Chronic endogenous glucocorticoid treatment induces hippocampal volume loss – an in vivo MRI study in rats at 7T.

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Introduction

Numerous MRI studies have reported atrophy of the hippocampus in normal aging (1) but also in several disorders, such as Cushing's disease, major depressive disorder, PTSD, and dementia (2-6). Most of these have been found to be associated with disturbances of the HPA axis, elevated glucocorticoid levels, and cognitive impairment (2, 3, 6, 7). In histological studies, glucocorticoids were found to induce reduced hippocampal layers, dentate granule and CA3 pyramidal cells (8-10), and dendritic atrophy of hippocampal neurons (11-12), associated with memory impairment (13). In a previous study, however, we found no hippocampal volume loss in adrenalectomized rats treated with dexamethasone, an exogenous glucocorticoid (14). It remains unclear, however, in what way adrenalectomy may have affected that result. The aim of this study was now to investigate the effects of chronic high-dose corticosterone, the endogenous rodent glucocorticoid, on (i) hippocampal volume and (ii) spatial memory in normal rats.

Material and Methods

Adult male Wistar rats (n=30, Charles River, UK) were either treated with the endogenous steroid hormone corticosterone (CORT, n=17, 218±8 g body weight; 400 µg/mL in <2% ethanol; C2505, Sigma-Aldrich, UK), or with vehicle (VEH, n=13, 217±11 g body weight; <2% ethanol) in the drinking water. Three weeks later rats underwent the Morris Water Maze (MWM) test to test for spatial memory deficits, followed by *in vivo* MRI (7T Bruker Biospec, Germany) 6 to 9 weeks later. The amount of drinking water was monitored, and the body weight (BW) measured weekly. Plasma corticosterone levels were explored by EIA (IDS Ltd., UK). Postmortem, the thymus-to-body weight ratio (THY/BW) was determined. For MR volumetry (MRV), 40 coronal slices (TR=4096.8 ms, TE=43.8 ms, RARE factor 4, 6 averages, resolution: 0.068x0.068x0.6 mm^3) were acquired. Both hippocampi (HC; from approx. -2.12 to -7.04 mm from Bregma) were manually outlined according to the Paxinos and Watson (15) rat brain atlas using the manufacturer's software (14, 16) (ROItool, Bruker). Volumes were calculated by multiplying the area with interslice distance. Evaluation of the MWM data (subgroup of rats; CORT, n=7, VEH, n=5) was done by using Ethovision (Nolders, Netherlandes), followed by a one-way repeated measure ANOVA. For group comparison, univariate ANOVA was done for plasma CORT, BW, THY/BW, and MANCOVA for RHV and LHV with BW before treatment as covariate. Two-tailed Spearman's rank correlation was used to investigate for significant associations between hippocampal volumes and plasma CORT levels. Significance was considered if $p < 0.05$.

Results

Alterations of the corticosteroid milieu were reflected in significantly reduced BW ($F(1,28)=70.729$, $p<0.001$), THY/BW ($F(1,28)=8.568$, $p<0.01$), and plasma CORT levels ($F(1,28)=114.226$, $p<0.001$) in CORT compared to VEH rats. MANCOVA revealed an overall significant group difference ($F(2, 26)=9.112$, $p=0.001$) with significantly reduced RHV ($F(1,27)=13.342$, $p=0.001$) and LHV ($F(1,27)=18.898$, $p<0.001$) in rats treated with CORT in comparison with VEH rats. There was an overall positive correlation between hippocampal volume (right, left and total) and plasma CORT levels (Spearman's rank correlation; right HV $r=0.454$, $p=0.006$; left HV $r=0.511$, $p=0.002$; total HV $r=0.487$, $p=0.003$). With regards to the MWM test, preliminary analysis of a subgroup of rats revealed significantly increased latency to locate the platform in the CORT group compared to VEH rats ($p<0.05$).

Conclusion

The main finding of 7-8% bilateral hippocampal volume loss is in line with previously described histological findings, such as significantly reduced hippocampal layers, dentate granule and CA3 pyramidal cells (8-10), and dendritic atrophy of hippocampal neurons (11, 12), and the vast majority of MRI studies in stress-related disorders (2, 4-6). Therefore, our finding supports the hypothesis that elevated stress levels of corticosterone directly result in hippocampal volume loss which may underlie the memory impairment. However, this finding has to be interpreted with care as brain volume normalization has not been done yet which may confound regional atrophy measures (14). Preliminary analysis of the MWM data suggests chronic CORT treatment to induced memory impairment in accordance with several reports in rats and humans (2, 13, 17). More detailed analysis of the MWM data of all rats is currently pending. It will allow for investigation of any correlations between hippocampal volume and memory impairment to probe whether memory impairment is a direct consequence of hippocampal volume loss.

References

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