loss (*7*), as opposed to our in vitro data showing Aβ-induced, microglial CD40-mediated neuronal degeneration. This difference may be due to other cell types (including astroglia and cerebral vascular cells) and extracellular systems available in vivo in the central nervous system that are likely to mitigate the tendency to neuronal death induced by Aβ and CD40L. In our experimental models, the CD40-mediated neurotoxic pathway appears to be activated quite early in the pathogenic cascade, suggesting that therapeutic agents that block the CD40 signaling pathway may suppress neurodegeneration in AD.

References and Notes

1. L. Meda *et al.*, *Nature* **374**, 647 (1995).
2. C. K. Combs, D. E. Johnson, S. B. Cannady, T. M. Lehman, G. E. Landreth, *J. Neurosci.* **19**, 928 (1999); R. N. Kalaria, *Curr. Opin. Hematol.* **6**, 15 (1999).
3. U. Schonbeck *et al.*, *J. Biol. Chem.* **272**, 19569 (1997); G. D. Sempowski, P. R. Chess, R. P. Phipps, *J. Immunol.* **158**, 4670 (1997); K. Karmann, C. C. Hughes, J. Schechner, W. C. Fanslow, J. S. Pober, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4342 (1995).
4. See supplementary data at www.sciencemag.org/feature/data/1041359.
5. All analyses were performed with SPSS for Windows, release 7.5.1. Data were analyzed with analysis of variance (ANOVA) followed by post hoc comparisons of means where appropriate with Bonferonni's method. Single mean comparisons were performed with the *t* test for independent samples. Alpha levels were set at 0.05 for each analysis.
6. Web figures are available at www.sciencemag.org/feature/data/1041359.
7. Tg APP_{sw} mice are the 2576 line crossed with C57B6/SJL as described [K. K. Hsiao *et al.*, *Science* **274**, 99 (1996); K. K. Hsiao *et al.*, *Neuron* **15**, 1203 (1995)]. To further characterize adult mice, we analyzed CD40 and TNF-α protein expression by protein immunoblot (densitometric signal ratio to actin) in brain lysates from 12-month-old Tg APP_{sw} or control littermates. CD40 and TNF-α protein levels were significantly ($P < 0.01$) increased in Tg APP_{sw} mice compared with control littermates (CD40, mean = 1.01 ± 0.03 SEM compared with 0.75 ± 0.03; TNF-α, 1.04 ± 0.01 compared with 0.81 ± 0.04).
8. Soluble $A\beta_{1\text{-}40}$ was quantified in the culture media of Tg APP_{sw} or control littermate–derived microglia 48 hours after plating with the Aβ40 enzyme-linked immunosorbent assay (ELISA) kit (QCB, Hopkinton, MA), in strict accordance with the manufacturer's instruction. Data showed a significant ($P < 0.001$) increase in soluble $A\beta_{1\text{-}40}$ from Tg APP_{sw} microglia (mean = 291.90 ± 21.81 SEM pg of $A\beta_{1\text{-}40}$ per milligram of total cellular protein) compared with control littermate–derived microglia (23.41 ± 7.75).
9. G. Ruggiero *et al.*, *J. Immunol.* **156**, 3737 (1996); D. Hollenbaug *et al.*, *J. Exp. Med.* **182**, 33 (1995); R. W. Denfeld *et al.*, *Eur. J. Immunol.* **26**, 2329 (1996).
10. M. Huberman *et al.*, *J. Neuroimmunol.* **52**, 147 (1994).
11. A. Klegeris, D. G. Walker, P. L. McGeer, *Brain Res.* **747**, 114 (1997).
12. G. M. Castillo, C. Ngo, J. Cummings, T. N. Wight, A. D. Snow, *J. Neurochem.* **69**, 2452 (1997).
13. J. Tan *et al.*, *J. Neuroimmunol.* **97**, 77 (1999).
14. J. Xu *et al.*, *Immunity* **1**, 423 (1994).
15. A. Schneider, J. Biernat, M. von Bergen, E. Mandelkow, E. M. Mandelkow, *Biochemistry* **38**, 3549 (1999); C. Sturchler-Pierrat *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13287 (1997); N. D. James *et al.*, *Neurobiol. Aging* **17**, 235 (1996); L. S. Higgins, J. M. Rodems, R. Catalano, D. Quon, B. Cordell, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4402 (1995); I. Genis, A. Fisher, D. M. Michaelson, *J. Neurochem.* **72**, 206 (1999).
16. T. Uchihara, H. Akiyama, H. Kondo, K. Ikeda, *Stroke* **28**, 1948 (1997).
17. F. Mach *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1931 (1997).
18. R. J. Ellis *et al.*, *Neurology* **46**, 1592 (1996).
19. V. H. Oleana, A. Salehi, D. F. Swaab, *Neuroreport* **9**, 1451 (1998); J. Rogers, J. Luber-Narod, S. D. Styren, W. H. Civin, *Neurobiol. Aging* **9**, 339 (1988).
20. V. Henn *et al.*, *Nature* **391**, 591 (1998).
21. Q. X. Li *et al.*, *Blood* **84**, 133 (1994).

22. We are grateful to D. Roskamp and R. Roskamp for their generous support, which made this work possible. We thank T. Reading for his support. R.A.F. is an investigator of the Howard Hughes Medical Institute. We thank P. Ricciardi-Castagnoli for providing the murine microglial cell lines (N9), J.-Y. Bonnefoy for providing the human CD40L, H. Yu for assistance in maintaining the CD40L-deficient mice, and A. Placzek for assistance in mice genotyping.

7 April 1999; accepted 10 November 1999

Cognitive Modularity and Genetic Disorders

S. J. Paterson,[1,2] J. H. Brown,[1,2] M. K. Gsödl,[1] M. H. Johnson,[3] A. Karmiloff-Smith[1]*

This study challenges the use of adult neuropsychological models for explaining developmental disorders of genetic origin. When uneven cognitive profiles are found in childhood or adulthood, it is assumed that such phenotypic outcomes characterize infant starting states, and it has been claimed that modules subserving these abilities start out either intact or impaired. Findings from two experiments with infants with Williams syndrome (a phenotype selected to bolster innate modularity claims) indicate a within-syndrome double dissociation: For numerosity judgments, they do well in infancy but poorly in adulthood, whereas for language, they perform poorly in infancy but well in adulthood. The theoretical and clinical implications of these results could lead to a shift in focus for studies of genetic disorders.

Many theorists use the adult neuropsychological model of impaired and intact cognitive modules to explain genetic disorders. Adults whose brains have developed normally but who later suffer brain damage are often left with a pattern of dissociations between different cognitive modules. They may function normally in several domains but, for instance, display impaired performance solely in the domain of face processing or in the domain of syntax. This leads theorists to claim that human (adult) cognition is characterized by independently functioning modules that subserve different domains and can be differentially impaired. When similar dissociations are found in children with genetic disorders, the use of the same adult model is either explicitly recommended (*1, 2*) or implicit in the argumentation from an uneven performance profile to underlying brain structure (*3–5*). It is also assumed that if the phenotypic outcome in a genetic disorder presents an uneven cognitive profile, then this will also characterize the initial state in infancy. In other words, it is claimed that atypically developing children start out with a fractionated pattern of impaired and intact modules. This claim is then used to bolster theoretical arguments in favor of the prespecified modular structure of the human mind/brain.

A syndrome that has attracted much attention in this respect is Williams syndrome (WS). WS is a rare neurodevelopmental disorder caused by a submicroscopic deletion on chromosome 7q11.23. Although a number of genes have now been identified in the deleted region, original claims as to their cognitive function in WS (*6*) have been challenged by our more recent work (*7*). WS occurs in 1 in 20,000 live births. Its clinical features include several physical abnormalities accompanied by mild to moderate mental retardation and a specific personality profile. The interest in WS among cognitive neuroscientists stems from the very uneven cognitive profile displayed in the phenotypic outcome (*8*). Many theorists have used this uneven pattern of abilities and impairments in WS to support claims about the existence of innately specified cognitive modules, some of which are spared (language and face processing) and others impaired (number and visuospatial processing) (*4, 5*). This view tends to ignore the dynamics of development [see (*8*) for discussion]. With the use of new data from infants, we argue that one cannot assume that the infant starting state can be directly inferred from the phenotypic outcome.

To assess our hypotheses and to explore the infant origins of the cognitive outcome in WS, we specifically chose two cognitive domains with different phenotypic end states. By middle childhood or adulthood, individuals with WS

[1]Neurocognitive Development Unit, Institute of Child Health, University College, London WC1N 1EH, UK. [2]Psychology Department, University College, London WC1E 6BT, UK. [3]Centre for Brain and Cognitive Development, Birkbeck College, London WC1E 7JL, UK.

*To whom correspondence should be addressed. E-mail: a.karmiloff-smith@ich.ucl.ac.uk

have serious impairments in numerosity judgment but display surprising proficiency in vocabulary. If the use of the adult neuropsychological model were justified and consistent patterns of deficit and proficiency persisted over developmental time, then one would predict that WS infants should display far better performance on a vocabulary task than on a number task. We tested this prediction in two experiments. In both experiments, infants were assessed using a replica of the basic Fagan visual-preference viewing box (*9*) and were seated in a special infant chair.

The order of the two experiments was randomized across infants. Those with WS formed the experimental group. We had three control groups, all matched with the WS group on sex and socioeconomic status. One control group consisted of infants with another abnormal phenotype—Down syndrome (DS)—of equivalent chronological and mental age. If infants in the DS group performed differently from those in the WS group in either of the two experiments, then mental retardation alone could not explain the WS results. Two groups of normally developing infants were also tested, one matched on mental age to control for level of general intelligence (the MA control group), and one matched on chronological age to control for equivalent length of experience (the CA control group).

For both experiments, the WS infants were all screened genetically and clinically before inclusion in the study. A FISH (fluorescent in situ hybridization) test for the elastin gene deletion had been carried out, and all WS infants were positive. This was supplemented by a clinical evaluation confirming the facial dysmorphology and other physical features typical of WS. We ascertained from their records that all DS control infants were full trisomy 21 and none were mosaic. The normal controls were included only if their mental age as assessed on the Bayley-II (*10*) was at the appropriate chronological age.

Experiment 1 was a test of sensitivity to numerical changes in a series of displays of pictures of everyday objects. A total of 65 infants took part. Infants were familiarized to pairs of stimuli containing arrays of changing sets of two objects in different configurations. They were then tested on a pair with one stimulus containing two objects and the other containing three objects [see (*11*) for details of spatial layout and spatial extent]; this task was based on well-established techniques used with normal infants (*12, 13*). Each infant was shown six familiarization trials. After familiarization with sets of two, the infant was presented with one card displaying new objects but the old numerosity (two) and another card displaying new objects but a novel numerosity (three). Each pair of stimuli was presented twice (three objects on left side, three on right side, for 5 s each). The side on which the novel numerosity first appeared was randomized, and experimenter B, who recorded the looking times to the old and novel numerosities, was blind to the position of each card.

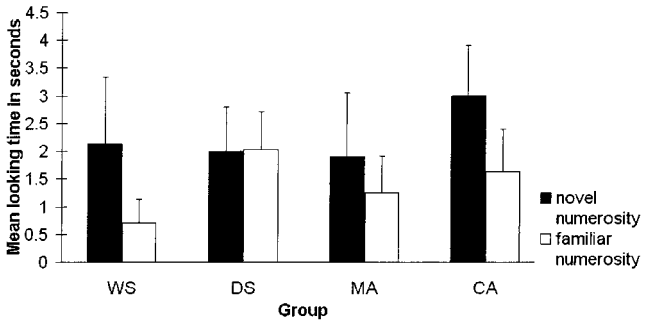

Fig. 1. Results of experiment 1: Mean looking time to old numerosity versus new numerosity as a function of atypical and normal groups. Thirteen infants with WS (mean chronological age 31.0 months, SD 5.39, range 24 to 36 months; mean mental age 16.9 months, SD 2.81, range 12 to 21 months) were tested. Twenty-two infants with DS (mean chronological age 30.0 months, SD 4.9, range 24 to 36 months; mean mental age 15.6 months, SD 2.43, range 12 to 20 months) formed the atypical control group. Sixteen infants were matched on mental age (MA) to the atypically developing infants (mean chronological age 15.4 months, SD 2.52, range 12 to 20 months; mean mental age 15.1 months, SD 2.66, range 11 to 21 months), and 14 infants were matched on chronological age (CA) to the clinical populations (mean chronological age 30.4 months, SD 5.3, range 24 to 36 months; mean mental age 30.4 months, SD 5.32, range 25 to 40 months). The atypical infants and normal MA controls were matched on the Mental Scale of the Bayley Scales of Infant Development II (*10*). The mean looking times to the novel and familiar numerosities were calculated for each infant. Outliers were removed from the analyses (*14*). The looking times were entered into a repeated-measures analysis of variance (ANOVA), with group (WS, DS, MA control, CA control) as the between-subjects factor and numerosity (novel or familiar) as the within-subjects factor. An effect of numerosity [$F(1, 57) = 34.21$, $P < 0.001$] and of group [$F(3, 57) = 5.94$, $P < 0.001$] was found. A significant group × numerosity interaction was also found [$F(3, 57) = 5.99$, $P < 0.001$]. Post hoc t tests comparing the mean scores for novel and old numerosities for each group revealed a significant difference in looking time between numerosities for the WS, CA control, and MA control groups (WS $t = 4.2$, df = 11, $P < 0.001$; CA control $t = 3.87$, df = 13, $P < 0.002$; MA control $t = 2.18$, df = 14, $P < 0.05$). There was no such difference for the DS group. A one-way ANOVA was carried out to examine whether the magnitude of these differences varied between groups, with difference (novel numerosity minus old numerosity) as the dependent variable [$F(3, 60) = 5.99$, $P < 0.001$]. Post hoc tests were then carried out to compare the magnitude of difference between looking times to novel and old numerosities for each of the groups. The WS and DS groups differed significantly [Tukey's honestly significant difference (HSD), $P < 0.05$], as did the DS and CA infants (Tukey's HSD, $P < 0.05$). The magnitude of the difference between the DS and MA infants was not significant.

If infants were merely sensitive to changes in the types of objects, they would show no preferential looking in the test phase when the numerosity changed. However, if during familiarization they had become sensitive to the constant numerosity (two) of each of the displays, then they should look significantly longer when, in the test phase, one of the pair displayed a new numerosity, even though both of the display cards showed interesting new objects. Given the serious impairments in numerosity judgment in the end states of both WS and DS, it was predicted that if initial states can be directly derived from end states, then infants with both syndromes would show impairment on this task and at best perform like their MA controls.

Figure 1 shows the results for the four groups, with the data from outliers removed (*14*). Relative to both MA and CA controls, WS infants showed no deficit in their sensitivity to different numerosities. By contrast, the DS infants were impaired relative to all other groups (their looking times to the old and novel numerosities were nearly equal). The WS proficiency in infancy exists even though WS children and adults show serious impairment in number tasks.

Experiment 2 assessed vocabulary development. Seventy-one infants (63 of whom also participated in experiment 1) took part, divided into the same four groups: the WS experimental group, the DS control group, and the CA and MA normal control groups. The task measured whether infants would look longer at a picture that is accompanied by the sound of its verbal label (matching) than at one that is not labeled (nonmatching); the task was based on well-established techniques used with normal infants (*15, 16*). Infants were presented with a series of pairs of colored photographs of everyday objects. Each pair of stimuli was presented twice (matching on left side, matching on right side). Just before opening the display to the infant, experimenter A said in a loud, clear voice, using the highly pitched intonation of "motherese": "Look, look at the X" (the name of the matching stimulus) and repeated this three times while the stimuli were on display. The named card was randomly assigned to the left or right compartment, and experimenter B was blind to the position of the named card. Unlike experiment 1, there was no familiarization phase. Experimenter B measured the looking times for each of the test displays. In other respects, experiment 2 was identical to experiment 1.

It was predicted that if initial states can be directly derived from end states, then infants

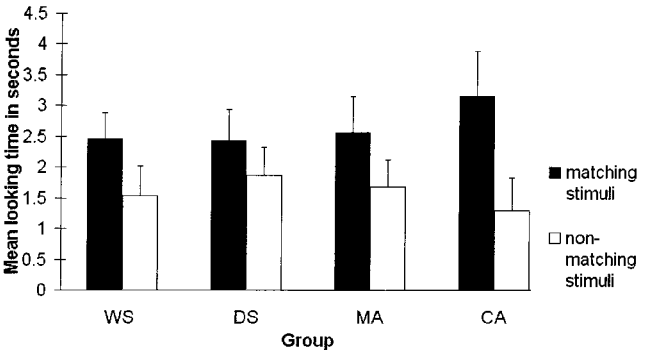

Fig. 2. Results of experiment 2: Mean looking time to named (matching) stimulus versus unlabeled (nonmatching) stimulus as a function of atypical and normal groups. Seventy-one infants were tested: 15 with WS (mean chronological age 30.4 months, SD 4.79, range 24 to 36 months; mean mental age 16.5 months, SD 2.39, range 12 to 20), 22 with DS (mean chronological age 29.7 months, SD 5.06, range 24 to 36 months; mean mental age 15.6 months, SD 2.36, range 12 to 20 months), 17 MA controls (mean chronological age 15.4 months, SD 2.33, range 12 to 20; mean mental age 15.1 months, SD 2.48, range 12 to 21 months), and 17 CA controls (mean chronological age 30.0 months, SD 5.13, range 24 to 36 months; mean mental age 30.6 months, SD 5.14, range 25 to 40 months). Mental age matching was again based on the Mental Scale of the Bayley Scales of Infant Development II (*10*). A repeated-measures ANOVA showed an effect of looking to the matching or nonmatching stimulus [$F(1, 64) = 87.79$, $P < 0.001$] on the mean looking time to each stimulus, but no effect of group (WS, DS, MA control, CA control). A significant interaction between group and match was also found [$F(3, 64) = 6.64$, $P < 0.001$]. Post hoc t tests examining the differences within each group between looking times to the matching and nonmatching stimuli revealed significant differences in all groups (WS $t = 4.65$, df = 13, $P < 0.001$; DS $t = 3.21$, df = 20, $P < 0.004$; MA control $t = 4.06$, df = 15, $P < 0.001$; CA control $t = 6.41$, df = 16, $P < 0.001$). A one-way ANOVA was carried out to examine whether the magnitude of these differences varied between groups, with difference (matching stimuli minus nonmatching stimuli) as the dependent variable [$F(3, 67) = 5.58$, $P < 0.001$]. Post hoc tests were then carried out to compare the magnitude of the difference between looking times to matching and nonmatching stimuli for each of the groups. The CA group was significantly different from all other infant groups (Tukey's HSD, $P < 0.05$). There was no difference between the WS and DS infants.

with WS should perform more like the CA controls and be well in advance of the DS controls, who should perform like the MA controls. Figure 2 displays the mean looking times to matching and nonmatching stimuli by group, with data from outliers removed (*17*). The findings show that despite their documented vocabulary proficiency in the end state, infants with WS are seriously delayed and as impaired in infancy as those with DS. This impairment in WS infants exists even though children and adults with WS end up with very good vocabularies, whereas those with DS remain seriously delayed.

The use of the neuropsychological model for understanding genetic disorders has hitherto only been criticized on theoretical grounds (*8, 18*). Our results now challenge the use of this model through empirical findings from the direct study of infant clinical populations. The results from both the domains of number and language show that researchers cannot rely on phenotypic outcomes to make generalizations about impaired or intact modules in the initial state. Our results point to a new type of developmental double dissociation illustrated by WS: For numerosity judgment, WS patients do well in infancy but poorly in adulthood, whereas for language, WS patients do poorly in infancy but well in adulthood. The data highlight how essential it is to trace cognitive dysfunctions back to their origins in infancy and not solely to focus on middle childhood and beyond.

Our findings should alter the way in which future studies of atypical development are carried out, with far more emphasis being placed on determining cognitive profiles in infancy. The identification of infant starting states also has crucial implications for clinical practice and the timing of cognitive remediation. With respect to number development, for instance, DS infants may lack the initial domain-specific prerequisites, whereas for WS infants it is the subsequent process of learning that explains phenotypic outcomes in the end state. Such critical differences in the initial state could in no way have been inferred from the impaired end states for numerosity judgment in each of the syndromes. Our findings also have implications for the idea that genes can be directly related to behavior—that is, the hypothesis that psychological function can be solely derived from phenotypic steady states in middle childhood and beyond. Finally, from a theoretical perspective, our results strongly suggest that cognitive scientists, neuroscientists, and philosophers of mind cannot use the purported sparing or impairment of a cognitive function in middle childhood or adulthood to support the claim that cognitive modules are innately specified in infancy. Where modules do exist in the adult end state, they are likely to be the product of a developmental trajectory (in both the normal and atypical cases), and not its starting point (*19, 20*).

References and Notes

1. C. M. Temple, *J. Child Psychol. Psychiatry* **38**, 27 (1997).
2. S. Baron-Cohen, in *Handbook of Mental Retardation and Development*, J. A. Burack, R. M. Hodapp, E. Zigler, Eds. (Cambridge Univ. Press, Cambridge, 1998), p. 335.
3. A. M. Leslie, *Curr. Dir. Psychol. Sci.* **1**, 18 (1992).
4. U. Bellugi, P. Wang, T. L. Jernigan, in *Atypical Cognitive Deficits in Developmental Disorders: Implications for Brain Function*, S. Broman and J. Graffman, Eds. (Erlbaum, Hillsdale, NJ, 1994), pp. 23–56.
5. S. Pinker, *Science* **253**, 530 (1991).
6. J. M. Frangiskakis *et al.*, *Cell* **86**, 59 (1996).
7. M. Tassabehji *et al.*, *Am. J. Hum. Genet.* **64**, 118 (1999).
8. A. Karmiloff-Smith, *Trends Cognit. Sci.* **2**, 389 (1998).
9. J. F. Fagan, *J. Exp. Child Psychol.* **9**, 217 (1970). The Fagan Box had a hinged display stage (85 cm long and 29 cm high), with two compartments to hold the stimulus cards. The center-to-center distance between the compartments was 30.5 cm, and on all trials the display stage was situated about 30.5 cm above the infant's head. In the center of the stage was a peephole 0.625 cm in diameter, through which an observer could see the visual fixations of the infant. The stage was illuminated using a fluorescent lamp out of the infant's view. It was centered directly over the infant, such that the infant could no longer see the parent. The stimuli were then placed in two compartments simultaneously by experimenter A and, once the infant's attention was attained by talking or by shaking a rattle, the familiarization or test trials began. Looking times were measured by experimenter B. Experimenter B held a stopwatch in each hand and timed the infant's looking to the left versus the right stimulus item by observing the corneal reflection of each stimulus in the infant's pupil. A beeper was set to a fixed length for the familiarization and test trials and signaled when each trial was to end. Between each familiarization or test trial, experimenter A pulled back the display stage from the infant's view, recorded the data called out by experimenter B, changed the stimuli, obtained the infant's attention again, centered the infant's gaze, and finally closed the stage, exposing the next stimuli to the infant. The roles of experimenters A and B were randomly changed between subjects. Reliability using this procedure has been shown to be high [R. A. Haaf, M. Brewster, C. M. de Saint Victor, P. H. Smith, *Infant Behav. Dev.* **12**, 211 (1989); L. M. O'Neill, S. W. Jacobson, J. L. Jacobson, *Infant. Behav. Dev.* **17**, 465 (1994)].
10. N. Bayley, *Bayley Scales of Infant Development* (Psychological Corporation, San Antonio, TX, ed. 2, 1993).
11. The stimuli for experiment 1 were six colored pictures of pairs of objects mounted onto white cards (17.7 cm by 17.7 cm). Different objects were used for each pair (airplanes, cats, dogs, cars, and so forth) in different positions on the card. To ensure that infants were not simply reacting to differences in mass and density [see analysis in M. W. Clearfield and K. S. Mix, *Psychol. Sci.* **10**, 408 (1999)], we were particularly careful to design stimuli pairs of different sizes taking up different spatial extents in various locations on the cards, and to ensure that the display of three stimulus items covered no more space than that of two stimulus items. This ensured that infants focused on the numerosity of the discrete displays rather than on their spatial extent.
12. P. Starkey, E. Spelke, R. Gelman, *Cognition* **36**, 97 (1990).
13. M. S. Strauss and L. E. Curtis, *Child Dev.* **52**, 1146 (1981).
14. Outliers were found with a box-and-whisker plot. The main body of the plot represented values between the 25th and 75th percentile. Participants whose looking time was more than 1.5 standard deviations above or below these values were excluded from further analyses, in accordance with the conventional procedure [P. R. Kinnear and C. D. Gray, *SPSS for Windows Made Simple* (Psychology Press, Hove, UK, ed. 3, 1999), p. 98]. The WS group and the MA controls involved one outlier each, the DS group two outliers, and the CA group none.
15. R. Golinkoff, K. Hirsh-Pasek, K. M. Cauley, L. Gordon, *J. Child Lang.* **14**, 23 (1987).
16. G. Schafer and K. Plunkett, *Child Dev.* **69**, 309 (1998).
17. Outliers were treated as in experiment 1 [see (*14*)].

The WS, DS, and MA control groups all involved one outlier, and the CA control group none.
18. D. V. M. Bishop, *Q. J. Exp. Psychol. A* **50**, 899 (1997).
19. A. Karmiloff-Smith, *Beyond Modularity: A Developmental Perspective on Cognitive Science* (MIT Press, Cambridge, MA, 1992).
20. J. Elman *et al.*, *Rethinking Innateness: A Connectionist Perspective on Development* (MIT Press, Cambridge, MA, 1996).
21. Supported by the Down's Syndrome Association (S.J.P.) and the UK Medical Research Council (A.K.-S.). We thank the Williams Syndrome Foundation and the Down's Syndrome Association for help in recruiting participants, P. Quinn and J. Spencer for providing training in the use of the Fagan Box, and S. Hesketh for assistance with initial pilot testing.

17 August 1999; accepted 20 October 1999

Mediation by a CREB Family Transcription Factor of NGF-Dependent Survival of Sympathetic Neurons

Antonella Riccio,[1] Sohyun Ahn,[1] Christopher M. Davenport,[1] Julie A. Blendy,[2] David D. Ginty[1]*

Nerve growth factor (NGF) and other neurotrophins support survival of neurons through processes that are incompletely understood. The transcription factor CREB is a critical mediator of NGF-dependent gene expression, but whether CREB family transcription factors regulate expression of genes that contribute to NGF-dependent survival of sympathetic neurons is unknown. CREB-mediated gene expression was both necessary for NGF-dependent survival and sufficient on its own to promote survival of sympathetic neurons. Moreover, expression of Bcl-2 was activated by NGF and other neurotrophins by a CREB-dependent transcriptional mechanism. Overexpression of Bcl-2 reduced the death-promoting effects of CREB inhibition. Together, these data support a model in which neurotrophins promote survival of neurons, in part through a mechanism involving CREB family transcription factor–dependent expression of genes encoding prosurvival factors.

Nerve growth factor (NGF), a member of the neurotrophin family of growth factors, is a target-derived survival factor for developing sympathetic neurons (*1*). CREB [cyclic adenosine monophosphate (cAMP) response element binding protein] is a mediator of the nuclear response to neurotrophins in PC12 cells and cortical neurons (*2*–*5*). Moreover, exposure of distal axons of sympathetic neurons grown in compartmentalized cultures to NGF results in robust phosphorylation of CREB on its transcriptional regulatory site, serine 133 (*6*). These observations support the idea that a target-derived NGF signal is propagated retrogradely from distal axons to CREB within nuclei of developing sympathetic neurons to control gene transcription. Whether CREB-dependent gene expression is required for survival of sympathetic neurons is unclear. Moreover, the identity of NGF-sensitive genes that contribute to growth and survival of sympathetic neurons is unknown.

Neonatal sympathetic neurons are completely dependent on NGF for survival in vivo and in vitro. To determine whether CREB-mediated gene expression is necessary for NGF-dependent neuronal survival, we monitored survival of sympathetic neurons after expression of either of two distinct inhibitors of CREB. One CREB inhibitor, A-CREB, is a potent and selective inhibitor of CREB DNA binding activity (*4*). The other, CREBm1, binds to CREB binding sites in DNA but is not activated because the transcriptional regulatory residue, serine 133, is mutated to alanine (*7*). Microinjection of expression vectors encoding either A-CREB or CREBm1, but not vector alone, led to nearly complete apoptotic death of sympathetic neurons grown in the presence of NGF, as assessed by Hoechst

[1]Department of Neuroscience, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205–2185, USA. [2]Department of Pharmacology, University of Pennsylvania Medical Center, Philadelphia, PA 19104–6084, USA.

*To whom correspondence should be addressed. E-mail: dginty@jhmi.edu

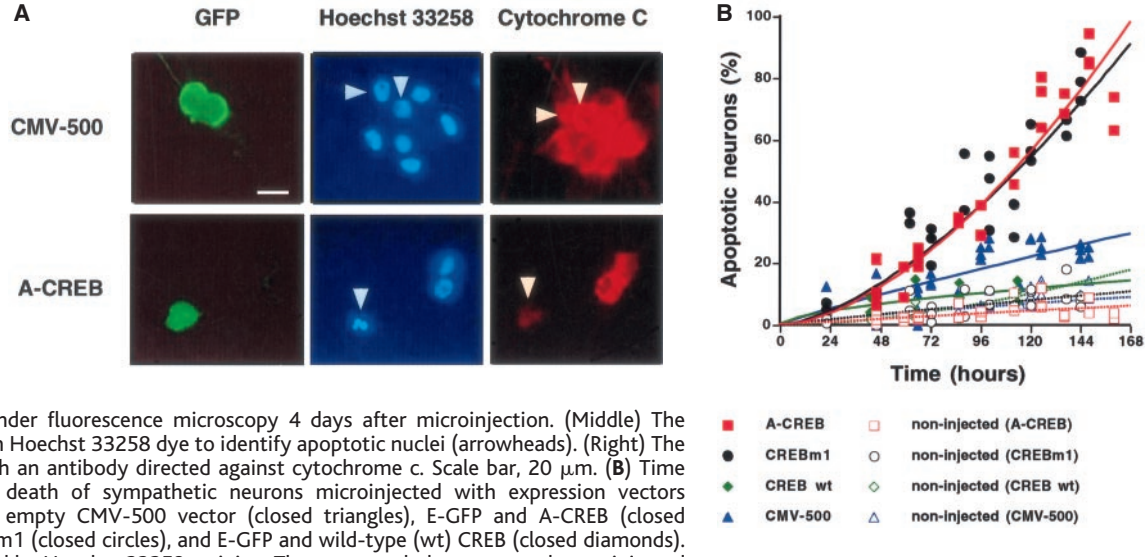

Fig. 1. Requirement of a CREB family transcription factor for survival of sympathetic neurons grown in the presence of NGF. Six to nine DIV sympathetic neurons were microinjected with expression vectors encoding GFP and either a CMV-500 expression vector or expression vectors encoding either A-CREB or CREBm1 (*23*). (**A**) Apoptotic cell death of sympathetic neurons expressing A-CREB. (Left) Neurons photographed under fluorescence microscopy 4 days after microinjection. (Middle) The same neurons stained with Hoechst 33258 dye to identify apoptotic nuclei (arrowheads). (Right) The same neurons stained with an antibody directed against cytochrome c. Scale bar, 20 μm. (**B**) Time course of apoptotic cell death of sympathetic neurons microinjected with expression vectors encoding E-GFP and the empty CMV-500 vector (closed triangles), E-GFP and A-CREB (closed squares), E-GFP and CREBm1 (closed circles), and E-GFP and wild-type (wt) CREB (closed diamonds). Cell viability was measured by Hoechst 33258 staining. The open symbols represent the noninjected cells from the same plates. Each data point represents the value from one individual plate of microinjected neurons for which viability of 50 or more microinjected or noninjected neurons was determined.