Berg, E. W. Meijer, *Science* **266**, 1226 (1994); R. H. Jin, T. Aida, S. Inoue, *Chem. Commun.* **1993**, 1260 (1993).
3. G. R. Newkome *et al.*, *Chem. Commun.* **1996**, 2737 (1996).
4. J. M. J. Fréchet, *Science* **263**, 1710 (1994).
5. G. R. Newkome *et al.*, *Angew. Chem. Int. Ed. Engl.* **34**, 2023 (1995); G. R. Newkome, *J. Heterocycl. Chem.* **33**, 1445 (1996).
6. S. C. Zimmermann, F. Zeng, D. E. C. Reichert, S. V. Kolotuchin, *Science* **271**, 1095 (1996).
7. Structural analogs of dendrimers (dendrigrafts) were built with large molecular building blocks such as oligomers and polymers through a "graft-upon-graft" synthetic strategy [D. A. Tomalia, D. M. Hedstrand, M. S. Ferritto, *Macromolecules* **24**, 1435 (1991); R. Yin, D. R. Swanson, D. A. Tomalia, *Polym. Mat. Sci. Eng.* **73**, 277 (1995)]. Some arborescent polymers exhibiting the same dendrigraft molecular topology have also been described [M. Gauthier and M. Moller, *Macromolecules* **24**, 4548 (1991); M. Gauthier, W. Li, L. Tichagwa, *Polym. Mat. Sci. Eng.* **73**, 232 (1995)].
8. L. Lochmann, K. L. Wooley, P. T. Ivanova, J. M. J. Fréchet, *J. Am. Chem. Soc.* **115**, 7043 (1993).
9. G. R. Newkome *et al.*, *Angew. Chem. Int. Ed. Engl.* **33**, 666 (1994).
10. N. Launay, A.-M. Caminade, R. Lahana, J.-P. Majoral, *ibid.*, p. 1589; N. Launay, A.-M. Caminade, J.-P. Majoral, *J. Am. Chem. Soc.* **117**, 3282 (1995); M. Slany *et al.*, *ibid.*, p. 9764; C. Larré, A.-M. Caminade, J.-P. Majoral, *Angew. Chem. Int. Ed. Engl.* **36**, 596 (1997).
11. We thank CNRS for financial support.

21 May 1997; accepted 5 August 1997

Infants' Memory for Spoken Words

Peter W. Jusczyk and Elizabeth A. Hohne

Infants' long-term retention of the sound patterns of words was explored by exposing them to recordings of three children's stories for 10 days during a 2-week period when they were 8 months old. After an interval of 2 weeks, the infants heard lists of words that either occurred frequently or did not occur in the stories. The infants listened significantly longer to the lists of story words. By comparison, a control group of infants who had not been exposed to the stories showed no such preference. The findings suggest that 8-month-olds are beginning to engage in long-term storage of words that occur frequently in speech, which is an important prerequisite for learning language.

The latter half of the infant's first year is a critical point in acquiring a language. During this period, language learners take their first steps toward acquiring a vocabulary. Young infants already possess many of the speech perception capacities required for learning words (*1*). Infants younger than 6 months distinguish a wide range of speech contrasts (*2*) and have some capacity to compensate for differences in voices (*3*) and speaking rates (*4*). Moreover, after 6 months, infants demonstrate some recognition of the particular phonetic and prosodic characteristics of their native language (*5*). Finally, 7.5-month-olds have another important prerequisite for lexical development: the ability to segment fluent speech into word-sized units (*6*–*8*).

As important as these speech perception capacities are, the acquisition of vocabulary requires many other capacities, such as the ability to learn meanings and to attach them to the appropriate verbal labels. Most critically, vocabulary acquisition requires some long-term retention of both meanings and verbal labels. Yet this latter aspect of vocabulary acquisition has not been well documented. In contrast to the considerable information that exists about early speech perception capacities (*9*), it is surprising that so little is known about infants' long-term memory for the sound patterns of words. The present investigation explores this critical prerequisite for vocabulary acquisition.

P. W. Jusczyk, Departments of Psychology and Cognitive Science, Johns Hopkins University, Baltimore, MD 21218, USA.
E. A. Hohne, AT&T Labs, Holmdel, NJ 07733–3030, USA.

To investigate infants' long-term retention of information about the sounds of words, we visited 15 8-month-old infants in their homes 10 times each during a 2-week period. On each occasion, while seated in a chair, infants heard 30 min of prerecorded speech consisting of three short stories for young children (*10*, *11*). In addition to recording the three stories, each talker also recorded a list of 72 content words, in citation form. Thirty-six of these words consisted of the most frequently repeated content words in the three stories (*12*). The remaining 36 words were foil words that never occurred in the stories. Each of these foils was chosen to match as closely as possible one of the story words with respect to the overall frequency of occurrence in English and to sound properties such as stress pattern and phonetic characteristics (such as vowel qualities and manner of articulation of consonants). Each of the 36 story words was randomly assigned to one of three test lists. Then the same words were randomly assigned once again to three more lists to create a total of six different story-word lists. The same procedures were followed with the foil words to create six different lists of these items. Examples of typical story-word and foil lists are shown in Fig. 1.

Two weeks after the last of the 10 home visits, each infant was brought to the laboratory so that her or his retention of the sound patterns of words in the stories could be examined. Our hypothesis was that if infants attended to and stored in memory the sound patterns of frequently occurring words in the stories, they might show subsequent recognition of these sound patterns by demonstrating listening preferences for these items over unfamiliar items. The head-turn preference procedure—a widely used computer-automated method in infant speech research (*6*, *7*, *13*, *14*)—was selected for testing the infants (*15*, *16*).

Mean times of listening to the story-word and foil lists were calculated for each infant, based on the infant's fixations of the flashing light on each trial (*17*). As shown in Fig. 2 (left), the infants had significantly longer mean listening times for the lists with the story words than for the lists with the foils [$t(14) = 3.44$, $P < 0.005$]. This listening preference for the lists of story words is consistent with the prediction that the infants extracted, encoded, and retained information about the sound patterns of frequently occurring words in the stories. However, despite the efforts made to equate the story-word and foil lists in critical aspects such as their phonetic characteristics and frequency of occurrence in the language, it is possible that the infants' prior experience with these words in the stories is not the crucial factor responsible for the listening preferences observed. Per-

Examples of Test Materials

Story-Word List	Foil Word List
sneeze	aches
elephant	apricot
ants	sloth
gray	jaunt
vine	ox
python	lanterns
peccaries	caribous
back	front
laugh	burp
out	change
best	beach
jungle	camel

Fig. 1. Examples of typical story-word and foil lists.

haps the story words were simply more intrinsically interesting than the foil words. If so, infants might show a preference for these items even without any prior exposure to the words in the stories.

To check this possibility, we tested an additional group of 15 9-month-old infants with the same methods and materials, but the infants had no prior period of exposure to the stories. As shown in Fig. 2 (right), infants without experience listening to the stories did not display any preferences for the lists of story words [$t(14) = -0.42$, $P > 0.60$]. If anything, these infants listened slightly longer to the foil words. Thus, there is no indication that the story words are simply more interesting to listen to than the foil words (*18*).

The present findings have important implications for understanding the course of language acquisition. These results indicate that 8- to- 9-month-olds engage in long-term encoding and retention of information about the sound patterns of words that occur frequently in fluent speech, even when there is little contextual support from the surrounding environment (that is, a prerecorded voice on audio tape). Long-term storage of sound patterns of words is obviously a prerequisite for acquiring vocabulary. Additionally, storage of sound patterns may provide the necessary database for drawing inferences about the types of phonetic and prosodic patterns that recur frequently in the native language.

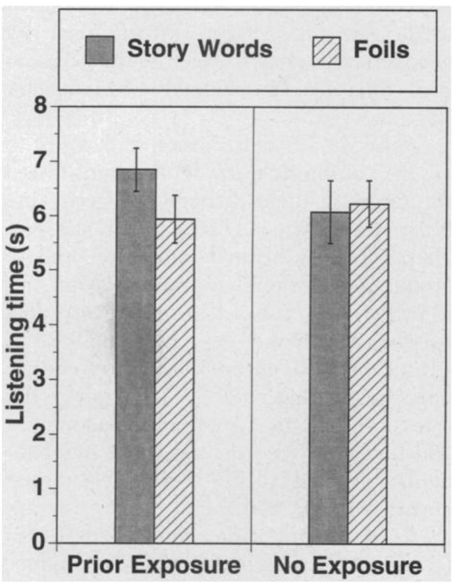

Fig. 2. (Left) Mean listening times and standard error bars for infants who, as 8-month-olds, had heard the stories on 10 different occasions before being tested. (**Right**) Comparable mean listening times and standard error bars for infants who were tested on the story-word and foil lists without any prior exposure to the stories.

The present study also provides further confirmation that infants at this age are beginning to segment words from fluent speech (*6*). The fact that the initial exposure to the words occurred in fluent speech contexts and subsequently led to listening preferences for citation versions of the words suggests that infants not only attended to and stored the sound patterns of words that recurred in the stories, but also that they have some means of representing these patterns and compensating for different acoustic-phonetic forms of the same words. These findings about the abilities of infants to store the sound patterns of words complement others that pertain to the concepts picked out by words. For example, infants as young as 3 months can segment the visual world into categories and internally represent these very rudimentary concepts (*19*). They also make inferences about the properties of physical objects (*20*). These overlapping developments in the abilities to segment, encode, and retain information about both sounds and object categories form the foundation for learning words. Both labels (that is, sound patterns) and concepts (that is, meanings) are required for building a lexicon. Word learning depends on associating underlying categories or concepts with the internalized representations of the sound patterns of words. The fact that infants retain information about sound patterns over a 2-week interval suggests that this process sometimes begins by first storing a familiar sound pattern, then attaching a meaning to it (as well as by subsequently learning the verbal label that goes with an already known meaning or concept). Finally, infants' close attention to and retention of sound patterns that occur frequently in the input suggests that this aspect of language acquisition can be characterized as a form of innately guided learning: that is, infants are primed to learn some things very rapidly and to learn them in a particular way (*9*, *21*).

REFERENCES AND NOTES

1. R. N. Aslin, P. W. Jusczyk, D. B. Pisoni, in *Handbook of Child Psychology*, D. Kuhn and R. Siegler, Eds. (Wiley, New York, in press), vol. 2; C. T. Best, in *Advances in Infancy Research*, C. Rovee-Collier and L. P. Lipsitt, Eds. (Ablex, Norwood, NJ, 1995), vol. 9, pp. 217–304.
2. P. D. Eimas, E. R. Siqueland, P. Jusczyk, J. Vigorito, *Science* **171**, 303 (1971); P. D. Eimas, *Percept. Psychophys.* **16**, 513 (1974); *ibid.* **18**, 341 (1975).
3. P. K. Kuhl, *J. Acoust. Soc. Am.* **66**, 1668 (1979).
4. P. D. Eimas and J. L. Miller, *Science* **209**, 1140 (1980).
5. P. W. Jusczyk, A. Cutler, N. Redanz, *Child Dev.* **64**, 675 (1993); P. W. Jusczyk, A. D. Friederici, J. Wessels, V. Y. Svenkerud, A. M. Jusczyk, *J. Mem. Lang.* **32**, 402 (1993); P. K. Kuhl, K. A. Williams, F. Lacerda, K. N. Stevens, B. Lindblom, *Science* **255**, 606 (1992); J. F. Werker and R. C. Tees, *Infant Behav. Dev.* **7**, 49 (1984).
6. P. W. Jusczyk and R. N. Aslin, *Cogn. Psychol.* **29**, 1 (1995).
7. J. R. Saffran, R. N. Aslin, E. L. Newport, *Science* **274**, 1926 (1996).
8. J. L. Morgan, *J. Mem. Lang.* **35**, 666 (1996).
9. P. W. Jusczyk, *The Discovery of Spoken Language* (MIT Press, Cambridge, MA, 1997).
10. The stories were *The Lion's Bed*, by Diane Redfield Massie; *"Stand Back," Said the Elephant*, *"I'm Going to Sneeze,"* by Patricia Thomas; and *The Little Gray Kitten*, by Mary Laurence Turnbull.
11. Pilot work suggested that varying the voice of the reader and the ordering of the stories better engaged infants' attention. Accordingly, versions of all the stories were recorded on audiocassette tapes by five different female talkers (none of whom visited the homes). Each talker recorded the stories in two different orders. On a given day, an infant heard a set of stories by a single talker. While the infant was listening to the stories, a research assistant also flipped through a series of cartoon drawings that were based on the stories. The talker and story ordering were varied every day, so that across the 10 visits, each infant was exposed to all 10 talker/story-order combinations.
12. On any given day, the words from these lists had occurred in the stories an average of 13 times.
13. D. G. Kemler Nelson *et al.*, *Infant Behav. Dev.* **18**, 111 (1995).
14. P. Halle and B. de Boysson-Bardies, *ibid.* **19**, 463 (1996).
15. Each infant sat on a caregiver's lap in the middle of a three-sided enclosure. On the center panel of the enclosure directly facing the infant was a green light, mounted at eye level, that could be flashed to attract the infant's attention to midline. A video camera, situated behind the panel and below the green light, recorded the test session through a peephole in order to check reliability in measuring head turns. A red light and a hidden loudspeaker were mounted on each side panel. An experimenter seated behind the center panel observed the infant through a small hole. She initiated trials and recorded the infant's looking times by operating a response box linked to a PDP 11/73 computer. Computer software was responsible for the selection and randomization of stimuli and for the timing and termination of trials.
16. A test trial began with the flashing of the green light on the center panel. When the infant oriented to the center panel, the green light was extinguished through the response box, and a red light on one of the side panels began to flash. When the infant oriented in the direction of the flashing red light, the experimenter initiated a speech sample and began recording the infant's looking time by pressing a button on the response box. Whenever the infant looked away, the experimenter pressed another button on the response box that stopped the timer. If the infant looked away for more than two consecutive seconds, the computer terminated the trial. Both the experimenter and the caregiver used foam earplugs and listened to loud masking music over sound-insulated headphones throughout the duration of the experiment to prevent them from hearing the stimulus materials. Each session began with a preparatory phase in which infants were presented with four practice trials consisting of lists of recorded words. Two of the lists included words from the stories, but not ones used in the test lists. The other two lists consisted of foils matched to the story words in the practice lists. The purpose of this phase was to familiarize the infants with the lights on the sides of the testing booth, and to ensure that they were capable of making the required orienting response. The loudspeaker from which the stimuli were emitted varied randomly from trial to trial. After the preparatory phase, the test phase began and continued until an infant completed all 12 test trials. For a given infant, the test and practice lists were always produced by the same talker (one of the five who had produced the stories). Thus, three of the infants were tested with each of the five voices.
17. That is, the times that infants spent looking away from the light on a trial were subtracted from the total trial duration to obtain this measure.

18. To explore further whether the differences in listening preferences between the two groups of infants were attributable to prior experience with the stories, the data from the two groups of infants were combined for a 2 (test group) × 2 (list type) analysis of variance. Only the interaction between the test group and list type was significant [$F(1, 28) = 5.60$, $P < 0.03$], confirming that the preference for the story-word lists occurred only when infants had had prior exposure to the stories.
19. P. D. Eimas and P. C. Quinn, *Child Dev.* **65**, 903 (1994); J. M. Mandler and L. McDonough, *Cogn. Dev.* **8**, 291 (1993); P. C. Quinn, P. D. Eimas, S. L. Rosenkrantz, *Perception* **22**, 463 (1993).
20. R. Baillargeon and J. DeVos, *Child Dev.* **62**, 1227 (1991); E. Spelke, *Cognition* **50**, 431 (1994); ______, K. Breinlinger, J. Macomber, K. Jacobson, *Psychol. Rev.* **99**, 605 (1992).
21. P. Marler, *Dev. Psychobiol.* **23**, 557 (1990).
22. Supported by a research grant from NIH to P.W.J. (HD15795). We thank A. M. Jusczyk, N. Redanz, D. Dombrowski, B. Boyle, and S. Elsis for their help in recruiting and testing participants and M. Brent, P. Smolensky, G. Ball, R. Tincoff, T. Nazzi, D. Houston, and two anonymous reviewers for helpful comments that they made on early versions of the manuscript. The parents of all infant participants gave informed consent.

22 July 1997; accepted 19 August 1997

LKLF: A Transcriptional Regulator of Single-Positive T Cell Quiescence and Survival

Chay T. Kuo, Margaret L. Veselits, Jeffrey M. Leiden*

Mature single-positive (SP) T lymphocytes enter a "resting" state in which they are proliferatively quiescent and relatively resistant to apoptosis. The molecular mechanisms regulating this quiescent phenotype were unknown. Here it was found that the expression of a Kruppel-like zinc finger transcription factor, lung Kruppel-like factor (LKLF), is developmentally induced during the maturation of SP quiescent T cells and rapidly extinguished after SP T cell activation. LKLF-deficient T cells produced by gene targeting had a spontaneously activated phenotype and died in the spleen and lymph nodes from Fas ligand–induced apoptosis. Thus, LKLF is required to program the quiescent state of SP T cells and to maintain their viability in the peripheral lymphoid organs and blood.

Single-positive ($CD4^+$ or $CD8^+$) mature T cells circulate through the blood and peripheral lymphoid organs in a quiescent or resting state until they encounter their cognate antigen bound to a major histocompatibility molecule (MHC) on the surface of an appropriate antigen-presenting cell (*1*). Engagement of the T cell antigen receptor (TCR) by the peptide antigen–MHC complex leads to T cell activation, a process that involves the highly orchestrated expression of more than 100 new genes and concomitant cell cycle progression and proliferation (*2*). Activated T cells acquire a characteristic set of cell surface markers and are more sensitive to apoptosis (*3, 4*). Many activated T cells die in the peripheral lymphoid organs from activation-induced cell death, an apoptotic pathway that has been postulated to protect the host against autoimmune disease (*5*). Several transcription factors, including activation protein–1 (AP1), nuclear factor of activated T cells (NFAT), nuclear factor–kappa B, (NF-κB), and the cAMP response element binding protein (CREB), are known to be important positive regulators of activation-specific T cell gene expression (*6, 7*). However, it was not known if specific transcription factors are also required to program or maintain the quiescent state in resting SP T cells (*8*).

The erythroid Kruppel-like factor (EKLF) is an erythroid-specific zinc finger transcription factor that binds to CACCC sequence motifs in the promoter of the β-globin gene and regulates the terminal stages of erythroid development (*9*). To identify EKLF-related genes that might play a similar role in T cell development, we screened an embryonic mouse cDNA library under low-stringency hybridization conditions with a probe derived from the zinc finger region of EKLF (*10*). We identified three additional KLF family members, BKLF, GKLF, and LKLF (*11*). To determine whether any of these other KLF proteins might participate in T cell development, we hybridized Northern (RNA) blots and mouse tissue sections to probes specific for each cDNA. LKLF was expressed in the lung, the vasculature, the heart, skeletal muscle, kidney, and testis as well as in the lymphoid organs of the mouse (Fig. 1A) (*12*). Within the thymus, LKLF was expressed exclusively in lymphoid cells in the thymic medulla, a region that contains mature SP thymocytes (Fig. 1B). LKLF was also expressed in large amounts in lymphoid cells in the white pulp of the spleen (*12*). To more precisely characterize LKLF expression in the hematopoietic lineages, we did Northern blot analyses on purified populations of hematopoietic cells (Fig. 1A). LKLF was expressed in both $CD4^+$ and $CD8^+$ SP thymocytes and splenocytes, but it was undetectable in less mature double-positive (DP) $CD4^+CD8^+$ thymocytes (Fig. 1A). LKLF was also expressed in $B220^+$ immunoglobulin M (IgM)–positive splenic B cells and in bone marrow macrophages (Fig. 1A). LKLF mRNA and protein were decreased significantly after the TCR-mediated activation of quiescent splenic T cells (Fig. 1, C and D). Degradation of the LKLF protein preceded disappearance of the mRNA, suggesting that both LKLF mRNA and protein are regulated after T cell activation. This developmentally and activation-regulated pattern of expression suggested that LKLF might play an important role in regulating the function of resting SP T cells in vivo.

We used homologous recombination in murine embryonic stem (ES) cells to produce a targeted mutation of the *LKLF* gene (Fig. 2A). The resulting null mutation deleted the entire *LKLF* gene. Heterozygous ($LKLF^{+/-}$) mutant ES cell clones produced with a phosphoglycerate kinase-*neomycin* resistance (PGK-*neo*^{r}) targeting vector (Fig. 2A) were retransfected with a PGK-*hygromycin* resistance (PGK-*hygro*^{r}) targeting vector (Fig. 2A) to produce three independently derived homozygous ($LKLF^{-/-}$) ES cell clones. The genotypes of these clones were confirmed by Southern (DNA) blot analysis (Fig. 2B). Each clone contained single *neo*^{r} and *hygro*^{r} integrations. By Northern blot analysis, each of the homozygous clones lacked detectable LKLF mRNA (Fig. 2C).

$LKLF^{+/-}$ ES cell clones were used to produce chimeric mice, which transmitted the targeted allele through the germ line (*13*). Heterozygous (*$LKLF^{+/-}$*) mice were phenotypically normal and were bred to produce LKLF-deficient animals. Mice homozygous for the LKLF mutation died between embryonic days 12.5 and 14.5 as a result of intra-amniotic and intra-embryonic hemorrhages (*13*). To analyze the role of LKLF in lymphoid development and function, we injected three independently derived $LKLF^{-/-}$ ES cell clones and two $LKLF^{+/-}$ ES cell clones into recombinase activating gene 2–deficient ($RAG\text{-}2^{-/-}$) blastocysts (*14*) to produce *$LKLF^{+/-}RAG2^{-/-}$* and *$LKLF^{-/-}RAG2^{-/-}$* chimeric mice. Because mature B and T cells cannot develop in the absence of RAG-2, all B and T cells in such chimeric mice are derived from the $LKLF^{-/-}$ ES cells. Thus, the $RAG\text{-}2^{-/-}$ chimera system provides a stringent test for the in-

Departments of Medicine and Pathology and The Committee on Genetics, University of Chicago, Chicago, IL 60637, USA.

*To whom correspondence should be addressed. E-mail: jleiden@medicine.bsd.uchicago.edu