ORIGINAL ARTICLE

Early Life Stress and Inherited Variation in Monkey Hippocampal Volumes

David M. Lyons, PhD; Chou Yang, MA; Anne M. Sawyer-Glover, RT(R)(MR); Michael E. Moseley, PhD; Alan F. Schatzberg, MD

Background: Opportunities for research on the causes and consequences of stress-related hippocampal atrophy are limited in human psychiatric disorders. Therefore, this longitudinal study investigated early life stress and inherited variation in monkey hippocampal volumes.

Methods: Paternal half-siblings raised apart from one another by different mothers in the absence of fathers were randomized to 1 of 3 postnatal conditions that disrupted diverse aspects of early maternal care (n=13 monkeys per condition). These conditions were previously shown to produce differences in social behavior, emotional reactivity, and neuroendocrine stress physiology. Hippocampal volumes were subsequently determined in adulthood by high-resolution magnetic resonance imaging.

Results: Adult hippocampal volumes did not differ with respect to the stressful postnatal conditions. Based on paternal half-sibling effects, the estimated proportion of genetic variance, ie, heritability, was 54% for hippocampal size. Paternal half-siblings with small adult hippocampal volumes responded to the removal of all mothers after weaning with initially larger relative increases in cortisol levels. Plasma cortisol levels 3 and 7 days later, and measures of cortisol-negative feedback in adulthood were not, however, correlated with hippocampal size.

Conclusions: In humans with mood and anxiety disorders, small hippocampal volumes have been taken as evidence that excessive stress levels of cortisol induce hippocampal volume loss. Results from this study of monkeys suggest that small hippocampi also reflect an inherited characteristic of the brain. Genetically informed clinical studies should assess whether inherited variation in hippocampal morphology contributes to excessive stress levels of cortisol through diminished neuroendocrine regulation.

Arch Gen Psychiatry. 2001;58:1145-1151

From the Departments of Psychiatry and Behavioral Science (Drs Lyons and Schatzberg and Mr Yang) and Radiology (Ms Sawyer-Glover and Dr Moseley), Stanford University Medical School, Stanford, Calif.

SMALL HIPPOCAMPAL volumes are found in adult humans with recurrent major depression[1,2] and posttraumatic stress disorder.[3,4] Childhood stress increases the risk of developing these mood and anxiety disorders,[5] and small hippocampal volumes are evident in adult survivors of childhood maltreatment.[6,7] Hippocampal morphology is altered by stress in carefully controlled studies of rodents,[8-11] and small hippocampal volumes in humans have been taken as evidence that stress-related disorders induce hippocampal volume loss.[2,11,12] An alternative possibility that cannot be dismissed in the absence of prospective longitudinal research is that small hippocampal volumes are inherited and predispose toward the development of psychiatric disorders that are triggered or aggravated by stress.[3,11]

Aside from a limited number of reports on inherited variation in autonomic activity[13] and cerebrospinal fluid monoamine levels,[14,15] primate research on stress neurobiology has focused on maternal deprivation.[16] Rhesus macaque monkeys raised without mothers exhibit increased brain dopamine and norepinephrine sensitivity,[17,18] exaggerated hypothalamic-pituitary-adrenal (HPA) responses to stress,[19,20] altered regulation of autonomic activity,[21] fragmented sleep patterns,[22] depression-like behavior,[18] and excessive consumption of alcohol.[23] Ecologically informed research on maternal availability has likewise identified untoward effects on primate postnatal development. Bonnet macaque monkeys raised by their mothers in variable foraging-demand conditions are impaired in social and emotional development.[24,25] In early adulthood, these same monkeys exhibit elevated cerebrospinal fluid levels of monoamines, somatostatin, and corticotropin-releasing factor (CRF).[26,27]

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

MATERIALS AND METHODS

MATERIALS

Forty infants sired by fathers that had no contact with their offspring were distributed randomly in groups each composed of 3 or 4 mother-infant pairs. All monkeys were of Guyanese origin (*Saimiri sciureus*) and were born and maintained at the Stanford University Primate Facility, Stanford, Calif. One monkey from the low-foraging demand condition (see "Experimental Design and Procedures" section) was excluded for reasons unrelated to the study. Twelve fathers and 30 mothers produced the 39 monkeys that constituted the study cohort. Twenty-one mothers each contributed 1 offspring, and 9 mothers produced with different fathers on separate occasions 2 offspring. Two fathers each sired a single offspring, 3 fathers each sired 2 offspring, 2 fathers each sired 3 offspring, 3 fathers each sired 4 offspring, 1 father sired 5 offspring, and 1 father sired 8 offspring. All procedures were conducted in accord with and as required by the Animal Welfare Act, and approved by Stanford University's Administrative Panel on Laboratory Animal Care.

EXPERIMENTAL DESIGN AND PROCEDURES

Four natal groups were randomly assigned to each of the following 3 rearing conditions when infants were 10 weeks old (age range, 8-13 weeks).

1. *Low-foraging demands.* Thirteen infants (7 males and 6 females) and their 13 mothers were maintained from 10 to 21 weeks post partum in low-foraging demand conditions. Each group received 600% by weight of their normal daily food intake buried in foraging boards.[37] All 80 holes in the foraging boards contained abundant amounts of food.
2. *High-foraging demands.* Thirteen infants (7 males and 6 females) and 13 mothers were maintained from 10 to 21 weeks post partum in high-foraging demand conditions where each group was provisioned with 120% of their daily food intake buried in the foraging boards. Many holes in the foraging boards contained little or no food, so more time and effort were required to find food.
3. *Intermittent social separations.* Thirteen infants (6 males and 7 females) and 13 mothers fed from standard foodhoppers were separated intermittently for 5 sessions each lasting 5 hours in duration. Every other week from 13 to 21 weeks post partum, each infant was removed one at a time, placed in a cage adjacent to unfamiliar monkeys, and temporarily deprived of all contact with members of the natal group.

After completion of these postnatal protocols at 21 weeks of age, all monkeys were housed in standard conditions. Social behavior, emotional reactivity, and increases in plasma cortisol levels elicited by removing all mothers after weaning were examined at 36 weeks.[39] Shortly thereafter monkeys were housed with 2 or 3 animals of the same sex from different rearing conditions. Approximately 4 years later in early adulthood (age range, 3.6-5.9 years) a neuroendocrine challenge was administered to test cortisol-negative feedback regulation of the HPA-axis response to exogenous stimulation by CRF.[40] Then 5 weeks later hippocampal volumes were determined by MRI.

BRAIN IMAGE ACQUISTION AND ANALYSIS

Magnetic resonance imaging was performed using a 1.5-T (General Electric Medical Systems, Milwaukee, Wis) system. Monkeys were scanned under anesthesia induced by a subcutaneous injection of a combination of 20 mg/kg of ketamine hydrochloride, 4 mg/kg of xylazine hydrochloride, and 0.04 mg/kg of atropine sulfate. Body temperatures were maintained within the normal range using a cushioned heating pad. Earplugs provided protection from noises generated by the scanner.

The first scan for each monkey was acquired in the sagittal plane with a 2-dimensional sequential spoiled gradient acquistion pulse sequence: repetition time, 18 milliseconds; echo time, 4 milliseconds; flip angle, 30°; 1 signal averaged; acquisition matrix, 256×128 pixels; voxel size, $0.5 \times 1.0 \times 4.0$ mm; and slice thickness, 4 mm. This initial localizer scan was used to standardize head tilt and rotation by assuring that 2 external markers (vitamin E capsules in the meatus of each ear) were aligned in both the coronal and axial planes. The head was repositioned as required, and another sagittal

Studies of stress and hippocampal plasticity in primates have produced conflicting results. Maternal deprivation in rhesus macaque monkeys increases dentate gyrus non–phosphorylated neurofilament protein levels, a neuronal marker of vulnerability in Alzheimer disease, Huntington disease, Parkinson disease, and amyotrophic lateral sclerosis.[28] Hippocampal cell damage in adult vervet monkeys is induced by sustained and fatal stress[29] or long-term treatment with cortisol implanted in adult hippocampus.[30] Yet elderly adult pigtailed macaque monkeys treated long-term with oral cortisol do not respond with diminished volumes or neuron numbers in any hippocampal cell field.[31] Rhesus macaque monkeys raised in social isolation also fail to show altered hippocampal volumes as determined in vivo at 18 months of age by magnetic resonance imaging (MRI).[32]

Here we test for early stress effects and inherited variation in hippocampal volumes in 39 squirrel monkeys. Paternal half-siblings raised apart from one another by different mothers in the absence of fathers were randomized to conditions that disrupted diverse aspects of early maternal care. In one condition on 5 occasions offspring were briefly separated from groups composed of 3 or 4 mother-infant pairs. Intermittent separations consistently elicit short-term elevations in cortisol levels with baseline cortisol levels restored soon after the subsequent social reunions.[33-35] In 2 other postnatal rearing conditions, differences in maternal availability were produced by manipulating the effort required to find food. Body weights and amounts of food consumed in each foraging condition were not significantly different, but high-foraging demand mothers stopped carrying their

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

localizer scan was performed. Head pitch was then standardized against the midsagittal image, with the final scan acquired in the coronal plane. The final scan used for hippocampal measurements (**Figure 1**) was a 3-dimensional inversion recovery prepared fast spoiled gradient acquistion pulse sequence: repetition time, 12 milliseconds; echo time, 3 milliseconds, inversion time, 300 milliseconds; flip angle, 15°; 4 signals averaged; acquistion matrix, 256 × 224 pixels; voxel size, 0.31 × 0.36 × 1.00 mm; and slice thickness,1 mm.

Image processing was performed offline with ANALYZE software (Biomedical Imaging Resource; Mayo Foundation, Rochester, Minn) as previously described for human studies of hippocampal volumes.[2] To minimize interscan variation a Histogram Match function in ANALYZE was used to normalize gray-scale pixel values for each brain against a single standard. A trained human rater blind to each monkey's identity then measured hippocampal volumes on each brain side.

Stereological methods were used with ANALYZE software to generate unbiased estimates of hippocampal volumes. Sampling parameters were set to yield at least 150 "hits" per measurement, a number previously shown to generate reliable determinations.[41] For sampling purposes, a rigid grid was superimposed on each brain image with grid placement randomly determined by ANALYZE. All grid points falling directly on hippocampal gray matter were identified by the trained human rater. From these determinations ANALYZE generated an unbiased estimate of hippocampal volumes based on the Cavelieri Principle.

Rules for identifying hippocampal volumes were adapted from human protocols.[2,42] The most posterior coronal slice for volumetry was identified when gray matter hippocampus first appeared adjacent to the trigone of the lateral ventricle. Hippocampal gray matter in all coronal slices anterior to this location was bordered superiorly by the fornix-fibria white matter junction, inferiorly by parahippocampal gyrus white matter, medially by subarchnoid spaces of the ambient cistern, and laterally by the cerebrospinal fluid–filled lateral ventricle or temporal horn white matter. The most anterior coronal slice used for volumetry fell at the head of the hippocampus medial to the amygdala in the coronal plane. One or two 1-mm slices anterior to this location were excluded from determinations of hippocampal volumes due to the lack of reliable boundaries for distinguishing amygdala from hippocampus.

To adjust for variation in overall brain size, brain volumes were defined and subsequently measured as all gray and white matter in both hemispheres, including the midbrain superior to the pons. The superior border of the pons was choosen for demarcation because it is easily recognized on MRIs of brain.[2] Based on the measurements of 2 trained raters independently scoring the same 13 monkey brains, interrater reliabilities expressed as intraclass correlations ranged from 0.90 to 0.97 (left hippocampus, 0.94; right hippocampus, 0.90; and overall brain size, 0.97).

DATA ANALYSIS

Sex, paternity (offspring grouped by father), and rearing condition main effects for adult hippocampal volumes were examined with repeated-measures analysis of variance (ANOVA) using least squares estimates from general linear models (Systat, Evanston, Ill). Sex, paternity, and rearing condition were between-subjects factors, and hippocampal volume on each brain side was considered the within-subjects factor. Paternity was not analyzed as a random factor because the error terms for sex and rearing condition could not be generated from appropriate interactions in the unbalanced factorial design.[39] This did not influence the analysis of paternity since the same error term is used regardless of whether paternity is random or fixed.[43]

Quantitative estimates of heritability (h^2)were generated from 1-way ANOVAs used to evaluate paternal half-sibling effects.[44] From separate ANOVAs for each measure of interest, the between-father mean square minus the within-father mean square was divided by 3.25 (average number of offspring per father), multiplied by 4 (paternal half-siblings share, on average, 25% of their genome by common descent), and divided by the total variance. Heritabilities resemble intraclass correlations adjusted for genetic relatedness. Under the null hypothesis of no hereditary effect, within- and between-father components of variance are equivalent, and the resulting F ratios approximate 1. As the between-father component of variance increases relative to within-father variance, F ratios grow larger in the half-sibling analysis and the null hypothesis is rejected. All test statistics were evaluated with 2-tailed probabilities ($\alpha < .05$), and descriptive statistics are presented as mean ± SD.

infant earlier, and these infants displayed modest prolonged increases in cortisol levels throughout the high-demand condition.[36,37]

Following completion of each 12-week condition at 21 weeks of age, all mothers were removed from natal groups after weaning at 36 weeks. At this stage of development squirrel monkeys are no longer reliant on maternal care. Sexual maturity is achieved at 3 years, and the squirrel monkey life span is approximately 21 years.[38] As previously described elsewhere in greater detail,[39] social behavior, emotional responses, and increases in cortisol levels elicited by removing all mothers after weaning were examined at 36 weeks of age for the study cohort. In early adulthood at 5 years we tested for differences in cortisol-negative feedback regulation of the HPA-axis response to exogenous stimulation by CRF.[40] Then 5 weeks later hippocampal volumes were determined by high-resolution MRI.

RESULTS

Analysis of adult hippocampal volumes revealed a brain side main effect ($F_{1,24}=6.78$, $P=.02$). Right hippocampal volumes were 4% larger than hippocampal volumes on the left side of the brain. Neither sex nor rearing condition differences were discerned, and none of the brain side interactions was significant, but the repeated measures ANOVA uncovered a paternity main effect ($F_{11,24}=2.47$, $P=.03$).

Certain fathers produced monkeys with large hippocampi, other fathers produced monkeys with smaller hippocampi, and similar hippocampal volumes were

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

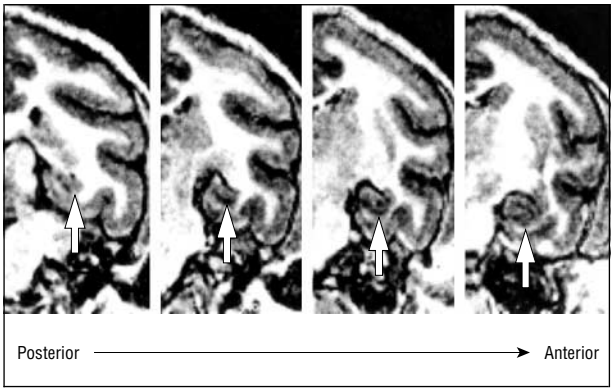

Figure 1. Representative images of adult squirrel monkey hippocampus (arrows) at 2-mm intervals in the coronal plane.

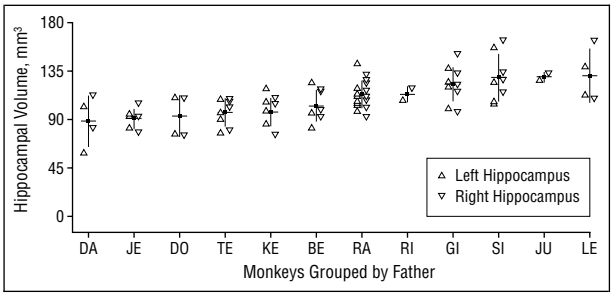

Figure 2. Adult hippocampal volumes grouped according to each monkey's father (average n=3.25 offspring per father). Error bars signify mean±SD.

found among monkeys that shared a common father (**Figure 2**). Right and left hippocampal volumes were correlated with one another (**Figure 3**), and these measures were added together to create a total hippocampal volume score. The estimated proportion of genetic variance, ie, heritability, was 54% for total hippocampal volume size ($F_{11,27}=2.58$, $P=.02$). Heritabilities for the right ($h^2=49\%$, $F_{11,27}=2.34$, $P=.04$) and left hippocampal volumes ($h^2=52\%$, $F_{11,27}=2.45$, $P=.03$) were not significantly different. The distribution of total hippocampal volumes was Gaussian in the sample of 39 monkeys (**Figure 4**), suggesting contributions from multiple additive genetic effects on hippocampal size.

Following completion of the rearing conditions at 21 weeks of age, all mothers were removed well after weaning at 36 weeks post partum. Plasma cortisol levels in their offspring 1 day later were 146% higher than the preseparation levels assessed with identical procedures (197±9 µg/dL vs 485±27 µg/dL). Significant differences in these relative elevations in cortisol levels were produced by the prior rearing conditions ($F_{2,24}=18.11$, $P\leq.001$), but these rearing-related differences were not associated with significant differences in hippocampal size (**Figure 5**).

Paternal half-siblings that responded with larger relative 1-day increases in cortisol levels had smaller adult hippocampal volumes after correcting for sex, rearing condition, and overall brain size ($r=-0.58$, $df=10$, $P=.048$; **Figure 6**). Half-sibling group differences in hippocampal volumes were not correlated with differences in overall brain size, and the relation between cortisol and hippocampal volume was absent when the 1-day cortisol

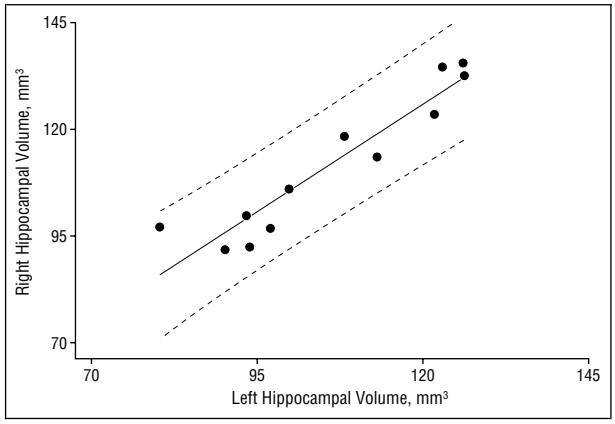

Figure 3. Correlation between mean right and mean left hippocampal volumes grouped according to each monkey's father (average n=3.25 offspring per father). Dashed lines signify 95% confidence intervals.

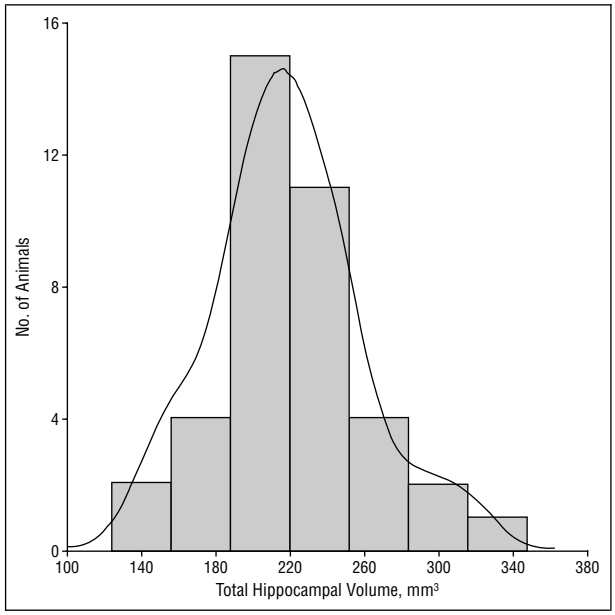

Figure 4. Distribution of total hippocampal volumes in 39 monkeys.

measures were analyzed as absolute cortisol concentrations. Average cortisol levels remained higher than baseline 3 and 7 days after the removal of all mothers, but these cortisol levels were not correlated with differences in adult hippocampal size. Half-sibling group differences in hippocampal volumes were also not correlated with subsequent measures of cortisol negative feedback in early adulthood.

COMMENT

In previous studies the subset of monkeys that we separated intermittently prior to weaning differed in assessments of social behavior, emotional reactivity, and cortisol-induced suppression of CRF-stimulated secretion of adrenocorticotropic hormone.[39,40] Rearing-related differences in these monkeys were not found in adult hippocampal volumes. Rhesus monkeys raised in social isolation also fail to show altered hippocampal volumes as determined in vivo by MRI,[32] despite striking changes in

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

other brain systems and numerous aspects of behavior.[17-23] Maltreated children with posttraumatic stress disorder do not differ from healthy children in hippocampal volumes,[45] but show elevated baseline urinary free cortisol and catecholamine concentrations.[46] Chronic alcohol abuse is known to produce hippocampal atrophy in humans[47] and rats,[48] but alcohol abuse was not common in the studies of children with posttraumatic stress disorder, and was absent altogether in monkeys. Early life stress without alcohol abuse may have minimal effects on hippocampal volumes determined in human and nonhuman primates by high-resolution MRI.

Paternal half-sibling group differences were apparent in monkey hippocampal volumes. Certain fathers produced offspring with large hippocampi, other fathers produced offspring with smaller hippocampi, and similar adult hippocampal volumes were found in monkeys that shared a common father. Since paternal half-siblings were raised apart from one another by different mothers in the absence of fathers, phenotypic similarities within half-sibling groups cannot be attributed to shared family environments. Based on a standard half-sibling analysis the estimated heritability was 54% for total hippocampal size. This estimate is not inflated by including in the sample 9 maternal half-sibling pairs.[39]

High heritabilities for overall brain size have been reported in humans[49-51] and rhesus macaque monkeys,[52] but little is known about the genetics of variation in regional brain morphologies. Neuroimaging research has identified in human twins a genetic basis for differences in surface features of cerebral cortex,[49,53] corpus callosum size,[51,54] and volumetric measures of other subcortical structures.[50] Our findings support preliminary evidence in humans[55] indicating that individual differences in hippocampal volumes are in part determined by genes. The Gaussian distribution of hippocampal volumes in monkeys suggests a polygenic trait, and not the effects of genetic epistasis nor a single major gene. Inherited variation in volumes may reflect heritable differences in hippocampal cell numbers,[56,57] or differences in physiological factors related to in vivo tissue perfusion.

Paternal half-siblings with small adult hippocampal volumes responded to the removal of all mothers after weaning with larger relative 1-day elevations in cortisol levels. In humans with mood and anxiety disorders small hippocampal volumes have been taken as evidence that excessive stress levels of cortisol induce hippocampal volume loss.[2,11,12] But in monkeys large rearing-related differences in cortisol levels elicited by removing all mothers after weaning did not produce differences in hippocampal size. An alternative explanation for the observed correlation is that small hippocampal volumes are inherited and predispose toward excessive stress levels of cortisol through diminished neuroendocrine regulation. The hypothesis that genes affect cortisol levels by acting on aspects of hippocampal morphology is consistent with evidence that the hippocampus plays a role in suppressing the HPA-axis stress response.[58,59] Various measures of cortisol negative feedback in monkeys were not, however, correlated with hippocampal size.

A limitation of our finding heritable differences in monkey hippocampal volumes is that heritabilities are

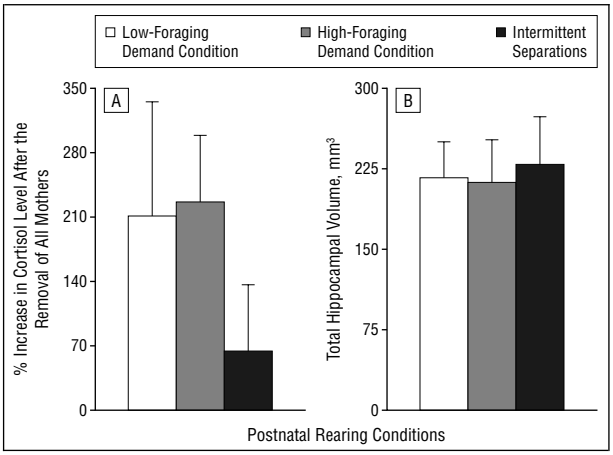

Figure 5. Postnatal rearing effects on the relative increase in cortisol levels elicited by removing all mothers and subsequent adult hippocampal volumes (n=13 monkeys per condition). Error bars signify mean±SD.

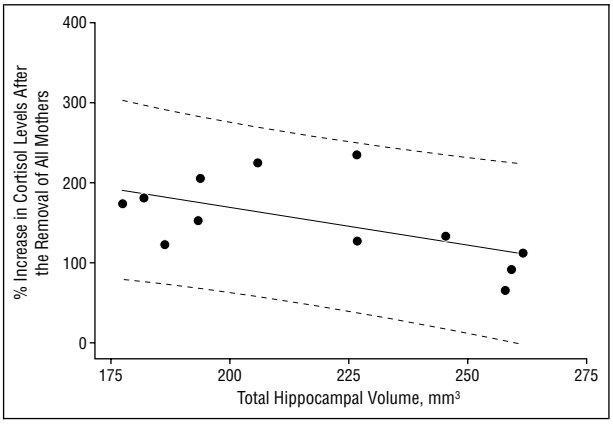

Figure 6. Correlation between mean increases in cortisol levels elicited by removing all mothers after weaning and mean total adult hippocampal volumes grouped according to each monkey's father (average n=3.25 offspring per father). Dashed lines signify 95% confidence intervals.

specific to the population and circumstance in which they are assessed. Genetically diverse populations in homogeneous environments demonstrate larger heritabilities than do inbred populations in diverse environments.[44] Very few parents that produced the study cohort shared a common mother or father, but extended family pedigrees could not be determined from monkey breeding colony records. The generality of our findings should therefore be tested in studies of other populations.

The absence of postnatal rearing effects must likewise be considered with caution. Rodent research has convincingly demonstrated that stress or glucocorticoids alone induce altered regulation of hippocampal serotonin receptor expression,[60] apical dendritic atrophy of CA3 pyramidal cells,[8] suppression of neurogenesis, and decreased survival of newborn granule cells.[10] Our failure to uncover neuroimaging-based differences does not rule out the possibility that microstructural plasticity occurs in the primate hippocampus.[28-30] There are, in fact, excellent reasons to expect subcellular plasticity in monkeys.

Following completion of the rearing conditions and well after weaning at 36 weeks of age, the monkeys we

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

separated intermittently responded to the removal of all mothers with smaller increases in cortisol levels, fewer distress peep-calls, and more time spent near peers.[39] Monkeys from the low-foraging and high-foraging demand conditions did not differ on any of these measures. In early adulthood at 5 years of age, only the intermittently separated monkeys showed signs of enhanced negative feedback regulation of the HPA-axis response to stimulation by exogenous CRF.[40] These findings parallel studies indicating that in rats brief postnatal intermittent social stress diminishes emotionality and HPA-axis reactivity throughout adolescence and adulthood.[61,62] In rats blunted HPA-axis stress responses are mediated by enhanced negative feedback regulation resulting from increased glucocorticoid receptor expression in adult hippocampus.[62] Experience-dependent augmentation of glucocorticoid receptor densities might likewise account for enhanced negative feedback described elsewhere for the intermittently separated monkeys.[40]

A final aspect of this study that warrants comment concerns the lack of long-lasting foraging demand effects on squirrel monkey brain and behavior. Squirrel monkey mothers in the high-foraging demand condition consistently exhibit increased cortisol levels relative to mothers in the low-foraging demand condition where food is easy to find.[37] High-foraging demand condition mothers stop carrying infants earlier, but continue to demonstrate otherwise normal nursing patterns.[37] By selectively accelerating certain aspects of development, squirrel monkey mothers apparently spare their offspring from abnormal outcomes previously reported for bonnet macaque monkeys raised by mothers under variable-foraging demands.[25-27] The high-foraging demand condition does not subsequently alter social or emotional aspects of behavior, HPA-axis stress physiology, or adult hippocampal volumes.

Accepted for publication June 26, 2001.

This work was supported in part by the Nancy Pritzker Network, New York, NY, and Public Health Service grant MH47573 from the National Institute of Mental Health, Bethesda, Md.

We thank Blake Mobley and Nadia Sachs for assistance with MRI processing, and Robert Sapolsky, PhD, and Bruce McEwen, PhD, for thoughtful comments on this research.

Corresponding author: David M. Lyons, PhD, Department of Psychiatry and Behavioral Science, 1201 Welch Rd, Medical School Laboratory Surge Bldg, Room P104–Mail Code 5485, Stanford University School of Medicine, Stanford, CA 94305-5485 (e-mail: dmlyons @stanford.edu).

REFERENCES

1. Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. *Am J Psychiatry.* 2000;157:115-118.
2. Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci.* 1999;19:5034-5043.
3. Gurvits TV, Shenton ME, Hokama H, Ohta H, Lasko NB, Gilbertson MW, Orr SP, Kikinis R, Jolesz FA, McCarley RW, Pitman RK. Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biol Psychiatry.* 1996;40:1091-1099.
4. Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Am J Psychiatry.* 1995;152:973-981.
5. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH, Nemeroff CB. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA.* 2000;284:592-597.
6. Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med.* 1997;27:951-959.
7. Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse: a preliminary report. *Biol Psychiatry.* 1997;41:23-32.
8. Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience.* 1995;69:89-98.
9. McEwen BS. Possible mechanisms for atrophy of the human hippocampus [review]. *Mol Psychiatry.* 1997;2:255-262.
10. Gould E, Tanapat P. Stress and hippocampal neurogenesis [review]. *Biol Psychiatry.* 1999;46:1472-1479.
11. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry.* 2000;57:925-935.
12. Bremner JD. Does stress damage the brain [review]? *Biol Psychiatry.* 1999;45:797-805.
13. Suomi SJ. Genetic and maternal contributions to individual differences in rhesus monkey biobehavioral development. In: Krasnegor NA, Blass EM, Hofer MA, Smotherman WP, eds. *Perinatal Development: A Psychobiological Perspective.* New York, NY: Academic Press Inc; 1987:397-419.
14. Higley JD, Thompson WW, Champoux M, Goldman D, Hasert MF, Kraemer GW, Scanlan JM, Suomi SJ, Linnoila M. Paternal and maternal genetic and environmental contributions to cerebrospinal fluid monoamine metabolites in rhesus monkeys (*Macaca mulatta*). *Arch Gen Psychiatry.* 1993;50:615-623.
15. Clarke AS, Kammerer CM, George KP, Kupfer DJ, McKinney WT, Spence MA, Kraemer GW. Evidence for heritability of biogenic amine levels in the cerebrospinal fluid of rhesus monkeys. *Biol Psychiatry.* 1995;38:572-577.
16. Lyons DM. Primate models, overview. In: Fink G, ed. *Encyclopedia of Stress.* Vol 3. San Diego, Calif: Academic Press Inc; 2000:236-241.
17. Lewis MH, Gluck JP, Beauchamp AJ, Keresztury MF, Mailman RB. Long-term effects of early social isolation in *Macaca mulatta*: changes in dopamine receptor function following apomorphine challenge. *Brain Res.* 1990;513:67-73.
18. Kraemer GW. Psychobiology of early social attachment in rhesus monkeys: clinical implications [review]. *Ann N Y Acad Sci.* 1997;807:401-418.
19. Chapmoux M, Coe CL, Schanberg S, Kuhn C, Suomi SJ. Hormonal effects of early rearing conditions in the infant rhesus monkey. *Am J Primatol.* 1989;19:111-117.
20. Higley JD, Suomi SJ, Linnoila M. A longitudinal assessment of CSF monoamine metabolite and plasma cortisol concentrations in young rhesus monkeys. *Biol Psychiatry.* 1992;32:127-145.
21. Mason WA, Mendoza SP, Moberg GP. Persistent effects of early social experience on physiological responsiveness. In: Ehara A, Kimura R, Takenaka O, Iwamoto M, eds. *Primatology Today.* New York, NY: Elsevier Science Inc; 1991:469-471.
22. Kaemingk K, Reite M. Social environment and nocturnal sleep: studies in peer-reared monkeys. *Sleep.* 1987;10:542-550.
23. Fahlke C, Lorenz JG, Long J, Champoux M, Suomi SJ, Higley JD. Rearing experiences and stress-induced plasma cortisol as early risk factors for excessive alcohol consumption in nonhuman primates. *Alcohol Clin Exp Res.* 2000;24:644-650.
24. Andrews MW, Rosenblum LA. The development of affiliative and agonistic social patterns in differentially reared monkeys. *Child Dev.* 1994;65:1398-1404.
25. Rosenblum LA, Andrews MW. Influences of environmental demand on maternal behavior and infant development [review]. *Acta Paediatr Suppl.* 1994;397:57-63.
26. Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci U S A.* 1996;93:1619-1623.
27. Coplan JD, Trost RC, Owens MJ, Cooper TB, Gorman JM, Nemeroff CB, Rosenblum LA. Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry.* 1998;55:473-477.

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014

28. Siegel SJ, Ginsberg SD, Hof PR, Foote SL, Young WG, Kraemer GW, McKinney WT, Morrison JH. Effects of social deprivation in prepubescent rhesus monkeys: immunohistochemical analysis of the neurofilament protein triplet in the hippocampal formation. *Brain Res.* 1993;619:299-305.
29. Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM. Hippocampal damage associated with prolonged and fatal stress in primates. *J Neurosci.* 1989;9:1705-1711.
30. Sapolsky RM, Uno H, Rebert CS, Finch CE. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *J Neurosci.* 1990;10:2897-2902.
31. Leverenz JB, Wilkinson CW, Wamble M, Corbin S, Grabber JE, Raskind MA, Peskind ER. Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J Neurosci.* 1999;19:2356-2361.
32. Sanchez MM, Hearn EF, Do D, Rilling JK, Herndon JG. Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Res.* 1998;812:38-49.
33. Stanton ME, Levine S. Brief separation elevates cortisol in mother and infant squirrel monkeys. *Physiol Behav.* 1985;34:1007-1008.
34. Coe CL, Glass JC, Wiener SG, Levine S. Behavioral, but not physiological, adaptation to repeated separation in mother and infant primates. *Psychoneuroendocrinology.* 1983;8:401-409.
35. Hennessy MB. Multiple, brief maternal separations in the squirrel monkey: changes in hormonal and behavioral responsiveness. *Physiol Behav.* 1986;36:245-250.
36. Levine S, Champoux M, Wiener SG. Social modulation of the stress response. In: Genazzani AR, Nappi G, Petraglia F, Martignoni E, eds. *Stress and Related Disorders From Adaptation to Dysfunction.* Pearl River, NY: Parthenon Publishing Group Ltd; 1991:121-128.
37. Lyons DM, Kim S, Schatzberg AF, Levine S. Postnatal foraging demands alter adrenocortical activity and psychosocial development. *Dev Psychobiol.* 1998; 32:285-291.
38. Brady AG. Research techniques for the squirrel monkey (*Saimiri* sp) [review]. *ILAR J.* 2000;41:10-18.
39. Lyons DM, Martel FL, Levine S, Risch NJ, Schatzberg AF. Postnatal experiences and genetic effects on squirrel monkey social affinities and emotional distress. *Horm Behav.* 1999;36:266-275.
40. Lyons DM, Yang C, Mobley BW, Nickerson JT, Schatzberg AF. Early environmental regulation of glucocorticoid feedback sensitivity in young adult monkeys. *J Neuroendocrinol.* 2000;12:723-728.
41. Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K. Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis [review]. *APMIS.* 1988;96:379-394.
42. Kates WR, Abrams MT, Kaufmann WE, Breiter SN, Reiss AL. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. *Psychiatry Res.* 1997;75:31-48.
43. Keppel G. *Design and Analysis: A Researcher's Handbook.* 2nd ed. Englewood Cliffs, NJ: Prentice-Hall International Inc; 1982.
44. Falconer DS, Mackay TFC. *Introduction to Quantitative Genetics.* 4th ed. London, England: Longman Group Ltd; 1996.
45. De Bellis MD, Keshavan MS, Clark DB, Casey BJ, Giedd JN, Boring AM, Frustaci K, Ryan ND. Developmental traumatology, part II: brain development. *Biol Psychiatry.* 1999;45:1271-1284.
46. De Bellis MD, Baum AS, Birmaher B, Keshavan MS, Eccard CH, Boring AM, Jenkins FJ, Ryan ND. Developmental traumatology, part I: biological stress systems. A. E. Bennett Research Award. *Biol Psychiatry.* 1999;45:1259-1270.
47. Sullivan EV, Marsh L, Mathalon DH, Lim KO, Pfefferbaum A. Anterior hippocampal volume deficits in nonamnesic, aging chronic alcoholics. *Alcohol Clin Exp Res.* 1995;19:110-122.
48. Bengoechea O, Gonzalo LM. Effects of alcoholization on the rat hippocampus. *Neurosci Lett.* 1991;123:112-114.
49. Bartley AJ, Jones DW, Weinberger DR. Genetic variability of human brain size and cortical gyral patterns. *Brain.* 1997;120:257-269.
50. Pennington BF, Filipek PA, Lefly D, Chhabildas N, Kennedy DN, Simon JH, Filley CM, Galaburda A, DeFries JC. A twin MRI study of size variations in human brain. *J Cogn Neurosci.* 2000;12:223-232.
51. Pfefferbaum A, Sullivan EV, Swan GE, Carmelli D. Brain structure in men remains highly heritable in the seventh and eighth decades of life. *Neurobiol Aging.* 2000;21:63-74.
52. Cheverud JM, Falk D, Vannier M, Konigsberg L, Helmkamp RC, Hildebolt C. Heritability of brain size and surface features in rhesus macaques (*Macaca mulatta*). *J Hered.* 1990;81:51-57.
53. Biondi A, Nogueira H, Dormont D, Duyme M, Hasboun D, Zouaoui A, Chantome M, Marsault C. Are the brains of monozygotic twins similar? a three-dimensional MR study. *AJNR Am J Neuroradiol.* 1998;19:1361-1367.
54. Tramo MJ, Loftus WC, Stukel TA, Green RL, Weaver JB, Gazzaniga MS. Brain size, head size, and intelligence quotient in monozygotic twins. *Neurology.* 1998; 50:1246-1252.
55. Sullivan EV, Pfefferbaum A, Swan GE, Carmelli D. Differential genetic contribution to variation in hippocampal and temporal horn volume: an MRI study of elderly twin men. *Soc Neurosci Abstracts.* 2000;26:1609.
56. Wimer RE, Wimer CC. A biometrical-genetic analysis of granule cell number in the area dentata of house mice. *Brain Res.* 1981;254:129-140.
57. Kempermann G, Kuhn HG, Gage FH. Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc Natl Acad Sci U S A.* 1997;94:10409-10414.
58. Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev.* 1991;12:118-134.
59. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis [review]. *Trends Neurosci.* 1997; 20:78-84.
60. Lopez JF, Chalmers DT, Little KY, Watson SJ. A.E. Bennett Research Award: regulation of serotonin1_A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biol Psychiatry.* 1998;43:547-573.
61. Levine S. Infantile experience and resistence to physiological stress. *Science.* 1957;126:405-406.
62. Meaney MJ, Diorio J, Francis D, Widdowson J, LaPlante P, Caldji C, Sharma S, Seckl JR, Plotskey PM. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress [review]. *Dev Neurosci.* 1996;18:49-72.

©2001 American Medical Association. All rights reserved.
Downloaded From: http://archpsyc.jamanetwork.com/ on 08/29/2014