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A randomized, placebo-controlled proof-of-concept, crossover trial of phenytoin for hydrocortisone-induced declarative memory changes

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Abstract

Background—Corticosteroid excess is associated with declarative memory impairment and hippocampal atrophy. These findings are clinically important because approximately 1% of the population receives prescription corticosteroids at any time, and major depressive disorder is associated with elevated cortisol levels and hippocampal atrophy. In animals, hippocampal changes with corticosteroids are blocked by phenytoin. The objective of the current study was to extend these preclinical findings to humans. We examined whether phenytoin attenuated the effects of hydrocortisone on declarative memory. Functional magnetic resonance imaging (fMRI) assessed task-related hippocampal activation.

Methods—A randomized, double-blind, placebo-controlled, within-subject crossover study was conducted in 17 healthy adult volunteers. Participants received hydrocortisone (2.5 days), phenytoin (3.5 days), both medications together, or placebo, with 21-day washouts between

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ES Brown served as the PI of the study and participated in the design of the study, supervision of data collection and manuscript writing. H Lu participated in the design of the study, data collection and analysis, writing and editing of the manuscript. D Denniston participated in data collection and editing of the manuscript. J Uh participated in data collection and analysis, writing and editing of manuscript. BP Thomas participated in data collection and analysis, writing and editing of manuscript. TJ Carmody participated in data analysis and writing and editing of the manuscript. RJ Auchus participated in data interpretation and writing and editing of manuscript. R Diaz-Arrastia participated in the design of the study and reviewed manuscript drafts. C Tamminga participated in the design of the study, supervised data collection and analysis, and edited manuscript drafts. All authors contributed to and have approved the final manuscript.

Conflict of Interest

No other authors have disclosures to make.

conditions. Differences between treatments were estimated using a mixed-effects repeated measures analysis.

Results—Fifteen participants had data from at least two treatment conditions and were used in the analysis. Basal cortisol levels negatively correlated with fMRI BOLD activation in the para-hippocampus with a similar trend observed in the hippocampus. Decrease in declarative memory with hydrocortisone was blocked with concomitant phenytoin administration. Relative to the placebo condition, a significant decrease in hippocampal BOLD activation was observed with hydrocortisone and phenytoin alone, and the two medications in combination. Declarative memory did not show significant correlations with hippocampal activation.

Limitations—The modest sample size, which limited our statistical power, was a limitation.

Conclusions—Findings from this pilot study suggest phenytoin attenuated effects of corticosteroids memory in humans, but potentiated the reduction in hippocampal activation.

Keywords

anticonvulsant; corticosteroid; fMRI; cognition; hippocampus

Introduction

Corticosteroids (a.k.a glucocorticoids) are commonly prescribed medications (Fardet et al., 2011) that are associated with a variety of neuropsychiatric side effects (Fardet et al., 2012; Wolkowitz et al., 2009). The hippocampus appears to be a primary target for corticosteroids in the brain (Brown, 2009). Animal data suggest that exposure to high levels of corticosteroids in stress paradigms or through corticosterone administration is associated with changes in the hippocampus including dendritic remodeling (Magarinos et al., 1997; Vyas et al., 2002). In non-human primates, most (Sapolsky et al., 1990; Uno et al., 1994; Uno et al., 1989), but not all (Leverenz et al., 1999), studies suggest that stress or exogenous corticosteroid administration are associated with atrophy of the hippocampus.

In humans, acute corticosteroid administration is associated with a reversible decline in declarative memory performance in adults (Brown et al., 2006; de Quervain et al., 2000; Newcomer et al., 1999; Wolkowitz et al., 1990) and children (Bender et al., 1988). The magnitude of memory change with corticosteroids may be related to specific gene polymorphisms (Kumsta et al., 2010). Chronic endogenous hypercortisolism in Cushing's syndrome is likewise associated with hippocampal atrophy and cognitive, particularly declarative memory, impairment (Starkman et al., 1992). At least partial recovery of brain atrophy and cognitive function occurs after treatment of Cushing's syndrome (Starkman et al., 1999), but the extent of recovery declines with age (Hook et al., 2007), and neuropsychiatric dysfunction remains a major source of morbidity in this disease. Chronic exogenous corticosteroid use is also associated with structural and functional hippocampal changes. We reported that patients receiving long-term prescription corticosteroid therapy had poorer declarative memory, decreased hippocampal volume, and decreased temporal lobe levels of N-acetyl aspartate as compared to controls with similar medical histories but minimal lifetime corticosteroid exposure (Brown et al., 2007; Brown et al., 2004).

Only two studies have examined the impact of exogenous corticosteroids on functional magnetic resonance imaging (fMRI) in humans. Decreased activity in both the hippocampus and prefrontal-cortex was observed in healthy controls scanned before and one hour after oral hydrocortisone (Oei et al., 2007). In another report using healthy controls, hydrocortisone administration was associated with decreased activity in the hippocampus and amygdala, reaching a peak response minimum at approximately 30 minutes post-

injection (Lovallo et al., 2010). Thus, hippocampal activation, as assessed by fMRI, appears to be decreased by acute administration of corticosteroids.

Stress and corticosteroids increase glutamate release in the hippocampus (Popoli et al., 2012). In animal models, the effects of stress or corticosteroids on the hippocampus are attenuated by agents that decrease glutamate release (e.g. phenytoin) or block the NMDA receptor (Magarinos et al., 1996). We reported significantly greater improvement in declarative memory with the NMDA receptor antagonist memantine than with placebo in patients receiving chronic corticosteroid therapy (Brown et al., 2008a). We observed significant improvement in declarative memory in corticosteroid-dependent patients receiving the glutamate release inhibitor lamotrigine.

In this report of a proof-of-concept study, we examine whether phenytoin prevents declarative memory changes with hydrocortisone (clinical aim). In addition, we explore the effect of hydrocortisone and phenytoin alone and in combination on hippocampal activation during a task (mechanistic aim).

Methods

The University of Texas Southwestern Medical Center Institutional Review Board (IRB) approved this study. All participants completed an IRB-approved written informed consent process at the Psychoneuroendocrine Research Program offices on the UT Southwestern campus. The study was registered at http://clinicaltrials.gov (NCT00591006).

Healthy volunteers (n=17) were recruited through flyers and other forms of advertising. Included were men and women age 18–50, vision corrected to at least 20–40, education of ≥ 12 years, and baseline Rey Auditory Verbal Learning Test (RAVLT) (Ryan et al., 1986) total words recalled score ≥ 40 (to exclude baseline cognitive impairment). Excluded were those with a history of schizophrenia, major depressive, bipolar, posttraumatic stress, schizoaffective, panic or eating disorders, or drug/alcohol abuse/dependence, seizures, brain surgery, multiple sclerosis, Parkinson's disease, taking CNS-acting medications, contraindications to phenytoin, hydrocortisone or MRI, significant medical conditions, or current tobacco use, pregnant/nursing women, prisoners, cognitive disorders, baseline 17-item Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960) score > 7 (to exclude those with clinically significant depressive symptoms at baseline), current suicidal ideation or history of a suicide attempt, and history of systemic or past 14 day inhaled corticosteroid use. Participants and all persons with participant contact were blinded to treatment order. The Structured Clinical Interview for DSM-IV (SCID) (First et al., 1995) was used to rule out exclusionary psychiatric illnesses. Declarative memory was assessed with the RAVLT (Ryan et al., 1986). Alternative versions of the RAVLT (different words) were used to minimize learning effects from repeated administration.

Participants received each of the four medication conditions (hydrocortisone + placebo, phenytoin + placebo, hydrocortisone + phenytoin, placebo + placebo) and four fMRI scans using a randomized, crossover design with a 21-day washout between medication conditions. Medication and placebo were purchased from Abram's Royal Pharmacy, Dallas, Texas. Three days prior to fMRI scans, participants took four capsules containing phenytoin (100 mg) or identical placebo by mouth at 2100 hours (400 mg/day) for a total of 3.5 days. Beginning 2 days prior to fMRI scans (day after initiating phenytoin or placebo), participants began taking four tablets containing hydrocortisone (20 mg) or placebo at 0900 hours and 2100 hours (160 mg/day) with the last dose at 0900 hours on the day of imaging. Thus, hydrocortisone was administered for a total of 2.5 days. Neuroimaging was performed at approximately 1300 hours after each exposure to medication. The RAVLT was

administered at baseline and after each medication course. Blood was drawn at approximately 1400 hours at baseline and after each scan to assess cortisol and phenytoin levels. The samples were sent to the Immunopharmacology Laboratory at National Jewish Medical and Research Center in Denver, Colorado for cortisol analysis and Quest Diagnostics for phenytoin levels. Side effects/adverse events were assessed at each visit. Participants were paid for their participation.

Scan acquisition

Imaging was performed on a 3 Tesla MRI system (Philips Medical Systems, Best, The Netherlands). A body coil was used for radiofrequency transmission and an eightchannel sensitivity encoding (SENSE) head coil was used for receiving. Foam padding stabilized the head and minimized motion.

The fMRI image acquisition used Blood-Oxygenation-Level-Dependent (BOLD) pulse sequence with the following parameters: TR=1500 ms, flip angle 70 degrees (to allow for T1 relaxation and to reduce inflow effect), TE=30 ms, SENSE factor 2, field-of-view 220x220 mm^2, matrix 64×64, 30 axial slices, voxel size 3.44×3.44×5 mm^3 no gap, duration 6 minutes/run. A T1-weight anatomic image was acquired using a Magnetization-Prepared-Rapid-Acquisition-of-Gradient-Echo (MPRAGE) sequence with the following imaging parameters: TR=2100 ms, TE=3.8 ms, TI=1100 ms, field-of-view 256×256×160 mm^3, matrix 256×256×160, voxel size 1×1×1 mm^3, duration 3 minutes and 57 seconds. In addition, we acquired an fMRI correction factor to account for potential alterations in brain vascular physiology due to hydrocortisone or phenytoin. The fMRI signal is negatively modulated by baseline blood oxygenation independent of neural activity (Lu et al., 2008). The modulation effect can be appreciated by considering that the fMRI signal is based on changes in venous blood oxygenation (Yv) from resting to activated conditions and that Yv cannot exceed 100%. Therefore, a lower resting Yv means an increase in activation, potentially generating a greater fMRI signal. Differences in this parameter across individuals or sessions are a major source of variation in fMRI signal amplitude (Cohen et al., 2002; Lu et al., 2008). Medications used in our study may alter Yv and present a confounding factor in assessment of neural activity using fMRI. This effect was corrected by measuring Yv in each session using a T2-Relaxation-Under-Spin-Tagging (TRUST) MRI technique (Lu and Ge, 2008). The imaging parameters were: voxel size 3.44×3.44×5mm^3, TR=8000ms, TI=1200ms, four TEs: 0ms, 40ms, 80ms and 160ms, measurement made in the superior sagittal sinus, duration 4.3 min.

Imaging Task

The fMRI experiments used a novelty detection task. Before entering the magnet, participants viewed two pictures, one indoor and one outdoor scene, for 20 seconds each. These were then considered as familiar pictures. For the in-scanner task, five fMRI runs were performed. Each run consisted of 40 stimulation trials with a stimulation period of 2 seconds followed by a fixation period of 4 seconds. During the stimulation period, one of four stimulus types (a familiar indoor, a familiar outdoor, a novel indoor, or a novel outdoor picture) was shown using a video projector located on the back of the magnet (visual angle=25°). Participants pressed a left-hand button for an indoor scene or right-hand button for an outdoor scene, regardless of familiarity. The purpose of the button press was to maintain attention. Each novel picture was used only once during the session. Additionally, periods of six-second fixation were introduced pseudorandomly (20/fMRI run) to vary the inter-trial interval.

Image processing

The fMRI images were preprocessed and analyzed using Statistical Parametric Mapping software (SPM5). All fMRI images were first realigned to correct for motion. The MPRAGE image was segmented into grey matter (GM) and white matter (WM) segments. The GM segment was normalized into Montreal Neurological Institute (MNI) space, and transformation parameters were applied to all images and the coil-sensitivity corrected MPRAGE image that was created during segmentation. After the normalization step, voxel size of all images was set to $3\times3\times3$ mm^3. All normalized functional images were then smoothed with an $8\times8\times8mm^3$ full width at half maximum (FWHM) Gaussian kernel.

The onset time for all pictures in the fMRI task was used as linear regressors for general linear model (GLM) analysis available in SPM5. A regressor for novel pictures and another for familiar pictures were specified in the GLM. Parameter estimates for the regressors were calculated and contrast images were generated for the novel pictures-familiar pictures contrast. The fMRI results presented were all based on the “novel pictures-familiar pictures” contrast.

Contrast images from individual subject analyses were then used in the group-level analysis with the flexible factorial statistical method in SPM5. Anatomic regions of interest (ROIs) in MNI space were created for the left and right hippocampus, and left and right para-hippocampal gyrus. These ROIs were created using WFU_PickAtlas software (Functional MRI Laboratory, Wake Forest University School of Medicine, NC). The average hemodynamic response function (HRF) for each regressor in the GLM design was then calculated from each ROI using a script from Marsbar toolbox (Brett et al., 2002). The peak stimulus response on HRF curve was seen between 4.5 and 9 seconds after stimulus onset, and these responses were summed to obtain integrated % signal change. The fMRI signal amplitude was corrected for Yv variance using a linear equation described previously (Lu et al., 2010): $S_{corr}= S_{orig}-Yv*slope$. The slope variable indicates the Yv modulation effect and represents the amount of signal variation for each unit of Yv difference when assuming that neural activity is identical. The slope was determined using the placebo/placebo data on a region specific basis.

Statistical Analysis

Of the 17 participants, two were excluded because data from at least two medication conditions were not available, leaving 15 evaluable participants. The participants received all four treatments except for three participants that each missed one treatment. The order of treatments was randomized by TJC (co-author) using a random number sequence and balanced such that every treatment followed every other treatment the same number of times. Statistical analysis was conducted for ROI results from the hippocampus and para-hippocampus. The fMRI signals, S_{corr}, of the left and right hemispheres were averaged for analysis as no significant lateralization in activation was observed. The differences between treatments were estimated with a mixed-effects repeated measures analysis (SAS Proc Mixed) to the S_{corr} measures. The model contained terms for treatment group, order of scans, and a term to allow for carry-over effects from prior treatment. Because of the cross-over design, all effects were within-subject. The effect sizes were computed by dividing the between-group difference estimated from the mixed-effects model by an estimate of the standard deviation of the raw data (Feingold, 2009; Raudenbush and Xiao-Feng, 2001). Standard deviation from the placebo/placebo condition was used to compute effect sizes.

Results

Demographic and baseline characteristics of the participants are given in Table 1. The sample was relatively young with normal baseline mood and declarative memory performance. Mean cortisol levels were higher during hydrocortisone administration (phenytoin + hydrocortisone 34.7±32.7, placebo + hydrocortisone 28.9±25.0, placebo + placebo 8.9±5.9, phenytoin + placebo 12.2±9.1). Phenytoin levels ranged from 2.6 to 15.8 (mean 8.6±3.5) during the phenytoin + hydrocortisone administration and 4.6 to 15.0 (mean 8.2±3.2) during the phenytoin + placebo administration. Phenytoin administration was not associated with significant differences in cortisol levels relative to placebo, and phenytoin levels did not correlate with cortisol levels.

Declarative memory data are provided in Table 2. Hydrocortisone was associated with a trend toward poorer performance than placebo on the RAVLT total (mean change −4.25, SE= 2.52 , p=.1, Cohen's *d* 0.38). RAVLT total performance when phenytoin was given along with hydrocortisone was not significantly different than placebo alone and that was significantly better than with hydrocortisone alone (Cohen's *d* 0.74).

In an effort to better understand physiologic mechanisms for the observed memory changes, we performed fMRI in conjunction with declarative memory testing. Baseline venous oxygenation was decreased by hydrocortisone, but not by phenytoin (Yv values: placebo + hydrocortisone 60.0±4.6%, phenytoin + hydrocortisone 61.1±5.7%, placebo + placebo 63.8±5.6%, phenytoin + placebo 65.6±3.4%). Paired t-tests revealed that placebo + placebo vs. placebo + hydrocortisone Yv values (p=0.03) and phenytoin + placebo vs. phenytoin + hydrocortisone (p=0.007) were significantly different but the phenytoin + placebo vs. placebo + placebo comparison was not (p=0.68).The corrected fMRI ROI data are provided in Table 3, and a whole brain group comparison image of the placebo + placebo > phenytoin + hydrocortisone, placebo + placebo > phenytoin + placebo, and placebo + placebo > placebo + hydrocortisone in Figure 1. Task-related hippocampal activation as assessed by S_{corr} showed the following pattern: placebo + placebo > phenytoin + placebo and also placebo + placebo > hydrocortisone + placebo (both large effect sizes). Thus, the signal was similarly lower in the hippocampus with either hydrocortisone or phenytoin alone as compared to placebo. The combination of hydrocortisone and phenytoin was associated (at the trend level) with even lower activation than either medication alone. In the para-hippocampus, phenytoin and hydrocortisone alone were associated with non-significantly lower activation than placebo, while the combination was associated with significantly lower activation than placebo (large effect size).

No significant correlations were observed between changes in BOLD activation and changes in RAVLT total scores (all p > .1), except for a trend for a negative relationship between change in RAVLT score and change in activation between phenytoin + hydrocortisone and placebo + placebo conditions (r=−0.510, p=.090). Cortisol level showed a significant negative correlation with BOLD activation in the para-hippocampus in the placebo + placebo condition (r=−0.541, p=.037), and a trend toward negative correlations in the hippocampus in the phenytoin + placebo (r=−0.475, p=.073) and placebo + placebo (r= −0.448, p=.094) conditions. Cortisol level was not associated with BOLD activation during either hydrocortisone administration condition (both p>.1). Phenytoin levels were not associated with BOLD activation during either phenytoin administration condition (both p>. 1)

All of the treatment conditions were well tolerated. Two adverse events were reported during the study. One participant, during the placebo + hydrocortisone condition, experienced difficulty breathing, tremors in his left hand and disorientation to time while

undergoing the fMRI procedure. The participant recovered in a few minutes, and was discontinued from the study. In another participant, mega cisterna magna was diagnosed as a benign and incidental finding on the MRI scans.

Discussion

We observed a reduction in declarative memory performance with hydrocortisone. Although this decline in RAVLT scores during hydrocortisone administration had a medium effect size, the findings did not reach statistical significance (p=0.1) perhaps due to the modest sample size in this pilot study. The finding is consistent with our prior research (Brown et al., 2006) and research by other investigators (de Quervain et al., 2000; Newcomer et al., 1999; Wolkowitz et al., 1990) suggesting reversible decline in declarative memory with brief corticosteroid administration. We also observed a reduction in baseline venous oxygenation with hydrocortisone. This finding suggests that hydrocortisone has effects on brain vasculature. Previous studies have reported a negative relationship between baseline cortisol levels and resting regional cerebral perfusion in the medial temporal lobe of patients with posttraumatic stress disorder (Bonne et al., 2003). In healthy controls, diurnal cortisol variability was negatively associated with hippocampal and para-hippocampal BOLD signal during a stressful stimulus (Cunningham-Bussel et al., 2009). We observed that basal cortisol levels were negatively associated with hippocampal activation during a task that was not stressful or emotionally charged, but, perhaps due to the overall increase in levels and differences in metabolism, this relationship disappeared following hydrocortisone administration. During hydrocortisone administration, a reduction in the BOLD signal was observed. These imaging results are consistent with two prior fMRI reports of decreases in hippocampal activation with hydrocortisone (Lovallo et al., 2010; Oei et al., 2007). The current study design used a longer exposure to hydrocortisone than did prior studies. Thus, the findings are novel in that we assessed the impact of hydrocortisone over repeated administration, not after a single dose.

Despite many decades of use for seizures, no previous studies have examined the effects of phenytoin on hippocampal activation. The findings suggest that phenytoin is associated with a decrease in hippocampal BOLD activation. This is similar to what has been observed in previous studies using the anticonvulsant lamotrigine (Brown et al., 2010; Deakin et al., 2008). No significant change in venous oxygenation was observed with phenytoin alone as compared to placebo. This is in contrast to the reduction in venous oxygenation with hydrocortisone. To our knowledge, prior studies have not examined the impact of phenytoin, as compared to placebo, on declarative memory. We found a non-significant decline in performance on the RAVLT compared to placebo when phenytoin was administered alone. Consistent with our findings, one report in healthy controls observed a non-significant decline in performance on a declarative memory task from baseline with phenytoin administration (Meador et al., 1995).

The primary aim of the study was to examine the effects of the combination of phenytoin and hydrocortisone on declarative memory. Based on the preclinical literature, we hypothesized that corticosteroid effects on human declarative memory could be prevented with phenytoin. Our findings support this hypothesis. RAVLT performance with the combination of phenytoin and hydrocortisone was significantly better than with hydrocortisone alone and was not significantly different from the placebo condition. The ability of phenytoin to prevent the effects of hydrocortisone on declarative memory is important because declarative memory impairment is perhaps the most common and clinically relevant feature of hippocampal insult or dysfunction. The finding is consistent with preclinical data suggesting that phenytoin prevents stress-induced hippocampal CA3 region apical dendritic atrophy in subordinate tree shrews (Magarinos et al., 1996). Thus, the

current data translate the pre-clinical data into humans and suggest that phenytoin can prevent the memory effects of hydrocortisone in humans as well as histological changes in the animal hippocampus. Improvement in memory was only observed when phenytoin was given in combination with hydrocortisone. This finding is consistent with what we observed with the glutamate release inhibitor lamotrigine. When administered to chronic corticosteroid-treated patients, lamotrigine was associated with a significant improvement in declarative memory as compared to placebo (Brown et al., 2008b)). However, in a group of patients with bipolar disorder, we observed a slight decline in declarative memory with lamotrigine alone (Osuji et al., 2008).

The memory findings in this report extend our previous research demonstrating improvement in declarative memory with lamotrigine in corticosteroid-treated patients (Brown et al., 2008b). Declarative memory changes appear to be both prevented and reversed by medications that, among other mechanisms, decrease glutamate release. Clinically, the current findings suggest that phenytoin may attenuate the effects of acute, high-dose corticosteroid therapy on declarative memory. However, we do not know whether phenytoin, like lamotrigine, reverses memory impairment associated with long-term corticosteroid therapy.

Our observation of an additive effect between phenytoin and hydrocortisone on BOLD signal but a counteracting effect between them on memory is one of the most intriguing findings of this study. We hypothesize the reason to be that each drug can alter (at least) two aspects of the brain physiology, baseline glutamate level and neuronal excitation, and the BOLD and memory performance changes reflect different aspects of these effects. Stress and administration of corticosteroids are associated with increases in baseline glutamate in the hippocampus (Chen et al., 1998; Ioannou et al., 2003; Lowy et al., 1993; Moghaddam et al., 1994). Higher-than-normal glutamate release in the hippocampus apparently has a negative impact on memory as studies have shown that normalization of glutamate release using an NMDA receptor antagonist [27] or glutamate release inhibitor (Brown et al., 2008b)) can restore memory performance. Therefore, we hypothesize that the reduction in memory performance due to hydrocortisone administration primarily reflects its enhancement effect on glutamate release. On the other hand, hydrocortisone also has the effect of reducing neuronal excitability (Hamzei et al., 2002). At the dose used in the present study, hydrocortisone binds primarily to the glucocorticoid receptor (GR), the activation of which is associated with decreased neuronal excitability (Prager et al., 2010). Decreased neuronal excitability is expected to decrease the BOLD signal, as we have observed in our experiment.

The effect of phenytoin on glutamate levels is opposite to that of hydrocortisone. Phenytoin is associated with a reduction in baseline glutamate release (Magarinos et al., 1996) and upregulation of gene expression associated with glutamate degradation (Mariotti et al., 2010). Therefore, the effect of phenytoin on memory is also expected to be opposite to that of hydrocortisone (i.e. restoring glutamate levels and memory performance). In terms of the effect of phenytoin on neuronal excitability, interestingly it is similar to the effect of hydrocortisone (i.e. decreasing excitability). Assuming that the neuronal excitability is directly related to the BOLD signal, phenytoin is expected to decrease hippocampal activation (Greenhill and Jones, 2010), as our experimental data have shown. In short, the two drugs seem to have opposite effects in terms of baseline glutamate level, but similar effects on neuronal excitability. Our data therefore suggest that the memory effects of the drugs are most reflective of their influence on baseline glutamate level whereas the alterations in BOLD fMRI are most reflective of the changes in neuronal excitability due to the drugs. It should be noted that a decrease in baseline glutamate level does not always cause memory improvement and an increase does not always cause memory impairment.

Instead, it is possible that brain's baseline glutamate level has an optimal level under which the memory performance is at its best. Therefore, both an increase (e.g. due to hydrocortisone alone) and a decrease (due to phenytoin alone) from that optimal level are detrimental (see Table 2). However, when the two are used in conjunction, the memory performance may be restored.

The current report found that changes in declarative memory did not correlate with changes in hippocampal BOLD activation. The literature on the relationship between task-related hippocampal activation and declarative memory performance is also mixed. A positive correlation between memory and hippocampal activation (Johnson et al., 2001) as well as no correlation (Petrella et al., 2007) are reported. Perhaps due to a compensatory mechanism, greater hippocampal activation in cognitive impaired subjects than in controls has also been reported (Schwarze et al., 2009; Thomaes et al., 2009) and is suggestive of a negative relationship between hippocampal BOLD signal and memory performance. Given these findings, one explanation for attenuation of the effects of hydrocortisone on the RAVLT and enhancement of its effect on BOLD signal by phenytoin is that declarative memory and the hippocampal BOLD signal are not closely related in the present intervention study using multiple medications. The improvement in memory performance associated with phenytoin administration could be due to the reduction in baseline glutamate release, resulting in a greater potential for neural response with a task. The BOLD signal did not reflect this change because the fMRI signal is additionally affected by the effect of phenytoin on vascular reserve. Therefore, the discrepancy between memory performance and BOLD signal could be due to a difference between neural-based signal and vasculature-based signal. Both findings are potentially important. Memory is an observable process mediated by the hippocampus that can impact daily functioning. On the other hand, the BOLD signal may reflect important mechanisms within the hippocampus. Future research should use techniques, such as magnetic resonance spectroscopy and basal cerebral blood flow measurement, which can measure basal glutamate concentrations and neural activity more directly and correlate these changes with declarative memory performance.

The study has several limitations. The sample size in this pilot study was modest limiting our ability to detect between-group differences. Thus, the findings must be interpreted with caution. However, the statistical power was increased by the crossover design in which the subjects served as their own controls. Perhaps due to the sample size in this study, hydrocortisone alone was associated with a reduction in RAVLT performance that did not reach statistical significance ($p=0.10$). The duration of exposure to hydrocortisone was subacute, but longer than in any other fMRI studies. Consequently, the findings might not be generalizable to settings with longer exposures to corticosteroids or phenytoin. In many cases the participants did not reach therapeutic phenytoin levels for seizures. However, the concentration needed to attenuate the effects of hydrocortisone on the hippocampus may not be the same as that needed for seizure control. Finally, it is important to note that phenytoin has a complex mechanism of action that, in addition to reducing glutamate release, includes decreasing sodium influx through inhibition of sodium channels, decrease in calcium influx, enhancement of ligand binding at sigma sites, and enhancement of GABA neurotransmission (Catterall, 1999; Tunnicliff, 1996). Thus, one cannot assume that all of the observed effects of phenytoin in this study were related to glutamate.

In summary, our findings suggest that this paradigm can be used to assess the effects of corticosteroids on the human hippocampus. These results also suggest that both declarative memory and hippocampal activation, as assessed with the BOLD signal, are sensitive to the effects of brief exogenous corticosteroid administration. In addition, the results extend findings with phenytoin in animal models to humans and suggest that phenytoin may attenuate the impact of acute corticosteroid exposure on declarative memory (the memory

process mediated by the hippocampus). Phenytoin has already demonstrated potential beyond seizure control (e.g. treatment of mania in bipolar disorder) (Mishory et al., 2000). The current findings suggest that phenytoin may also block the effects of stress or corticosteroids on declarative memory. Future research will include a larger trial of phenytoin to confirm these findings, use of other medications in the paradigm, use of other neuroimaging techniques, and exploration of hippocampal activation with hydrocortisone in clinical populations associated with elevated cortisol, such as major depressive disorder and Cushing's disease.

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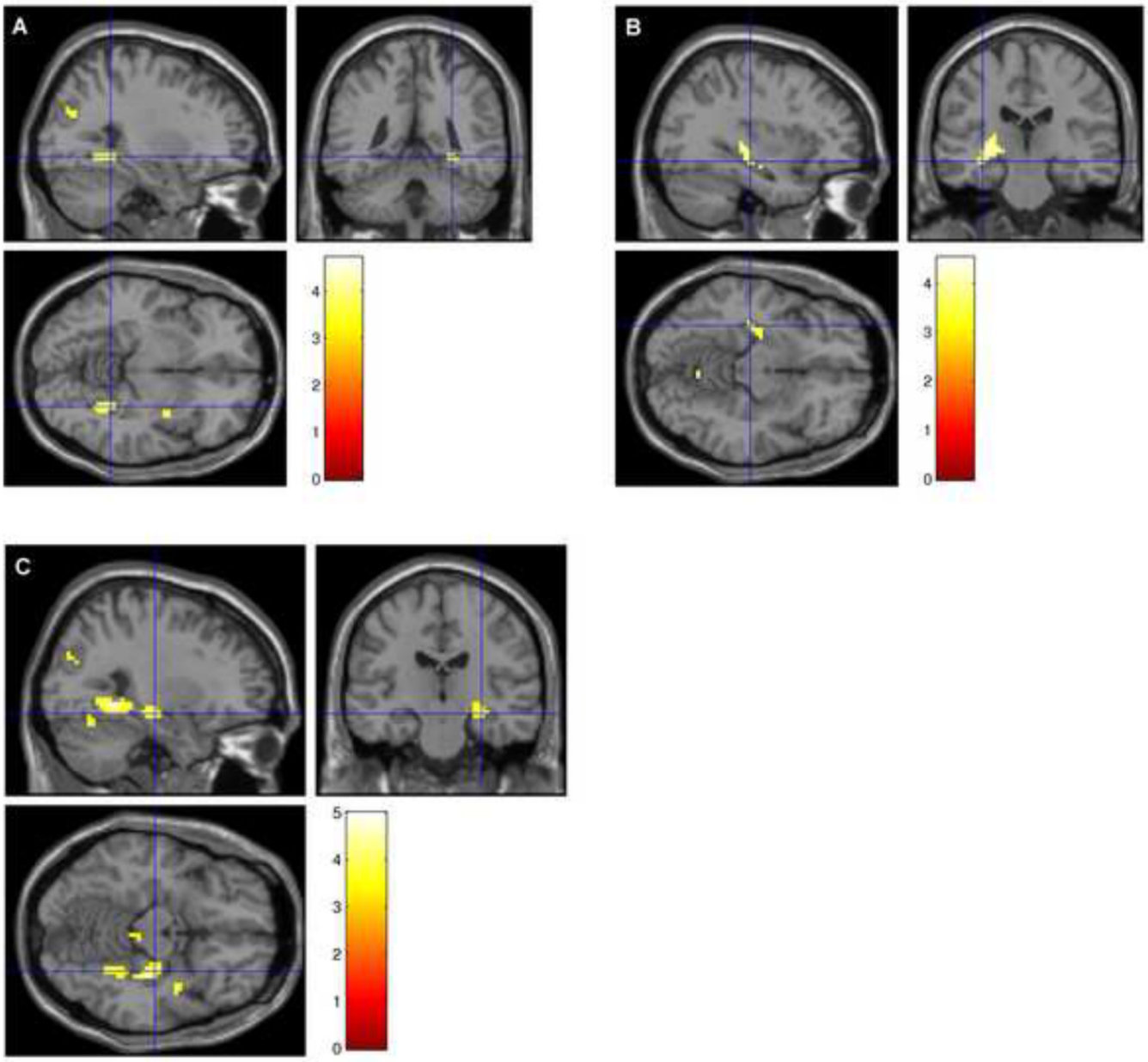

Figure 1.

Group subtraction results that survived an uncorrected threshold of p=0.001 for **A** Placebo + Placebo − Hydrocortisone + Placebo, **B** Placebo + Placebo − Phenytoin + Placebo, and C Placebo + Placebo − Phenytoin + Hydrocortisone.

Table 1

Baseline Demographic characteristics of participants (N =15)

Variable	Mean or % (N=15)	S.D
Age (years)	25.3	8.1
Education (years)	16.5	2.8
Female (%)	60.0	
Race		
Caucasian (%)	73.3	
African American (%)	6.7	
Hispanic (%)	13.3	
Native American (%)	6.7	
Right Handed (%)	83.3	
RAVLT Total T-Score	53.8	8.1

Table 2

Changes in Declarative Memory between Treatment Conditions

Measure	Difference	Mean	Std. Error	D.F.	t-Value	P-Value	Effect Size
RAVLT Total T-Score	Phen+HC-Pbo+HC	8.27	2.65	33.1	3.12	<.01	0.74
RAVLT Total T-Score	Phen+HC-Pbo+Pbo	4.02	2.52	33.8	1.59	.12	0.36
RAVLT Total T-Score	Phen+Pbo Pbo+Pbo	−3.46	2.37	32.7	−1.46	.15	0.31
RAVLT Total T-Score	Pbo+HC-Pbo+Pbo	−4.25	2.52	32.4	−1.69	.10	0.38

Table 3

Hippocampal and parahippocampal activation differences between treatment conditions

Brain Region	Difference	Mean	STD Error	DF	T-value	P-Val	Effect Size
Hippocampus	Phen+HC-Pbo+HC	−0.12	0.07	35.4	−1.83	.08	0.67
Hippocampus	Phen+HC-Pbo+Pbo	−0.28	0.06	34.9	−4.43	<.01	1.58
Hippocampus	Phen+Pbo-Pbo/Pbo	−0.17	0.06	33.0	−2.76	<.01	0.93
Hippocampus	Pbo+HC-Pbo+Pbo	−0.16	0.06	33.3	−2.55	.02	0.90
Para-Hippocampus	Phen+HC-Pbo+HC	−0.18	0.10	33.6	−1.84	.08	0.58
Para-Hippocampus	Phen+HC-Pbo+Pbo	−0.37	0.10	33.1	−3.81	<.01	1.17
Para-Hippocampus	Phen+Pbo-Pbo+Pbo	−0.15	0.09	31.1	−1.62	.11	0.47
Para-Hippocampus	Pbo+HC-Pbo+Pbo	−0.19	0.10	31.6	−1.93	.06	0.59