Preliminary Evidence That Hippocampal Volumes in Monkeys Predict Stress Levels of Adrenocorticotropic Hormone

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Background: Hippocampal volumes previously determined in monkeys by magnetic resonance imaging are used to test the hypothesis that small hippocampi predict increased stress levels of adrenocorticotropic hormone (ACTH).

Methods: Plasma ACTH levels were measured after restraint stress in 19 male monkeys pretreated with saline or hydrocortisone. Monkeys were then randomized to an undisturbed control condition or intermittent social separations followed by new pair formations. After 17 months of exposure to the intermittent social manipulations, restraint stress tests were repeated to determine test/retest correlations.

Results: Individual differences in postrestraint stress ACTH levels over the 17-month test/retest interval were remarkably consistent for the saline ($r_s = .82, p = .0004$) and hydrocortisone ($r_s = .78, p = .001$) pretreatments. Social manipulations did not affect postrestraint stress ACTH levels, but monkeys with smaller hippocampal volumes responded to restraint after saline pretreatment with greater increases in ACTH levels with total brain volume variation controlled as a statistical covariate ($\beta = -.58, p = .031$). Monkeys with smaller hippocampal volumes also responded with diminished sensitivity to glucocorticoid feedback determined by greater postrestraint ACTH levels after pretreatment with hydrocortisone ($\beta = -.68, p = .010$).

Conclusions: These findings support clinical reports that small hippocampi may be a risk factor for impaired regulation of the hypothalamic-pituitary-adrenal axis in humans with stress-related psychiatric disorders.

Key Words: ACTH, glucocorticoid feedback, hippocampus, HPA axis, stress

Hippocampal volumes are smaller in humans with stress-related psychiatric disorders compared to healthy controls (1,2). Studies of humans (3–5) and animal models (6,7) suggest that excessive stress levels of cortisol are a cause of hippocampal volume loss. Far less researched, but of equal importance, are indications that small hippocampi may also represent a risk factor for impaired regulation of the hypothalamic-pituitary-adrenal (HPA)-axis response to stress (8–12).

Opportunities to study the causes and consequences of hippocampal volume variation are limited in humans with stress-related psychiatric disorders. Therefore, we recently examined the effects of early experiences and inherited variation in squirrel monkey hippocampal volumes (13). Paternal half-siblings raised apart from one another by different mothers in the absence of fathers were randomized to three postnatal conditions at 10 weeks of age (see supplementary text online). After weaning, at 9 months of age, all monkeys were socially housed in identical laboratory conditions. Sexual maturity occurs at 2–3 years of age, and the average maximum lifespan for squirrel monkeys is 21 years (14). In early adulthood, at 5 years of age (range = 3.7–6.0 years), hippocampal volumes were determined from T1-weighted brain images (Figure 1A). Image acquisition and processing details are provided in the supplementary text online.

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Received September 26, 2006; revised March 7, 2007; accepted March 16, 2007.

Hippocampal volumes in the young adult monkeys did not differ significantly with respect to prior postnatal treatment-related differences in plasma cortisol levels at weaning (15). However, in keeping with studies of humans (16,17), significant heritabilities were discerned in monkeys by paternal half-sibling analysis of left and right hippocampal volumes considered separately or combined (15). Here we investigate in the same monkeys whether these hippocampal measures predict subsequent poststress levels of adrenocorticotropic hormone (ACTH) after pretreatment with saline or hydrocortisone. The dose of hydrocortisone used to assess glucocorticoid feedback is known to suppress stress-induced increases in squirrel monkey ACTH (18). Plasma ACTH levels are measured because endogenous cortisol cannot be distinguished from exogenous hydrocortisone. Only the males from our previous studies are examined because cyclical ovarian hormone effects on HPA-axis activity are difficult to control for in female monkeys.

Methods and Materials

At 8.5 years of age (range = 6.4 – 10.6 years), 19 pair-housed adult male squirrel monkeys were restrained for two separate 30-min sessions in a standard primate chair. Restraint is a well-studied psychological stressor in animal biomedical research (19). An intramuscular saline injection was given 60 min before the first restraint test. Seven days later, an intramuscular injection of 2.5 mg/kg hydrocortisone sodium succinate was given 60 min before the second and otherwise identical restraint test. All procedures were conducted in accordance with National Institute of Health guidelines, and were approved by Stanford University's Panel on Laboratory Animal Care.

Immediately after each stress test, a blood sample was collected and monkeys were returned to the home cage. Subsequent samples were collected 30 and 60 min later to provide poststress measures of recovery. Additional samples were also collected seven days before and seven days after the saline and

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2007.03.012

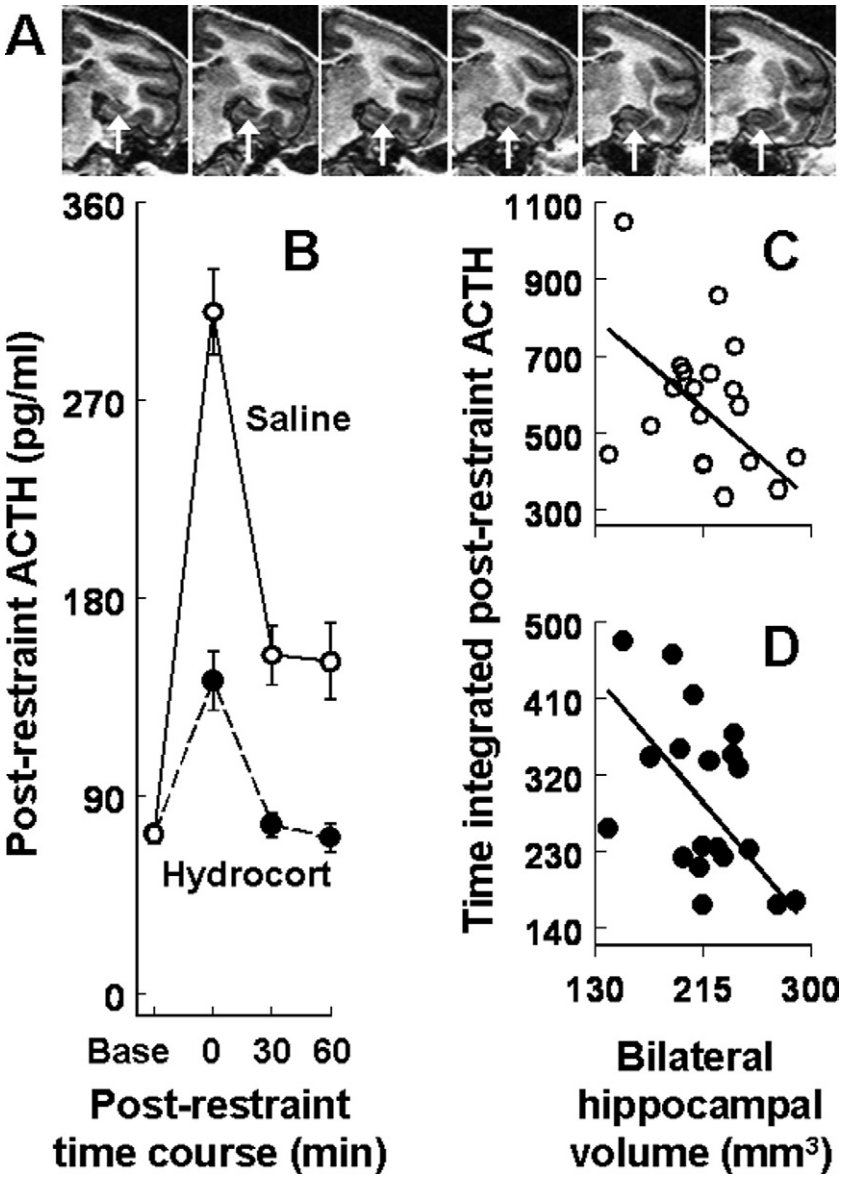

Figure 1. Hippocampal volume predicts postrestraint stress levels of ACTH. **(A)** Representative magnetic resonance images of squirrel monkey hippocampus (arrows) at 1 mm intervals in the coronal plane. **(B)** Postrestraint stress ACTH levels averaged across the 17-month test/retest interval for pretreatment with saline or hydrocortisone (mean ± SEM). Bilateral hippocampal volumes regressed on time integrated postrestraint ACTH levels in 19 adult male monkeys pretreated with **(C)** saline or **(D)** hydrocortisone. ACTH, adrenocorticotropic hormone.

hydrocortisone restraint stress tests to measure ACTH levels at baseline in home cage conditions. All samples were obtained as described elsewhere (supplementary text online) from manually restrained monkeys by femoral venipuncture between 13:30–14:30 hours to control for diurnal variation (20). Plasma ACTH levels were measured in duplicate with an established radioimmunoassay (21).

After the initial restraint stress tests, monkeys were randomized to the following adult treatment conditions. In one condition, 10 monkeys were exposed to six intermittent social separations that each lasted 3 weeks in duration. During each social separation session, monkeys were individually housed, and could see, hear, smell, but not touch other monkeys. After each intermittent separation, new pairs were formed and maintained for 9 weeks. New pair formations (22) and social separations (23) increase plasma cortisol levels in adult squirrel monkeys. In the undisturbed control condition, adult monkeys were housed with the same companion in stable same-sex pairs. Hippocampal volumes from 9 of 10 pair-housed control monkeys were available for analysis. Randomization to the adult conditions was stratified by prior postnatal condition to provide similar size samples in the factorial design (see supplementary figure online). Ten weeks after the final separation, when all of the monkeys were housed in pairs, restraint stress tests were repeated to determine test/retest correlations.

Time integrated postrestraint ACTH levels were determined with the trapezoidal rule to estimate the area under each monkey's saline and hydrocortisone curves. For each of these measures, Spearman correlations were used to evaluate the consistency of individual differences over the 17-month test/retest interval. The hypothesis that hippocampal volume predicts time integrated postrestraint ACTH levels after saline or hydrocortisone was assessed using linear least squares regressions with total brain volume variation controlled as a statistical covariate. Adult social manipulations and postnatal conditions were subsequently added to the analysis to statistically control for systematic experience-dependent effects. All test statistics were evaluated with two-tailed probabilities ($p < .05$).

Results

Individual differences in time integrated postrestraint ACTH levels were remarkably consistent over the 17-month test/retest interval for the saline (r_s = .82, p = .0004) and hydrocortisone (r_s = .78, p = .001) pretreatments. As expected from previous research (18), restraint stress after saline robustly increased ACTH levels and pretreatment with hydrocortisone suppressed postrestraint ACTH levels compared to pretreatment with saline (Figure 1B). Monkeys with greater time integrated postrestraint ACTH levels averaged across the test/retest interval for saline responded with greater time integrated postrestraint ACTH levels after hydrocortisone (r_s = .64, p = .006).

Monkeys with smaller hippocampal volumes (left and right combined) responded to restraint after saline pretreatment with greater time integrated ACTH levels averaged across the test/retest interval with total brain volume variation controlled as a statistical covariate ($\beta = -.58$, p = .031; Figure 1C). Monkeys with smaller hippocampal volumes also responded with diminished sensitivity to glucocorticoid feedback determined by greater time integrated postrestraint ACTH levels after hydrocortisone ($\beta = -.68$, p = .010; Figure 1D). A significant hippocampal volume main effect ($F(1,11) = 5.85$, $p < .034$) was also discerned with the postnatal and adult social manipulations examined in a single mixed factor ANOVA with postrestraint ACTH levels after saline and hydrocortisone included as repeated measures. Adult social manipulations and postnatal effects were not significant in the omnibus ANOVA for all of the monkeys but ACTH levels were, on average, 24 % greater during the follow-up retests compared to the initial test sessions ($F(1,11) = 5.26$, p = .043).

Discussion

These findings suggest that naturally occurring hippocampal volume variation in monkeys predicts consistent individual differences in poststress ACTH levels after saline or hydrocortisone pretreatment. Hippocampal lesions likewise increase the duration and peak levels of stress-induced HPA-axis activation and impair negative feedback determined by glucocorticoid administration in rodents (24–26). Impaired glucocorticoid feedback occurs in stress-related psychiatric disorders (27,28), but evidence for hippocampal regulation of the HPA-axis in humans (29,30) and monkeys (31) is limited to studies previously conducted at baseline in stress-free conditions.

Over the 17-month test/retest interval, poststress ACTH levels were increased on average by 24%. Despite this normative age-related increase, the relative rank order of individual differences was consistent over time. These observations concur with reports that aging increases the HPA-axis response to stress in humans (32) and monkeys (18), and correspond with 1-year test/retest correlations of ~.70 for human poststress ACTH levels (33). Longitudinal evidence further suggests that 3.5- and 5-year test/retest correlations are ~.90 for hippocampal volumes in humans between 14-83 years of age (34,35).

The results of this study should be interpreted in the context of potential limitations. Our findings for males may or may not hold true for females. Small samples diminished the power to detect heritable variation and related interactions between hippocampal volumes and experience-dependent changes in poststress ACTH levels. Saline and hydrocortisone pretreatments were not counterbalanced, but test-order acclimation is unlikely because several restraint sessions are needed for HPA-axis acclimation in monkeys (36,37). Exposure to adult social stress did not enhance the restraint stress response in contrast to studies of rats (38). Paired-housing of monkeys after each separation may have blocked the expected stress facilitation effect.

In summary, this study of monkeys suggests that small hippocampi may be a risk for impaired regulation of the HPA-axis response to psychological stress. Similar studies of humans are needed because HPA-axis dysregulation is often a feature of stress-related psychiatric disorders (27, 28). Identification of risk factors for stress-related psychiatric disorders may help to define patient populations most likely to benefit from preventative interventions.

This research was supported by the Nancy Pritzker Network for the Study of Depression, and Public Health Service grants DA16902, MH50604, and MH47573.

The authors declare no conflicting financial or other competing interests.

Supplementary material cited in this article is available online.

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