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Manual morphometry of hippocampus and amygdala in adults with attention-deficit hyperactivity disorder

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ABSTRACT

Previous studies have pointed to the involvement of limbic structures in the genesis of attention deficit hyperactivity disorder (ADHD). The present researchers manually segmented magnetic resonance images of 30 individuals with ADHD and 30 individually matched controls, focusing on amygdala and hippocampus volumes. Neither hippocampus nor amygdala volume differed significantly between individuals with and without ADHD. However, ADHD patients with higher hyperactivity scores had significantly smaller left amygdala volumes. This finding suggests that limbic alterations are significant in hyperactive symptoms in the pathophysiology of ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterised by inattention, impulsivity and hyperactivity. These symptoms are often accompanied by emotional instability, disorganised behaviour, impaired affect control and emotional hyper-reactivity (Philipsen et al., 2008). The symptom of inattention is more likely to persist into adulthood than impulsivity or hyperactivity (Wilens et al., 2009).

Previous studies have reported ambiguous results on changes in hippocampal volume in the presence of ADHD. Scholars have variously reported increased volume (Plessen et al., 2006), decreased volume (Bonath et al., 2016; Hoogman et al., 2017; Posner et al., 2014) or no difference (Amico et al., 2011; Perlov et al., 2008). The findings regarding the amygdala appear more consistent, with most studies reporting smaller volume (Bonath et al., 2016; Frodl and Skokauskas, 2012; Hoogman et al., 2017; Lopez-Larson et al., 2009; Sasayama et al., 2010) or similar volume (Amico et al., 2011; Perlov et al., 2008) in individuals with ADHD compared to healthy controls (HC). With treatment and time, changes seem to diminish from childhood to adulthood (Frodl and Skokauskas, 2012). In addition, a mega-analysis by the ENIGMA Working Group on subcortical structural changes found smaller accumbens, caudate, putamen and intracranial volumes in individuals with ADHD compared to controls (Hoogman et al., 2017). Maier et al. (2016) reported subtle global but not focal grey matter volume reductions.

Given the available evidence, this study was aimed at investigating the hippocampus and amygdala, which are two limbic regions important for affect generation and regulation (Richter-Levin, 2004; Sah et al., 2003). Manual morphometry was performed in HCs and adults with ADHD. With the lack of reliable research comparing automated and manual morphometric studies with post-mortem volume measures, manual morphometry is considered to be the gold standard even today (Focke et al., 2014; Schoemaker et al., 2016). Considering the heterogeneity of earlier results, working hypothesis was not directed.

2. Material and methods

2.1. Participants

This research study was part of the prospective, double-blind, placebo-controlled, multicentre COMPAS study (Number: 2006-000222-31, ISRCTN54096201; BMBF01GV0606). The ethics committee of the University Medical Center Freiburg approved the study (Faculty of Medicine Freiburg 217/06). The diagnostic procedure is described in a previous paper (Philipsen et al., 2014). Experienced senior consultants made ADHD diagnosis according to DSM-IV criteria. The psychometric instruments used included clinician-administered diagnostic interviews

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and self-report scales (CAARS-SL (Conners, 1999), ADHS-DC (Roesler, 2004), WURS-K (Retz-Junginger et al., 2002)). A cut-off of ≤30 in the short version of Wender Utah Rating Scale served as an exclusion criteria but following international guidelines the positive diagnosis was established clinically. All the patients had been stimulant free for at least 6 months and were not taking any psychiatric medications. The participants from the two study centres (Freiburg and Mannheim) were scanned in Freiburg. The HC group was recruited through public announcements. The psychometric tools used were the SKID-I, SKID-II (Wittchen et al., 1997), CAARS-SL (Conners, 1999), WURS-k (Retz-Junginger et al., 2002) and BDI (Beck et al., 1961).

The sample consisted of 30 participants (19 male, 11 female) with ADHD and 30 HCs matched according to age, gender and IQ using in-house software (see CP Kaller, unpublished toolbox). The groups were comparable in age (ADHD:35.77 ± 8.73[22−51]; HC:35.53 ± 8.30[22-53]) and premorbid verbal intelligence as assessed by the Multiple-Choice-Word-Test-B (Lehrl et al., 1995) (ADHD:122.37 ± 13.51[92−143]; HC:123.63 ± 13.80[93−145]). Total brain volume (TBV) was 1211.89 ± 117.33 ml in the ADHD group and 1240.44 ± 141.06 ml in the HC group.

2.2. Image acquisition and post-processing

A magnetisation-prepared rapid gradient echo T1-weighted anatomical scan was conducted (TR = 2200 ms, TE = 4.11 ms, FA = 12°, FOV = 256 × 256 mm^2, voxel size = 1 × 1 × 1 mm^3) on using a Siemens TIM Trio Magnetom (Erlangen, Germany) with a 12-channel head coil. Anatomical T1-weighted MPRAGE images were re-orientated using SPM8 (Statistical Parametric Mapping, Department of Cognitive Neurology, London; Friston et al., 2007). All the images were oriented in the axial position passing through the anterior and posterior commissural structures.

2.3. Manual morphometry

The images were processed using ITK-Snap software, which enables manual image segmentation (Yushkevich et al., 2006). The amygdala was segmented according to a protocol developed in the work of Convit, Pruessner and Yushkevich (Convit et al., 1999; Pruessner et al., 2000; Yushkevich et al., 2006). An anatomical atlas was also consulted (Duvernoy, 2005). A validated protocol was followed for segmentation of the hippocampus (Pruessner et al., 2000).

The hippocampal–amygdala border was traced from the sagittal and axial views. The amygdala was outlined from the coronal view, and the optic chiasm served as a landmark defining the most anterior portion of the amygdaloid area. The hippocampus tracing started with the most lateral section and proceeded medially until it was no longer visible. This sagittal outline was refined from the coronal view, starting with the most posterior portion and proceeding anteriorly to the hippocampal–amygdala border. The hippocampus was divided into the uncus and the body plus tail. The uncus was identified from the coronal view and verified from the sagittal view by the presence of recessus uncalis.

2.4. Intra-rater reliability and inter-rater reliability

Rater 1 twice traced the 30 HC group images (1-month pause between segmentations) to prove the reliability based on Cronbach's alpha. Rater 2 measured 20 of the images applying the same protocol. The raters were blind to the participants' identity. The inter-rater reliability was calculated as an intraclass-correlation (ICC).

2.5. Statistical analysis

The statistical analyses were performed using software R, version 3.2.2 (www.r-project.org). Group comparisons of demographic and psychometric data (age, IQ and psychometric scores) were made with two-sided independent-sample *t*-tests, and a group comparison of gender was made with Pearson's two-sided chi-squared test.

TBV and age are known to affect segment volume, so the researchers adjusted for their influence. A general linear model with age and TBV was computed for each of the four target variables: right and left amygdala (RA, LA) and hippocampus (RH, LH). Then, each value was corrected by the difference between the predicted value for the participants' actual age and TBV and the predicted value at an age of 35.65 years and a TBV of 1226 ml. Group comparisons of these corrected segment volumes were made using multivariate analysis of covariance (MANCOVA). The group effect on each individual segment volume was assessed in a post-hoc *t*-test. IQ and BDI were added as covariates in an additional MANCOVA model to test their influence.

Correlation analysis with ADHD-related psychometric scales – the WURS and CAARS, which has four prominent subscales (inattention, hyperactivity, impulsivity and problems with self-concept) - was performed, using Pearson's coefficient and controlling for family-wise errors by applying Holm's adjustment method. Holm's multiple testing correction dominates the unmodified Bonferroni correction (Holm, 1979). The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Intra-rater and inter-rater reliability

The intra-rater reliability proved to be excellent (RA: α = 0.936; LA: α = 0.958; RH: α = 0.944; LH: α = 0.943; all regions: α = 0.995). The inter-rater reliability was also very good (hippocampus: ICC = 0.831; amygdala: ICC = 0.713). The overall ICC for both regions was 0.957.

3.2. Volumetric findings

The groups showed no difference in segment volumes (RH: p = 0.781, ADHD: 2458.81 ± 262.39 ml, HC:2481.53 ± 359,91 ml; LH: p = 0.7588, ADHD:2283.18 ± 290.91 ml, HC:2258.12 ± 336.50 ml; RA: p = 0.2728, ADHD:1444.51 ± 153.24 ml, HC:1405.06 ± 120.70 ml; LA: p = 0.8919, ADHD:1311.08 ± 170.32 ml, HC:1305.76 ± 128.81 ml).

3.3. Correlation analysis

No significant correlations of childhood ADHD symptoms according to the WURS-k with amygdala or hippocampus volumes were found. There was a negative correlation between the LA volume and the CAARS hyperactivity score in the ADHD group (p = 0.046), adjusted for multiple tests (Fig. 1). No correlations in the HC group and across both groups were significant.

4. Discussion

This study is one of the largest manual morphometry studies on adults with ADHD focusing on the amygdala and the hippocampus. There was no evidence for changes in amygdala or hippocampal volume in ADHD. However, the LA volume of individuals with ADHD had a negative correlation with hyperactivity levels.

The lack of differences in hippocampus and amygdala volume is in line with the results of other studies investigating adults with ADHD (Perlov et al., 2008; Seidman et al., 2006). In children and adolescents with ADHD, however, smaller amygdala volumes and larger (Plessen et al., 2006) and smaller hippocampus volumes (Posner et al., 2014) have been reported. So far, the largest study on volume differences found smaller amygdala and hippocampus volumes in a sample of both children and adults with ADHD (Hoogman et al., 2017). The observed differences appear to be very small and might go unnoticed in smaller samples. With ongoing treatment and upon reaching adulthood, these differences seem to diminish (Frodl and Skokauskas, 2012), in line with a VBM study by this research group (Maier et al., 2016). The

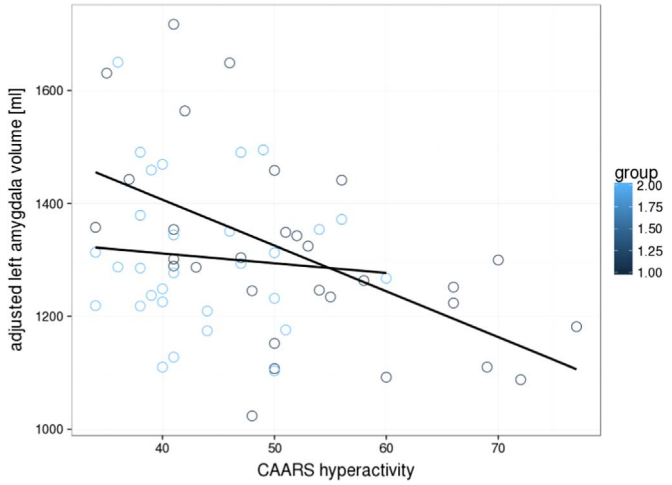

Fig. 1. Correlation CAARS-B (hyperactivity) and left amgydala volume adjusted for age and total brain volume (TBV) in the ADHD group. ADHD: attention deficit hyperactivity disorder, TBV: total brain volume, CAARS: Conners' Adult ADHD ranking scale.

normalisation of volume deficits over time might be linked to a compensatory learning mechanism affecting brain plasticity, to delayed brain maturation in ADHD (Shaw et al., 2007) or to both.

Stimulant medication may affect the volume of different brain areas (Nakao et al., 2011), so only patients who took no stimulant medication for at least 6 months before the scan were included in the present study. Nevertheless, the effects of earlier medication cannot be completely ruled out.

As well, in the absence of any group difference in segmented volumes, the possibility of more subtle structural differences cannot be ruled out. Small volume abnormalities in distinct amygdala subnuclei would have rather small effects on the volume of the entire amygdala. ADHD is a complex disorder with various subtypes with many aetiologies and possibly different pathomechanisms. The result of decreased LA volumes in patients with high hyperactivity levels, therefore, might hint that symptom domains are modulated in different ways by different brain circuits. Longitudinal multimodal imaging studies combining structural and neurochemical signals are needed to obtain more information about the precise pathophysiology, involved circuits, dynamic changes over the lifetime and possible dependency on therapeutic interventions.

5. Conflict of interest

Kathrin Nickel: no conflicts of interest, Ludger Tebartz van Elst: advisory boards, lectures, or travel grants within the last four years: Eli Lilly, Janssen-Cilag, Novartis, Shire, UCB, GSK, Servier, Janssen, and Cyberonics, Evgeniy Perlov: no conflicts of interest, Renate Jitten-Schachenmeier: no conflicts of interest, Daniel Beier: no conflicts of interest, Dominique Endres: no conflicts of interest, Peter Goll: received travel grant from GSK, Boston Scientific, Otsuka Pharma and Actelion, Alexandra Philipsen: reported serving on advisory boards, giving lectures, performing phase 3 studies, or receiving travel grants within the last 3 years from Eli Lilly and Co, Janssen-Cilag, MEDICE Arzneimittel Pütter GmbH and Co KG, Novartis, and Shire; and has authored books and articles on psychotherapy published by Elsevier, Hogrefe, Schattauer, Kohlhammer, and Karger. Simon Maier: no conflicts of interest.

Contributors

All authors have made substantial contributions to the conception and design.

LTvE, AP, EP and SM planned and designed the study.

SM, KN, DE, DB and PG conducted the MRI scans.

SM, KN, RJ-S conducted the manual morphometric analysis.

SM and LTvE supervised the manual morphometric analysis.

KN, AP, DE, PG, DB, RJ-S conducted the acquisition of non-MRI data.

SM, KN, LTvE, EP undertook the statistical analysis.

All authors contributed to the interpretation of results.

KN and SM drafted the manuscript.

All authors revised the manuscript for important intellectual content.

All authors have given final approval of the version to be published.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pscychresns.2017.07.001.

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