


# Effects of Acute Confinement Stress-induced Hypothalamic-pituitary Adrenal Axis Activation and Concomitant Peripheral and Central Transforming Growth Factor- $\beta$ 1 Measures in Nonhuman Primates

Chronic Stress  
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## Abstract

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a multifunctional cytokine with anti-inflammatory, immunosuppressive, and neuroprotective properties. The hypothalamic-pituitary-adrenal axis and immune system exert bidirectional influences on each other, via cortisol and TGF- $\beta$ 1, but the exact nature of the interaction is not well characterized. The current study examined the effects, in bonnet macaques (*Macaca radiata*), of two consecutive acute confinement stress periods in an unfamiliar room while mildly restrained, first without and then with dexamethasone pretreatment (0.01 mg/kg intramuscular). Preceding the confinement studies, a non-stress control condition obtained contemporaneous levels of cortisol and TGF- $\beta$ 1 in both plasma and cerebrospinal fluid to match the confinement stress studies. Subjects were reared under either normative or variable foraging demand conditions. Since there were no rearing effects at baseline or for any of the conditions tested—either for cortisol or TGF- $\beta$ —the study analyses were conducted on the combined rearing groups. The stress condition increased both plasma and cerebrospinal fluid cortisol levels whereas dexamethasone pretreatment decreased cortisol concentrations to below baseline levels despite stress. The stress condition decreased TGF- $\beta$ 1 concentrations only in cerebrospinal fluid but not in serum. Together, the data suggested that stress-induced reductions of a centrally active neuroprotective cytokine occur in the face of hypothalamic-pituitary-adrenal axis activation, potentially facilitating glucocorticoid-induced neurotoxicity. Stress-induced reductions of neuroprotective cytokines prompt exploration of protective measures against glucocorticoid-induced neurotoxicity.

## Keywords

transforming growth factor- $\beta$ 1, stress, cortisol, cytokine, dexamethasone, glucocorticoid, nonhuman primate, cerebrospinal fluid

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## Introduction

The interaction between the hypothalamic-pituitary-adrenal (HPA) axis and the immune system is intricate. The HPA axis plays an important role in the homeostatic maintenance of the bidirectional regulation of the central nervous and immune systems, which, upon exposure to stress, interact to mount an immune/inflammatory response.<sup>1,2</sup> Cytokines are the primary means by which the immune system affects the central nervous system

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(CNS).<sup>3</sup> It has been invariably debated that cytokines are produced in the periphery,<sup>4</sup> which through various mechanisms, influence the brain. However, it has been recently shown that the brain itself produces cytokines that could potentially be neuroprotective or neurotoxic.<sup>3</sup>

Among these cytokines, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is of particular interest. TGF- $\beta$ 1 is a pleiotropic cytokine, which coordinates multiple physiological processes.<sup>5</sup> TGF- $\beta$ 1 elicits its response by acting through serine-protease T $\beta$ R-I and T $\beta$ R-II receptors, subsequently activating signaling against decapentaplegic peptide (Smad) transcription factor cascade resulting in transcription of key target genes.<sup>5</sup> Sufficient evidence exists to support the role of TGF- $\beta$ 1 possessing potent immunosuppressive effects at very low concentrations.<sup>3,6,7</sup> In addition, considerable *in vitro* data indicate bidirectional regulation of the HPA axis and TGF- $\beta$ 1, in which each system decreases functional activity of the other.<sup>8–13</sup> Based on our previous findings showing a direct relationship between plasma TGF- $\beta$ 1 and the HPA axis under stress,<sup>14</sup> we sought to examine, in the current study, the relationship of TGF- $\beta$ 1 and the HPA axis in both the plasma and the cerebrospinal fluid (CSF) compartments under conditions of acute stress. Consistent with these considerations, we had shown that cortisol increases TGF- $\beta$ 1 peripherally but decreases gene expression of glial cell TGF- $\beta$ 1.<sup>15</sup> Based on this observation, we hypothesized that CSF TGF- $\beta$ 1 might decrease under conditions of acute stress.

It has been generally appreciated that TGF- $\beta$ 1 is neuroprotective.<sup>9,16,17</sup> Specific mechanisms have been postulated for the anti-inflammatory<sup>18</sup> and neuroprotective action of TGF- $\beta$ 1.<sup>19</sup> Specifically, TGF- $\beta$ 1 shields against NMDA receptor excitotoxicity by synthesis and release of type 1 plasminogen activator inhibitor (PAI-1) in astrocytes.<sup>5</sup> The anti-apoptotic activity of TGF- $\beta$ 1 is posited to be due to stabilization of calcium homeostasis and increasing the expression of anti-apoptotic proteins such as Bcl-2 (B-cell lymphoma-2) and Bcl-xl (B-cell lymphoma-extra large).<sup>5</sup> The anti-inflammatory effects are postulated to be due to inhibition of reactive oxygen species production.<sup>18</sup> The indirect neuroprotective effects of TGF- $\beta$ 1 are supported by *in vitro* studies in our laboratory that show gene expression of TGF- $\beta$ 1 and activation of its signaling pathway is significantly upregulated by hypoxia in vascular endothelial cells.<sup>20,21</sup>

A functional linkage between the HPA axis and TGF- $\beta$ 1 is consistent with findings of a strong positive correlation between peripheral cortisol and TGF- $\beta$ 1 in bonnet macaques exposed to moderate stress.<sup>14</sup> The biologic specificity of this relationship is highlighted in an interesting comparative study of squirrel monkeys (*Saimiri sciureus*), a New World monkey where cortisol levels are 10-fold higher than those of either Old World monkeys or

humans.<sup>22</sup> Activated monocytes from squirrel monkeys showed a four-fold higher TGF- $\beta$ 1 response than did human cells, while other cytokines (tumor-necrosis factor- $\alpha$  and - $\beta$  and interleukin-2) were unaffected.<sup>22</sup>

The HPA axis regulates critical metabolic, biochemical and cellular responses to stress, and clinical investigations have shown important relationships between various human psychopathologies and HPA axis function.<sup>23–27</sup> Frequently, these relationships are revealed by HPA axis response to neuroendocrine challenge, such as the dexamethasone suppression test (DST).<sup>28</sup> Dexamethasone, a synthetic glucocorticoid, is used for testing the sensitivity of glucocorticoid negative feedback on the HPA axis. A nonhuman primate version of DST has been previously reported.<sup>28</sup>

Based on glial cell responses to glucocorticoids,<sup>15</sup> the hypothesis that central TGF- $\beta$ 1 may, in fact, decrease centrally despite stress-induced HPA axis activation has not, to our knowledge, been tested. Given the important neuroprotective, anti-apoptotic and anti-inflammatory effects of TGF- $\beta$ 1,<sup>16</sup> a significant physiological decrease in this cytokine levels during acute stress response could have potential therapeutic implications in facilitating attenuation of stress-induced neurotoxicity.

## Methods

### Subjects

Subjects were nine male ( $n=9$ , all males) bonnet macaques, all born in Downstate Medical Center's Primate Behavior Laboratory. Subjects were singly housed in cages measuring approximately  $0.9 \times 0.8 \times 0.8$  m in temperature- and humidity-controlled rooms under a 12:12 h light:dark cycle. Cages had a mobile back wall by which subjects could be quickly restrained at the front of the cage for injection. Subjects had *ad libitum* access to water and standard laboratory diet throughout the study. At all times, the "Principles of laboratory animal care" (National Institute of Health publication no. 85-23, revised 1996) were followed.

### Rearing

Of the nine subjects, four were reared normally reared, whereas five were reared under conditions of variable foraging demand (VFD). Briefly, bonnet mother-infant dyads are exposed to uncertain variations in food procurement. Foraging demand varied between easy access to food and difficult access to food in 2-week blocks for a total of 14 weeks. The reader is referred to a study by Andrews and Rosenblum<sup>29</sup> for further details. The four subjects that were raised normally were reared in their natal social groups, consisting of their mothers and

several other mothers with offspring of various ages, and an adult harum male. The remaining five subjects had been exposed to VFD rearing as infants as described previously.<sup>30</sup> The subjects weighted between 6 and 9 kg each.

Briefly, these subjects were reared normally for the first 10–12 weeks of life, after which they and their mothers were placed in social groups of 5–7 mother–infant dyads. The male subjects necessitate individually housing after full maturity to avoid injury and wounding. After social acclimatization, mother–infant dyads were exposed to conditions in which the effort necessary to obtain food varied between easy access (low demand) and more difficult access (high demand) in 2-week blocks for 16 weeks. Ample food was always available, and the growth and health of the VFD-reared infants were normal. After VFD rearing, all dyads were returned to standard ad libitum feeding, and infants were separated from their mothers at approximately a year later and peer-housed. The time from VFD exposure was about seven years in the VFD group. At the time of the present experiment, all subjects were adults, and groups were of comparable age—normally reared = 9.4 years (SE = 1.12), VFD-reared = 7.5 y (SE = 0.26);  $t(7) = 1.49$ ,  $p = .179$ .

### General Parameters of Sampling and Stress Application

Each subject was housed individually during the experiment. The time between the experimental conditions (stress exposure and dexamethasone + stress exposure) was about 7–11 days. All blood and CSF sampling occurred at the same time of day (12:35 p.m., 5.5 h after onset of colony room lighting). Personnel conducted the study with no knowledge of the subjects' rearing history.

### Baseline Sampling

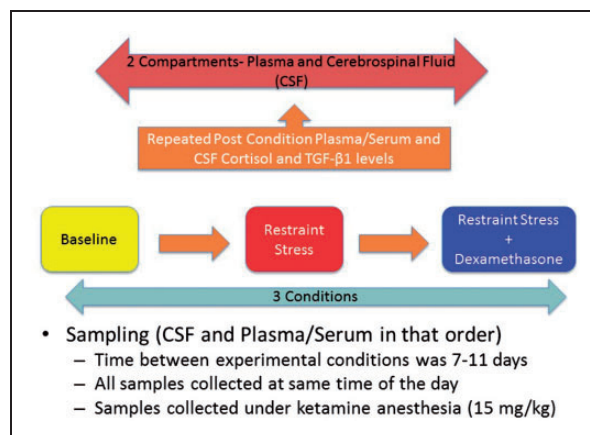
In order to minimize the influence of stress responses from the subjects for baseline measurements, on days of sampling, experimenters quickly entered the colony room, gently brought the selected subjects to the front of their cages using the squeeze mechanism, and administered 15-mg/kg ketamine intramuscular (IM). The squeeze mechanism was then released and the experimenters left the room. After 2–4 min, the anesthetized subjects were removed from their cages, and blood and CSF were sampled, in that order. Blood was drawn from the cephalic or saphenous vein, deposited in tubes with and without sodium heparin, and placed on ice. Cisternal CSF was sampled as previously described.<sup>31</sup> Plasma and serum were then separated by centrifugation at 4000 g for 15 min at 4°C.<sup>32</sup> Subjects thus were anesthetized within 5 min, and sampling was completed by 15 min. All samples were stored at –70°C until assayed.

### Exposure to Stress

Animal models of anxiety and stress have helped us with understanding the neurobiology of certain psychiatric illnesses.<sup>33</sup> Restraint and immobilization stress models are most commonly employed to induce stress-related biochemical, behavioral, and physiological changes in animals.<sup>33</sup> Subjects were trained to enter holding cages from their pens. On days of stress exposure, the selected subjects were taken from their home cages, transported to an unfamiliar room, placed individually in squeeze cages measuring  $0.7 \times 0.6 \times 0.8$  m (see Appendix for Figure 3), and gently squeezed to the front of the cage. Squeeze cages have mobile back walls and by pulling the back wall forward, the subject's mobility can be restricted, thus accomplishing our acute restraint stress model (see Appendix for Figure 3). Subjects were restrained to the extent that their ventral and dorsal sides made light contact with the front and back of the cage but complete rotation was possible.<sup>14</sup> Each application of this gentle restraint was evaluated throughout the experiment by at least three experimenters to ensure consistent application across subjects and sessions. Experimenters then left the room. Confinement was maintained for 30 min, at which time experimenters re-entered, ketamine (15 mg/kg) was immediately administered, and blood and CSF were sampled and stored as described above. The order of studies was that subjects first underwent baseline sampling, then the acute isolation/confinement stress (Stress), and finally, the dexamethasone pretreatment study (Stress + Dex) (see Figure 1 for flow chart).

### Dexamethasone Administration and Stress Exposure

In the current study, dexamethasone (Sigma, St. Louis, MO; 0.01 mg/kg IM) was administered in the home cage at 8:00 a.m. Dexamethasone is administered IM, at about 7 a.m. and 4–6 h later, cortisol levels are measured.<sup>28</sup>



**Figure 1.** Timeline for the experiment showing the three conditions and the two compartments.

It has been previously observed that maximum cortisol suppression occurs 4–6 h after dexamethasone administration.<sup>28</sup> Thus, the selected subjects were exposed to the stress paradigm 4 h following dexamethasone administration, and 30 min after that, CSF and blood were sampled under ketamine anesthesia.

### TGF- $\beta$ 1 Assay

Serum total TGF- $\beta$ 1 was measured by enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Minneapolis, MN) after acid activation of serum as described by the manufacturer. Prior to acid activation of serum, bioactive TGF- $\beta$ 1 was not detectable. In order to ascertain (a) acceptable interassay variability of <5–7%; and (b) that the samples did not contain any TGF beta inhibitors to account for low levels, we used (a) known quantities of control bio active TGF beta in each assay as well as samples from animals with known levels of high and low TGF beta as well as (b) we spiked low TGF beta samples with known amounts of TGF beta and made sure that there were no inhibitors that accounted with the low levels. While the deduced amino acid sequence of mature TGF- $\beta$ 1 from vervets has been shown to be 100% homologous to that of human TGF- $\beta$ 1,<sup>34</sup> we are unaware of similar comparisons of macaque and human TGF- $\beta$ 1. Thus, it should be kept in mind that cytokine levels reported by this assay are based on reactivity with human TGF- $\beta$ 1 and have not been specifically validated with macaques.

### Cortisol Assay

Plasma and CSF cortisol concentrations were determined by radioimmunoassay by the Clinical Chemistry Laboratory of Downstate Medical Center. Cortisol concentration was determined using competitive protein binding method described by Murphy.<sup>32</sup>

### Analyses

Distributions of variables were assessed for normality using normal probability plots and Kolmogorov–Smirnov statistics. After confirming normality, we constructed linear mixed effects (LME) models (on SPSS version 22), with condition (baseline vs. stress vs. DEX + stress) as the fixed effect, intercept as the random effect, and the dependent variables comprised CSF TGF- $\beta$ 1, plasma TGF- $\beta$ 1, CSF cortisol, and plasma cortisol. Models showing significant effect of condition were followed by post-hoc pairwise comparison with least significant difference correction. As we did not observe any effect of rearing on any of the three conditions, we pooled the data for both the rearing groups and performed the analyses with groups combined. All tests were two-tailed, with significance considered at  $p < .05$ .

**Table 1.** Total number of samples available for each testing condition.

Condition	Baseline (n = 9)	Stress (n = 9)	Dex + stress (n = 9)	Total
Plasma cortisol	9	9	9	27
CSF cortisol	7	7	9	23
Serum TGF- $\beta$ 1	8	8	9	25
CSF TGF- $\beta$ 1	7	6	8	21

TGF- $\beta$ 1: transforming growth factor- $\beta$ 1; CSF: cerebrospinal fluid; dex: dexamethasone.

### Results

Due to insufficient CSF samples, cortisol levels were measurable in seven subjects at baseline and under conditions of stress (Table 1). For CSF TGF- $\beta$ 1, we report analysis from seven, eight, and eight subjects at baseline, under stress, and under conditions of stress + dexamethasone, respectively, due to inadequate CSF samples. We did not have any missing samples for plasma cortisol analysis. However, for serum, we could measure TGF- $\beta$ 1 from eight samples each for baseline and stress conditions. The LME models used account for the missing values.

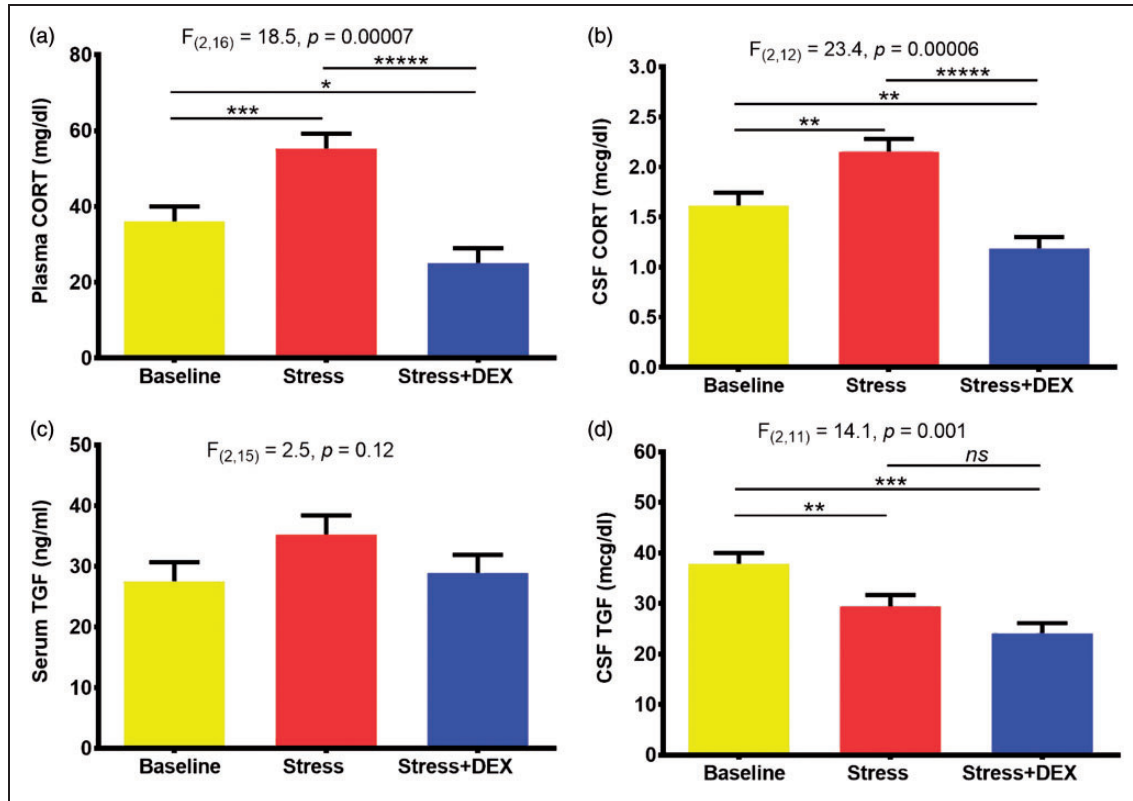
#### *Effect of Stress and Stress + Dexamethasone on Peripheral and Central Cortisol*

As shown in Figure 2, stress condition had a significant effect on both plasma and CSF cortisol levels. Exposure to stress and to DEX + stress substantially affected cortisol in both plasma and CSF in a similar fashion. The mean plasma cortisol concentration was 36.1 mg/dl at baseline (Figure 2(a)), which significantly increased to 53.3 mg/dl under stress (mean difference = 19.2 mg/dl,  $p = .001$ ). After exposure to stress following dexamethasone pretreatment, plasma cortisol decreased to 25.1 mg/dl, which was a significant reduction when compared to either baseline (mean difference = 11.0 mg/dl,  $p = .04$ ) or stress-alone conditions (mean difference = 30.2 mg/dl,  $F_{(2,16)} = 18.5$ ,  $p = .00007$ ). Similarly, in CSF (Figure 2(b)), when compared to a baseline value of 1.62  $\mu$ g/dl, stress significantly increased CSF cortisol to 2.15  $\mu$ g/dl (mean difference = .54  $\mu$ g/dl,  $p = .004$ ). As in plasma, cortisol levels in CSF were significantly reduced when dexamethasone was administered 4 h prior to stress onset, dropping to 1.19  $\mu$ g/dl when compared to either baseline (mean difference = .43  $\mu$ g/dl,  $p = .01$ ) or stress-alone conditions (mean difference = .97  $\mu$ g/dl,  $F_{(2,12)} = 23.4$ ,  $p = .00006$ ).

#### *Effect of Stress and Stress + Dexamethasone on Peripheral and Central TGF- $\beta$ 1*

In contrast to the synchrony of peripheral and central cortisol across experimental conditions, the effects on





**Figure 2.** (a) Bar diagram showing plasma cortisol under the three study conditions. Significant increase in serum cortisol from baseline (yellow) is observed under conditions of stress (red) and a reduction to below baseline condition after exposure to stress following pretreatment with dexamethasone (blue). Condition effect:  $F_{(2,16)} = 18.5, p = .00007$ . (b) Bar diagram showing CSF cortisol under the three study conditions. Significant increase is observed in CSF cortisol under conditions of stress (red) and a reduction to below baseline condition after exposure to stress following pretreatment with dexamethasone (blue). Condition effect:  $F_{(2,12)} = 23.4, p = .00006$ . (c) Bar diagram showing Serum TGF under the three study conditions. There is no significant difference between conditions. Condition effect:  $F_{(2,15)} = 2.5, p = .12$ . (d) Bar diagram showing CSF TGF- $\beta 1$  under the three study conditions. Significant reduction is observed in CSF TGF- $\beta 1$  under conditions of stress (red). Condition effect:  $F_{(2,11)} = 14.1, p = .001$ .

peripheral and central TGF- $\beta 1$  diverged substantially. It can be seen in Figure 2(c) that mean values of serum TGF- $\beta 1$  did not significantly differ in any condition ( $F_{(2,15)} = 2.5, p = .12$ ). However, as seen in Figure 2(d), CSF levels of TGF- $\beta 1$  decreased significantly to below baseline levels after exposure to stress (mean difference =  $8.5 \mu\text{g/dl}$ ,  $p = .01$ ), and stress following pretreatment with dexamethasone conditions when compared to baseline (mean difference =  $13.8 \mu\text{g/dl}$ ,  $p = .0003$ ). However, no significant difference in central TGF- $\beta 1$  was found between the stress vs. stress + dexamethasone conditions ( $p = .08$ ).

We examined the relationship between peripheral cortisol and TGF- $\beta 1$  at baseline, controlling for rearing, to explore any association between the two variables. There was a strong effect size ( $\eta_p^2 = 0.24$ ) though the effect was not significant ( $F_{(1,4)} = 1.30, p = .31$ ). We examined this putative relationship using Spearman's correlation and did not find any difference in the result ( $p = .31$ ).

We investigated the compartmental effects using LME models with condition, compartment (CSF vs. plasma), and condition  $\times$  compartment as fixed effects, intercept as a random effect, and TGF- $\beta 1$  as dependent variable. We found a significant compartment  $\times$  condition interaction ( $F_{(1,20)} = 8.8, p = .007$ ), such that stress significantly reduced TGF- $\beta 1$  in CSF as compared to plasma.

### Moderating Effect of VFD

Secondary LME models examining the moderating effects of rearing showed no effects of group or group  $\times$  conditions (all  $p$  values  $> .05$ ). Condition effects maintained significance as in the primary models (all  $p$  values  $< .05$ ). The small number of subjects per group may be underpowered to examine the effects of VFD; nonetheless, this exploratory analysis ruled out VFD rearing as a potential confound to the primary analyses.

## Discussion

The results of the current study demonstrate the complex relationship between circulating levels of glucocorticoids and production of TGF- $\beta$ 1 in the CNS versus the periphery. Whereas isolation/confinement for 30 min in an unfamiliar room induced significant increases in cortisol both in plasma and in CSF, contemporaneous TGF- $\beta$ 1 levels were unaffected in serum but fell significantly in CSF. Serum levels of TGF- $\beta$ 1 were unaffected by stress or DEX + stress, while stress-induced increases of cortisol in blood and CSF were suppressed by dexamethasone. In addition, administration of dexamethasone prior to the stress manipulation still produced a significant reduction in levels of CSF TGF- $\beta$ 1 despite suppression of cortisol below baseline. These are inferences which need further testing as TGF- $\beta$ 1 suppression might be influenced by either endogenous or exogenous glucocorticoids.

While prior evidence indicates bidirectional control of cortisol and TGF- $\beta$ 1 in peripheral systems,<sup>14</sup> our findings show a concomitant glucocorticoid increase and TGF- $\beta$ 1 decrease in CSF but not serum under stress. These data are in accord with findings of increased TGF- $\beta$ 1 within the CNS after adrenalectomy which is reversed by glucocorticoid administration.<sup>9</sup> A similar *in vitro* finding of glucocorticoid-induced reductions of TGF- $\beta$ 1 expression has been observed in cultured human fetal lung fibroblasts.<sup>35</sup> In light of TGF- $\beta$ 1's neuroprotective properties, suppression of this cytokine may be a mechanism by which endogenous or exogenous glucocorticoids potentially exert neurotoxic effects. By corollary, TGF- $\beta$ 1 agonists may attenuate the neurotoxic effects of stress.

Our results are in accordance with the Batuman laboratory where it was shown *in vitro* that TGF- $\beta$ 1 gene expression was upregulated by dexamethasone in peripheral T cells but downregulated in glial cells.<sup>15</sup> The divergent response observed in serum and CSF TGF- $\beta$ 1 in the current study are in keeping with prior studies in rhesus macaques showing differential physiological response in CNS versus the periphery. However, those studies compared adrenocorticotrophin levels in the plasma and CSF.<sup>36,37</sup> We have previously reported a strong positive relationship between peripheral cortisol and TGF- $\beta$ 1 levels under conditions of acute restraint stress.<sup>14</sup> This study differs from the study of Smith et al. as we report herein the relationship between peripheral (plasma) and central (CSF) TGF- $\beta$ 1 in relationship to the HPA axis in a previously unreported upon sample. This current study also diverges methodologically from our laboratory's previous one as the duration of acute stress was shorter (30 min) versus a longer stress period (90 min).<sup>14</sup> However, capture activates the HPA axis and TGF- $\beta$ 1, and cortisol changes were observed in the current study which validates the restraint duration.<sup>30</sup>

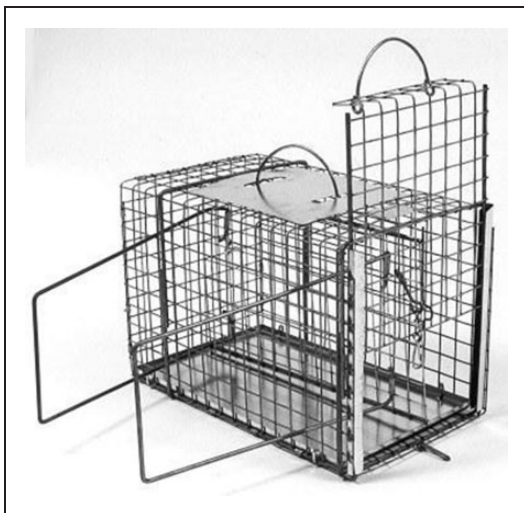
Interestingly, rearing group differences were not found on statistical analyses. This may be distinguished from other findings in our laboratory, in which somewhat more severe stress (90 min of closer confinement in an unfamiliar room) in larger samples resulted in higher concentrations of serum TGF- $\beta$ 1 and plasma cortisol in two-year-old VFD-reared subjects versus controls.<sup>14</sup> The current study is not powered to show rearing group differences and the negative effects should not serve to contradict previous positive effects. Furthermore, the sample size is small, and any heterogeneity provided by the VFD and non-VFD reared sample is not sufficient to obtain significant rearing effects. The primary purpose of the current study is to demonstrate compartmental effects of stress on TGF- $\beta$ 1 concentrations in nonhuman primates.

A further limitation is the absence of examination of the relationship of other cytokines and interleukins on the HPA axis. An extensive literature documents the interaction between various cytokines and interleukins and their effects on the HPA axis.<sup>38</sup> While some interleukins such as IL-1 have more potent effects on HPA axis activation, others such as IL-2 and TNF- $\alpha$  have weak or no effect on HPA axis activation.<sup>38</sup> It is beyond the scope of this paper to review the effects of other cytokines on the HPA axis and the reader is referred to other sources for more details.<sup>5,10,18,19,38,39</sup> We chose to limit our findings to study the relationship between TGF- $\beta$ 1 and its interaction with the HPA axis, as glucocorticoids play an important inhibitory role on hippocampal neurogenesis.<sup>40</sup> It has been observed that glucocorticoids inhibit dentate gyrus neurogenesis via an NMDA receptor dependent excitatory pathway.<sup>40</sup> Further, chronic stress has been shown to have inhibitory effects on granule cells through NMDA receptor inhibition.<sup>40</sup> While TGF- $\beta$ 1 is shown to have neuroprotective effects from glutamate-induced excitotoxicity,<sup>5,18</sup> apoptosis,<sup>5,18</sup> chemical hypoxia,<sup>18</sup> and reactive oxygen species,<sup>5,18</sup> further studies are needed to understand the neuroprotective role of TGF- $\beta$ 1 on hippocampal neurogenesis during stress conditions. Another limitation of our study is that the TGF- $\beta$ 1 assay which was used was based on reactivity to human cytokines. Further studies are required to develop specific assays that are validated in macaques.

The current study supports the hypothesis of an intimate relationship between cortisol and TGF- $\beta$ 1 during stress, and suggests that this relationship may be expressed in divergent regulatory pathways in the periphery vis-à-vis the CNS. The study design does not protect against an order effect, as the order of the procedures was not randomized. However, the primary result of the study—decreased CSF TGF beta in response to an acute stressor—is unchanged by the dexamethasone component of the study. In view of the reported role that central TGF- $\beta$ 1 may have in neuroprotection, and

because of the negative feedback on cortisol release exerted by TGF- $\beta$ 1, continued investigation of the cortisol–TGF- $\beta$ 1 relationship may yield important information. We therefore provide preliminary evidence for divergent compartmental effects for serum TGF- $\beta$ 1 (no change) and CSF TGF- $\beta$ 1 (decrease) in response to stress although future studies with additional subjects are required.

## Appendix



**Figure 3.** Primate restraint cage with mobile back door for confinement experiment.

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