-5 (GPT-Ad5) [J. R. Forsayeth and P. D. Garcia, *Biotechniques* **17**, 354 (1994)] and DEAE-Dextran (80 μg/ml; Pharmacia), and were brought to 5 ml in DMEM. Then 10 μg of each plasmid was added, after which the mixture was plated onto culture dishes and incubated for 2.5 hours in a 5% CO_2-enriched atmosphere at 37°C. At the end of the incubation, cells were rinsed with 2 ml of 10% dimethyl sulfoxide in phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free) for 2 min to stop transfection and to remove nonadherent cells, and 10 ml of DMEM supplemented with FBS (10%) was added. Cells were incubated overnight to recover. We routinely obtained 70 to 90% efficiency of transfection in RSFs, as determined with cytomegalovirus promoter–driven green fluorescent protein or β-galactosidase (β-gal) expression vector and detection by fluorescence or by in situ staining with 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal), respectively. Transfected cells were then replated onto 24-well plates coated with fibronectin (FN; 30 μg/ml) or anti-α5 (BIIG2; 20 μg/ml) or onto FN-coated plates with anti-α5 (4 μg/ml) in solution (*3*). Cells were incubated in DMEM supplemented with lactalbumin hydrolysate (0.2%) for 18 hours.
9. F. Kheradmand, E. Werner, P. Tremble, M. Symons, Z. Werb, unpublished data.
10. J. T. Bruder, G. Heidecker, U. R. Rapp, *Genes Dev.* **6**, 545 (1992).
11. P. Angel *et al.*, *Mol. Cell Biol.* **7**, 2256 (1987).
12. The human CL-1 promoter luciferase reporter construct was made by cloning the −517 to +63 nucleotide sequences of the 5′-flanking region of human CL-1 promoter, which contains a minimal promoter from human genomic DNA, by polymerase chain reaction–based cloning. A Hind III deletion site was engineered into the 3′ end of the promoter fragment. This fragment was then subcloned into the Kpn 1–Hind III site in PGL3 plasmid (Promega) to give the (−517 to +63) hCL-1/Luc construct. Transfected cells were lysed in luciferase lysis buffer (Promega), and 3 μg of protein as analyzed by the Bradford method was assayed for luciferase activity with a luminometer. Transfection efficiencies, determined by cotransfection with β-Gal construct (2 μg), were essentially similar.
13. A. Abo, M. R. Webb, A. Grogan, A. W. Segal, *Biochem. J.* **298**, 585 (1994); A. W. Segal and A. Abo, *Trends Biochem. Sci.* **18**, 43 (1993); X. Xu, D. C. Barry, J. Settleman, M. A. Schwartz, G. M. Bokoch, *J. Biol. Chem.* **269**, 23569 (1994).
14. H. M. Lander, *FASEB J.* **11**, 118 (1997); G. Bonizzi *et al.*, *Eur. J. Biochem.* **242**, 544 (1996); S. Miyamoto and I. M. Verma, *Adv. Cancer Res.* **66**, 255 (1995); A. Ray and K. E. Prefontaine, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 752 (1994); A. S. Baldwin Jr., *Annu. Rev. Immunol.* **14**, 649 (1996).
15. D. J. Van Antwerp, S. J. Martin, T. Kafri, D. R. Green, I. M. Verma, *Science* **274**, 787 (1996).
16. Nuclear protein extracts [D. S. Finbloom *et al.*, *Mol. Cell Biol.* **14**, 2113 (1994)] (5 μg) were incubated with a ^{32}P-labeled NF-κB–specific oligonucleotide (Promega) and subjected to electromobility shift analysis as described by the manufacturer.
17. J. A. West-Mays, K. J. Strissel, P. M. Sadow, M. E. Fini, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6768 (1995).
18. Z. Werb, *Cell* **91**, 439 (1997); L. M. Coussens and Z. Werb, *Chem. Biol.* **3**, 895 (1996).
19. B. Anand-Apte *et al.*, *J. Biol. Chem.* **272**, 30688 (1997); P. J. Keely, J. K. Westwick, I. P. Whitehead, J. D. Channing, L. V. Parise, *Nature* **390**, 632 (1997); F. Michiels, G. G. M. Habets, J. C. Stam, R. van der Kammen, J. G. Collard, *ibid.* **375**, 338 (1995).
20. T. H. Lin *et al.*, *J. Cell Biol.* **136**, 1385 (1997); P. E. Hughes *et al.*, *Cell* **88**, 521 (1997); K. M. Yamada and B. Geiger, *Curr. Opin. Cell. Biol.* **9**, 76 (1997); M. A. Schwartz, M. D. Schaller, M. H. Ginsberg, *Annu. Rev. Cell Dev. Biol.* **11**, 549 (1995).
21. ROS production was detected as a transient increase in catalase (200 U/ml)-sensitive oxidation of homovanillic acid [W. Ruch, P. Cooper, M. Baggiolini, *J. Immunol. Methods* **63**, 347 (1983)]. For each treatment, untransfected or Rac1N17-transfected fibroblasts (1×10^6) were plated on FN in seven replicates, and then stimulated with anti-α5 in solution (4 μg/ml) or with CD (2 μg/ml). In the next 2 hours, one plate was harvested every 20 min. For Rac1V12- and Rac1N17-transfected cells, the H_2O_2 production was measured over 2 hours, starting 24 hours after transfection. The results are the average of the integrated peak detected in at least three independent experiments.
22. RSFs were fixed in 4% paraformaldehyde, permeabilized with Triton-X100, and then stained with a polyclonal antibody to the p65 (Rel) component of NF-κB (Santa Cruz Biotech) and with a fluorescein-conjugated antibody to rabbit Ig.
23. Cell lysates (40 μg) were resolved by SDS–polyacrylamide gel electrophoresis (12% gel) and immunoblotted with polyclonal to IκB antibody (Santa Cruz Biotech). Supershift analysis was done by incubating nuclear extracts (5 μg) with antibody to the p50 component of NF-κB (1 μg; Santa Cruz Biotech) for 30 min at ambient temperature.
24. IL-1α was detected by slot immunoblotting (*3*) of medium (100 μl) after 18 hours of culture with 1.5×10^4 with a polyclonal antibody to rabbit IL-1α (Endogen).
25. We thank C. Damsky for the gift of anti-α5 (BIIG2) and critical reading of the manuscript and D. Williams-Herman for assistance in preparing the hCL-1/Luc construct. Supported by NIH grants DE10306 and AR20684 (Z.W.), a fellowship from the American Lung Association, a Mentored Clinician Scientist Award (HL03732) (F.K.), a Pew International Foundation Fellowship (E.W.), and an Institutional National Research Service Award (ES07106).

17 November 1997; accepted 11 March 1998

Modular Organization of Cognitive Systems Masked by Interhemispheric Integration

Kathleen Baynes,* James C. Eliassen, Helmi L. Lutsep,
Michael S. Gazzaniga

After resection of the corpus callosum, V.J., a left-handed woman with left-hemisphere dominance for spoken language, demonstrated a dissociation between spoken and written language. In the key experiment, words flashed to V.J.'s dominant left hemisphere were easily spoken out loud, but could not be written. However, when the words were flashed to her right hemisphere, she could not speak them out loud, but could write them with her left hand. This marked dissociation supports the view that spoken and written language output can be controlled by independent hemispheres, even though before her hemispheric disconnection, they appeared as inseparable cognitive entities.

One of the central challenges to cognitive neuroscience is to unmask the apparent unitary nature of perceptual, memorial, and cognitive systems. Neuropsychological analyses, functional brain imaging methodologies, and analyses of normal reaction times have contributed to revealing how seemingly unitary processes are made up of multiple components. Frequently these multiple components are distributed across the hemispheres but appear unified because of the integration that is possible through the corpus callosum. Examination of split-brain patients both before and after their surgery is another tool for the unmasking of such processes. Here we use this method to reveal a dissociation between the neural representations involved in spoken and written language. These results are consistent with the view that the brain processes enabling written language do not call upon brain representations responsible for phonological capacity.

V.J., a 44-year-old female with a high school education, has a normal developmental and educational history. Her mother, her only sister, and her only daughter are left-handed. No other member of her immediate family has a seizure disorder. Her first seizure occurred when she was 16-and-a-half. V.J. is deaf in her right ear as a result of injuries incurred during a seizure episode in her early 30's. She elected to undergo surgery to resect the corpus callosum after

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K. Baynes, Center for Neuroscience, University of California at Davis, Davis, CA 95616, and VA Northern California Health Care System, Martinez, CA 94553, USA.
J. C. Eliassen, Center for Neuroscience, University of California at Davis, Davis, CA 95616, USA.
H. L. Lutsep, Oregon Stroke Center, Oregon Health Sciences University, Portland, OR 97201, USA.
M. S. Gazzaniga, Center for Cognitive Neuroscience, Dartmouth College, Hanover, NH 03654, USA.

*To whom correspondence should be addressed at Center for Neuroscience, University of California at Davis, Davis, CA 95616, USA. E-mail: kbaynes@ucdavis.edu

suffering a severe burn during a drop-attack at age 41. After surgery, the neurological examination demonstrated minimal apraxia with 0/15 errors with the right hand and 4/15 errors with the left hand. No buccofacial apraxia was present. There was a trace of finger weakness on the left. Hence, there was minor impairment of the left hand only. Additional clinical details are reported elsewhere (*1*).

Since the total resection of her corpus callosum, V.J. has been unable to write or type at will. Before her surgery her dominant left hand wrote and carried out commands normally. After surgery she became agraphic and could not write simple notes or even sign her name. Within 6 months, she recovered the ability to sign her name although she remains dissatisfied with the appearance of her signature. Without lateralization procedures, she could copy single numbers and letters well with her left hand, but poorly with her right hand. She could write about 80% of the alphabet in uppercase and lowercase letters with her left hand, but only a few uppercase letters with her right hand (Fig. 1). Now, 3 years after surgery, V.J. can write a few whole words to auditory dictation with her left hand, but responses are often perseverative or paraphasic. In one session, 35 months after surgery, she correctly wrote only 2 of 73 items from the Johns Hopkins Dysgraphia Battery to oral dictation. Nonetheless, she was able to spell 69 of these items correctly out loud. Still, she continues to be unable to independently write notes or make shopping lists as she did before surgery.

The foregoing profile is consistent with the view that her motor-dominant left hand, now disconnected from her speech-dominant left hemisphere, was agraphic as a result of the cortical-cortical disconnection. However, what was unexpected was that the right hand controlled by the left hemisphere, which can still read and spell, was unable write simple words. To better assess the neural representation of spoken and written language in V.J., we used lateralized testing techniques. We discovered that V.J. could write more fluently only when stimuli were presented with the tachistoscope to her left visual field and her left hand responded out-of-view. Experiments reported here focus on using tachistoscopic presentations to better understand the neural representation of the language system that led to this profound change in writing ability.

Callosotomy was performed in two stages. In early January 1995 V.J. underwent anterior section. Before surgery, we tested her ability to read tachistoscopically displayed words out loud. V.J. was able to read words with equal facility in each visual field. When the same experiment was performed after her anterior section, V.J. remained able to read words with equal facility in either field (Table 1). This result was expected because visual information is passed between the hemispheres in the posterior section of the corpus callosum or splenium, which was still intact (*2*). Her seizures were not controlled, so the posterior section was undertaken.

Recovery from the posterior section of the callosum was marked by the unexpected inability to write to dictation with either hand. As expected, V.J. was unable to read aloud words lateralized to the left visual field (LVF), but easily read words lateralized to the right visual field (RVF), replicating the classic studies (*3*). However, unlike other cases, V.J. was able to write, completely or in part, words lateralized to the silent right hemisphere, but could write almost nothing lateralized to the speaking left hemisphere. Her left hand wrote about one-quarter of the words in each word class without error. More frequently, words were initiated correctly but were not completed, resulting in a pronounced serial-position effect in her left-hand responses (Table 2).

For comparison, this task was presented to two right-handed patients with complete callosotomies: J.W., a 42-year-old male, and D.R., a 51-year-old female. Both patients have normal left language dominance and have been reported elsewhere (*4*). As expected, they were both able to read out loud and write words lateralized to the RVF/left hemisphere (J.W.: wrote 100%, spoke 100%; D.R.: wrote 100%, spoke 100%) and were unable to read out loud or write words lateralized to the LVF/right hemisphere (J.W.: wrote 3%, spoke 4%; D.R.: wrote 0%, spoke 0%) (*5*). The dissociation between the motor output for the spoken and written word in V.J. stands out in comparison (Fig. 2).

To investigate the limits of the right hemisphere's ability to write, we selected a set of 40 pictures with names between three and six letters in length (*6*). These were all high-frequency concrete nouns. These pictures were presented to V.J. in a lateralized format. She saw and attempted to name out loud the entire picture set in one session and saw and attempted to write the names of the pictures in a second session. As expected, she named RVF/left hemisphere items accurately (95% correct), but wrote

Table 1. Percentage correct of nouns, verbs, adjectives, and adverbs read aloud by V.J. before surgery, after anterior collosotomy, and after complete callosotomy.

Field of display	Part of speech			
	Noun	Verb	Adjective	Adverb
	Before surgery			
LVF/right hem.	100	92	77	85
RVF/left hem.	92	92	100	85
	After anterior callosotomy			
LVF/right hem.	100	100	94	94
RVF/left hem.	94	100	94	88
	*Spoken responses after complete callosotomy**			
LVF/right hem.	0	0	0	0
RVF/left hem.	94	100	100	82
	Written responses after complete callosotomy†			
LVF/right hem.	24	24	29	18
RVF/left hem.	0	0	7	0

*Postsurgical written and spoken responses were collected on the same trial with written responses collected first followed by an attempt to name the stimulus item. Later, verbal responses were collected alone to be certain writing had not interfered with them. There were still no correct responses to LVF trials, and RVF percentages were in the same range (nouns, 94%; verbs, 82%; adjectives, 94%; and adverbs, 100%). †All written responses were collected with both hands out of view behind a shield, which allowed V.J. to view the stimuli but obscured her view of her responses. The hand corresponding to the visual field of the display was used for the response. A list of 68 words was created so that there were 17 nouns, 17 verbs, 17 adjectives, and 17 adverbs matched for length (nouns, mean 5 letters; verbs, mean 5.2 letters; adjectives, mean 4.9 letters; adverbs, mean 5.1 letters) and frequency [nouns: mean 545.3, range 254 to 1661; verbs: mean, 549.8, range 240 to 1513; adjectives: mean 519.9, range 247 to 1635; adverbs: mean 494.2, range 226 to 1712 (*12*)]. These words were presented to her one at a time for 116 ms in a random order counterbalanced by visual field to read aloud.

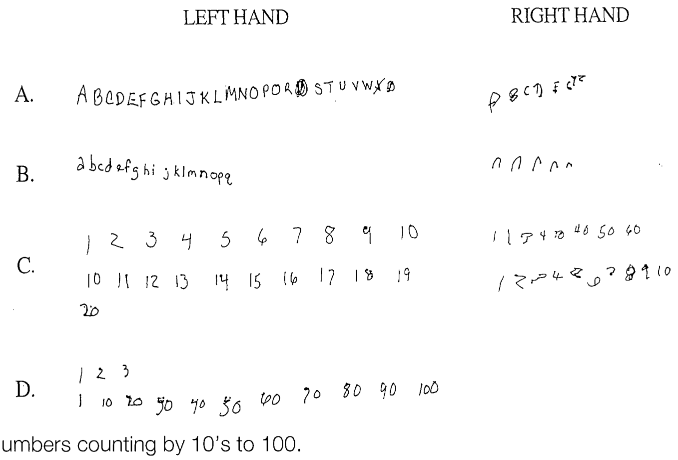


Fig. 1. Right and left hand free-field writing to the following commands. (**A**) Write the alphabet in capital (big) letters. (**B**) Write the alphabet in lowercase (small) letters. (**C**) Write the numbers from 1 to 20. When the right hand stopped writing at 5, the left hand picked up the pen and wrote 30, 40, 50, 60. Reminded to use only her right hand, V.J. made a second attempt and completed numbering up to 10. (**D**) Write the numbers counting by 10's to 100.

none of the names correctly (0% correct). For the LVF/right hemisphere presentations, V.J. named only four items correctly (10% correct) and was unable to write any of the names (0% correct). For three of the named items, she stated that she "saw" the pictures, which implies a loss of fixation and display of the picture to the talking left hemisphere. Most of her verbal responses to LVF/right hemisphere stimuli consisted of, "I didn't catch it," "Too fast," or "I didn't see it" (83%). Although she failed to write any names, twice she attempted to draw the pictured stimuli [see (*1*) for examples of drawn responses].

V.J. was also unable to write the names of 10 common objects palpated with either hand out-of-view (0% correct with either hand). In contrast, when asked to name the same items out loud under conditions that were otherwise the same, she was able to name all of the objects palpated with her right hand and none palpated with her left. The inability to write the names of objects or pictures indicates that V.J.'s right hemisphere has limited access to graphemic output. Although it can form and copy numbers, letters, and words, it cannot write the names from pictures of the objects or the objects themselves.

This inability to write the names of pictures or objects raised the question of whether the right hemisphere had any lexical or semantic knowledge of the words it did write. The same set of 68 words used for writing were matched with a set of pseudowords made by rearranging sublexical units of the original set, thereby controlling for length, letter frequency, and roughly for phonological similarity. The resulting 136 letter strings were presented tachistoscopically to each visual field for 150 ms. V.J. responded by pressing a button with the hand ipsilateral to the field of display to indicate real words. She completed this task easily and accurately with both hemispheres (RVF/left hemisphere $A' = 0.85$; LVF/right hemisphere, $A' = 0.90$). Of the split-brain patients tested on lexical decision, only J.W. equals her accuracy, and no other patient has superior LVF/right hemisphere accuracy (*7*).

Table 2. Percentage of words that had one, two, three, four, and five letters correctly written.

Visual field	Number of letters written correctly in order*				
	One	Two	Three	Four	Five
LVF	71	68	52	38	27
RVF	10	03	03	03	00

*Words included here contain no errors from left to right. Some items were written with errors or omissions (child → chird, or again → ag-n) and they were not counted beyond the first error or omission even if the correct number of omitted letters was indicated.

In another test of hemispheric knowledge of word meaning, a set of 32 pictures was selected (*8*) to form the basis of a matching task. All the pictures had names from three to six letters in length. They were presented to V.J. in four conditions: bilateral display of the same picture to both visual fields followed by a single picture lateralized to either the right or left visual field; bilateral display of a picture followed by a word lateralized to either visual field; bilateral display of a word followed by a word lateralized to either visual field; and bilateral display of a word followed by a picture lateralized to either visual field. Half of the unilateral items matched the bilateral displays and half did not. Bilateral displays lasted 500 ms, followed by a 100-ms pause, a 200-ms tone, and a 150-ms lateralized display. Conditions were presented at separate testing sessions at least 1 month apart. V.J.'s task was to respond with a button press by the hand ipsilateral to the display when the unilateral item matched the bilateral display.

This task was easy for V.J. in either visual field (Table 3). She accurately responded to same trials and made no false-positive responses. Her right hemisphere accurately recognized when a word and a picture matched one another, although it was unable to generate a written word in response to a picture trial.

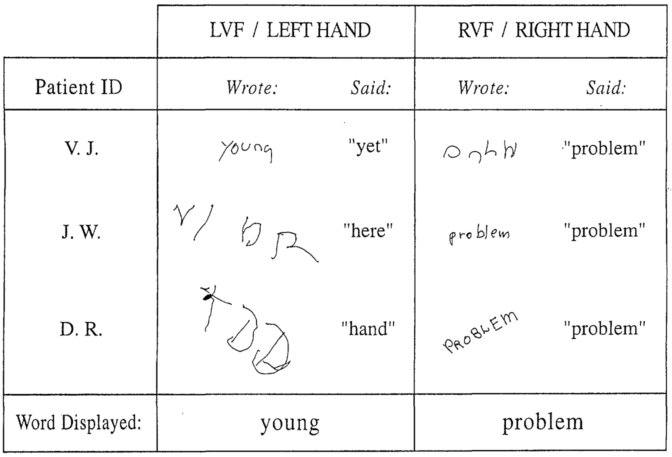
Finally, although her left hemisphere was able to translate from print to sound (that is, pronounce written words), it was not clear if V.J. would be able to perform oral spelling tasks. The same group of 68 words used in the first series of reading experiments was read to her and she was asked to spell those words aloud. She performed this task with only three errors. This finding demonstrates that her left hemisphere has a knowledge of spelling despite its impaired ability to form written letters and words.

V.J. appears to have bilateral representation of language as might be expected in a left-handed individual (*9*). However, her bilateral representation of language at the level of written and spoken output is modular in nature rather than diffusely distributed across the cerebral hemispheres. Other clinical data indicate that the relation between the dominant hand and hemisphere may signal an unusual distribution of language. Language problems, including difficulty with spoken speech, anomia, and agraphia, have been observed after callosotomy in patients whose dominant hand was ipsilateral to the speech-dominant hemisphere as determined by Wada examination (*10*). We suggest such patients may also be examples of anomalous language lateralization in terms of a qualitatively different distribution of language components rather than extremes on a continuum of left or right dominance. The distribution of language skills in V.J. indicates that questions relating language dominance and handedness may have been formulated incorrectly. Rather than looking for dichotomous or continuous distribution of language components, we should be developing models that incorporate modular language lateralization.

It has been difficult to determine wheth-

Table 3. Percentage of correct responses and reaction times for the same or different judgments for words and pictures.

Visual field	Condition			
	Word-word	Picture-word	Picture-picture	Word picture
	Accuracy (%)			
LVF	94	94	100	94
RVF	100	100	100	100
	Median reaction time (*ms*)			
LVF	599	737	585	676
RVF	690	518	632	610



	LVF / LEFT HAND		RVF / RIGHT HAND	
Patient ID	*Wrote:*	*Said:*	*Wrote:*	*Said:*
V. J.	young	"yet"		"problem"
J. W.		"here"	problem	"problem"
D. R.		"hand"	PROBLEM	"problem"
Word Displayed:	young		problem	

Fig. 2. Written and spoken responses to lateralized words by the split-brain subjects V.J., J.W., and D.R. J.W. and D.R. make accurate spoken and written responses to RVF/left hemisphere displays but can neither speak nor write accurately to material displayed in the other visual field. In contrast, V.J. can make accurate spoken responses to RVF/left hemisphere displays, but is only able to write when displays are in the LVF/right hemisphere.

er phonology and orthography are dependent systems that arose together or whether the two are independent, as would be expected if one is biologically determined and one is a human invention. This case illustrates that the neural substrate that supports writing processes can be separate from that which supports spoken speech—the result that would be expected if writing is an independent skill, an invention of the human species, that develops from, but is not part of, the inherited basis of spoken language. The innateness of our ability for spoken language guarantees that this skill develops with a well-established pattern and time course (*10*). Writing (and reading) are more problematic. Both skills require explicit instruction and practice and are a major focus of the public educational process. Many people grow to adulthood without mastering these functions. The percentage of functionally illiterate adults in the United States has been placed as high as 20% of the population (*11*). Spoken language may be innate, an "instinct" if you will, but writing does not develop without instruction. The pattern of lateralization observed here suggests it can have a more varied neural representation as well.

REFERENCES AND NOTES

1. J. C. Eliassen, thesis, University of California at Davis (1997).
2. M. S. Gazzaniga, *The Bisected Brain* (Appleton-Century-Crofts, New York, 1970); D. N. Pandya and B. Seltzer, in *Two Hemispheres—One Brain*, F. Lepore, M. Ptito, H. H. Jasper, Eds. (Liss, New York, 1986), pp. 47–73; J. J. Sidtis, B. T. Volpe, J. D. Holtzman, D. H. Wilson, M. S. Gazzaniga, *Science* **212**, 344 (1981).
3. R. W. Sperry, M. S. Gazzaniga, J. E. Bogen, in *Handbook of Clinical Neurology*, P. J. Vinken and G. W. Bruyn, Eds. (Wiley, New York, 1969), vol. 4, pp. 273–290; M. S. Gazzaniga and R. W. Sperry, *Brain* **90**, 206 (1967).
4. M. S. Gazzaniga, B. T. Volpe, C. S. Smylie, D. H. Wilson, J. E. LeDoux, *Brain* **102**, 805 (1979); M. S. Gazzaniga, J. J. Sidtis, B. T. Volpe, C. S. Smylie, *ibid.* **105**, 53 (1982); K. Baynes, M. J. Tramo, M. S. Gazzaniga, *Neuropsychologia* **30**, 187 (1992).
5. Patient J.W. has demonstrated the ability to read aloud and write the names of concrete words and pictures presented to his LVF. He has never been successful in responding to LVF presentations when stimulus lists varied word class [K. Baynes, M. C. Wessinger, R. Fendrich, M. S. Gazzaniga, *Neuropsychologia* **33**, 1225 (1995); M. S. Gazzaniga *et al.*, *Brain* **119**, 1255 (1996)].
6. J. L. Locke, *The Child's Path to Spoken Language* (Harvard Univ. Press, Cambridge, MA, 1993); L. R. Gleitman and E. Wanner, in *Language Acquisition: The State of the Art*, E. Wanner and L. R. Gleitman, Eds. (Cambridge Univ. Press, Cambridge, 1982), pp. 3–48, E. H. Lenneberg, *Biological Foundations of Language* (Wiley, New York, 1967).
7. K. Baynes and J. C. Eliassen, in *Right Hemisphere Language Comprehension: Perspectives from Cognitive Neuroscience*, M. Beeman and C. Chiarello, Eds. (Erlbaum, Mahwah, NJ, 1998), pp. 79–104.
8. J. G. Snodgrass and M. Vanderwart, *J. Exp. Psychol. Hum. Percept. Perform.* **6** (1980).
9. R. L. Carter, M. Hohenegger, P. Satz, *Neuropsychologia* **18**, 569 (1980); B. Milner, in *Advances in Neurology*, D. P. Purpura, J. K. Penry, R. D. Walters, Eds. (Raven, New York, 1975), vol. 8, pp. 299–321; D. W. Loring, K. J. Meador, G. P. Lee, D. W. King, *Amobarbital Effects and Lateralized Brain Function: The Wada Test* (Springer-Verlag, New York, 1992); J. Levy and M. L. Reid, *Science* **194**, 337 (1976); J. Herron, D. Galin, J. Johnstone, R. E. Ornstein, *ibid.* **205**, 1285 (1979).
10. K. J. Sass, R. A. Novelly, D. D. Spencer, S. S. Spencer, *J. Neurosurg.* **72**, 85 (1990); N. M. Sussman, R. C. Gur, R. E. Gur, M. J. O'Connor, *ibid.* **59**, 514 (1983).
11. M. J. Adams, *Beginning to Read: Thinking and Learning About Print* (MIT Press, Cambridge, MA, 1990).
12. W. N. Francis and H. Kucera, *Frequency Analysis of English Usage: Lexicon and Grammar* (Houghton-Mifflin, Boston, 1982).
13. Informed consent was obtained from V.J. in accordance with the regulations of the UC Davis Human Subjects Committee. We thank V.J. and her husband for their patience and cooperation. We also thank S. Nutik, E. Austin, J. Walker, and R. Berwald for sharing information regarding V.J.'s medical care. We express our appreciation to A. Caramazza and M. Miozzo for sharing the Johns Hopkins Dysgraphia Battery with us. Supported by the National Institute of Neurological Disorders and Stroke (grant P01 NS 17778) to the University of California at Davis.

9 January 1998; accepted 23 March 1998

Thymocyte Development in the Absence of Pre-T Cell Receptor Extracellular Immunoglobulin Domains

Bryan A. Irving, Frederick W. Alt, Nigel Killeen*

Immature thymocytes express a pre–T cell receptor (pre-TCR) composed of the TCRβ chain paired with pre-Tα. Signals from this receptor are essential for passage of thymocytes through a key developmental checkpoint in the thymus. These signals were efficiently delivered in vivo by a truncated form of the murine pre-TCR that lacked all of its extracellular immunoglobulin domains. De novo expression of the truncated pre-TCR or an intact αβTCR was sufficient to activate characteristic TCR signaling pathways in a T cell line. These findings support the view that recognition of an extracellular ligand is not required for pre-TCR function.

Mature αβ T lymphocytes bear on their surface a heterodimeric T cell receptor (TCR) that contains the protein products from rearranged TCRα and TCRβ loci. During development, rearrangement of the TCRβ locus occurs first, allowing immature $CD4^-CD8^-$ [double-negative (DN)] thymocytes to synthesize the TCRβ protein and express it on the surface in association with the pre-Tα (pTα) protein (*1*, *2*). Signals from this "pre-TCR" then induce the cells to differentiate into $CD4^+CD8^+$ [double-positive (DP)] cells and to undergo a rapid series of cell divisions. In the absence of pre-TCR function, this differentiation is blocked, and thymic cellularity is severely reduced (*3–12*).

Signaling by the pre-TCR and the mature receptor involves the CD3/ζ chains and depends on the concerted action of Src family and Syk/ZAP-70 tyrosine kinases (*4–6*). No ligand for the pre-TCR has yet been identified, but one might be expected, given the surface expression of the pre-TCR (*11*, *13*, *14*) and its structural resemblance to antigen receptors. Alternatively, surface expression of the TCRβ-pTα heterodimer might be sufficient to initiate the pre-TCR signaling process.

To distinguish between these possibilities, we generated transgenic mice expressing a truncated form of the pre-TCR heterodimer that lacked all of its extracellular immunoglobulin (Ig) domains. Truncated forms of TCRβ and pTα (β_T and $pT\alpha_T$, respectively) were constructed (Fig. 1A) (*15*) that have their normal extracellular Ig domains replaced with Flag or Myc epitope tags but still retain the cysteine residues and transmembrane domains required for heterodimer formation and assembly with the signal-transducing CD3 and ζ chains (*16*). To confirm that the modifications had not adversely affected assembly of the receptor (*17*), we transiently transfected the truncated chains, individually or together, by electroporation into a TCR-deficient thymoma (*18*). Because the cells do not express TCRβ, the expression of $pT\alpha_T$ alone did not rescue CD3 on the cell surface (Fig. 1B). In contrast, the introduction of β_T alone resulted in a slight but reproducible increase in surface expression of CD3, presumably through the formation of heterodimers with endogenous pTα. The expression of β_T and $pT\alpha_T$ together resulted

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B. A. Irving and N. Killeen, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143–0414, USA.
F. W. Alt, Howard Hughes Medical Institute and Department of Genetics and Pediatrics, Children's Hospital and Center for Blood Research, Harvard Medical School, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: nigel@itsa.ucsf.edu