The Neurotrophic Effect of Antidepressant Drugs on Hippocampal Volume

A Subfield Analysis

Dani Beck

Master of Philosophy in Psychology
Cognitive Neuroscience
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Author: Dani Beck

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IV
Summary

Name of author: Dani Beck

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Background. Research investigating potential brain structural differences between depressed cohorts and healthy controls have found significant volumetric differences localised to the hippocampus. Specifically, studies have shown a reduction in volume in subregions of the hippocampus in sufferers of major depressive disorder (MDD). In animal studies, antidepressants have been implicated as potentially acting to counter hippocampal volume loss and aiding neurotrophic effects, but findings from human studies are sparse. Research aims. The current cross sectional study aimed to investigate hippocampal subfield volume differences between adult remitted MDD (rMDD) subjects and healthy controls. Moreover, the study investigated whether antidepressants had a neurotrophic effect on hippocampal subfield volumes. Method. Magnetic Resonance Imaging (MRI) data of 191 rMDD subjects and 77 healthy controls were processed using a new automated segmentation tool, implemented in FreeSurfer 6.0, for hippocampal segmentation. Results. Our analysis revealed a main effect across hippocampal subfields, indicating smaller volumes present in rMDD subjects localised to the granular cells of the dentate gyrus (GC_DG), CA4, parahippocampus, and molecular layer (ML), while larger subfield volumes were present in the presubiculum bilaterally. The current paper also demonstrates hippocampal subfield volume differences associated with medication status. Specifically, the findings revealed a main effect in previously medicated rMDD subjects, with hippocampal subfield volume observed as larger in the rMDD group. Conclusions. The study adds to the literature suggesting that hippocampal volume reductions are present in individuals with a history of depression. The results implicate the use of antidepressant drugs as neurotrophically enhancing hippocampal subfield volume, potentially partly through reversing neurogenesis suppression. Longitudinal studies are needed to determine any direct effects of antidepressants on hippocampal subfield volumes.

Keywords: depression, hippocampal subfield volume, antidepressants, neurotrophic effect, neurogenesis
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1. Introduction

The prevalence of major depressive disorder (MDD) is globally on the rise, currently affecting an estimated 350 million people worldwide (World Health Organisation, 2016). According to the National Institute of Mental Health (2016), symptoms manifest themselves both mentally (prolonged sadness, irritability, anxiety, loss of interest, problems with concentration, suicidal thoughts, feelings of guilt) and physically (fatigue, aches, increase or decrease in appetite, weight, or sleep). The diagnostic and statistical manual of mental disorders (DSM-V; American Psychiatric Association, 2013) requires that five or more of these symptoms are met for a duration of at least two weeks in order for an individual to be diagnosed with depression. In many cases, depression leads to emotional and cognitive dysfunction for many sufferers of the disorder, including poorer performance on mental tasks as well as memory impairment (Burt, Zembar, & Niederehe, 1995; Hubbard et al., 2016; Shelton & Kirwan, 2013) and structural changes in the brain (Schmaal et al., 2016a; Schmaal et al., 2016b). With depression predicted to be one of three leading causes of burden of disease by 2030 (Mathers & Loncar, 2006), it is crucial to investigate its pathophysiology further. One method of doing so involves the use of brain-imaging studies. By improving our knowledge of the involved neural circuits and tracking structural changes in specific regions of the brain, neuroimaging studies can potentially help us better understand risk and ontogenetic factors involved in depression, which in turn may lead to the implementation of more effective prevention and treatment strategies.

Despite numerous neuroimaging studies in the past, our understanding of the neuroanatomical foundation of depression remains fractional. Research investigating potential structural differences between participants with depression and healthy controls have found significant volumetric differences for several brain structures (Schmaal et al., 2016a; Schmaal et al., 2016b). In their investigation of potential structural differences, significantly thinner cortices have been found in the frontal and temporal lobes of patients with MDD (Schmaal et al., 2016a), while significant volumetric differences have been found in the hippocampus (Schmaal et al., 2016b). Reports of hippocampal volume reduction in depressed patients is not a new revelation in research however, with previous studies having reported similar associations over the duration of the past two decades (Bijanki, Hodis, Brumm, Harlynn, & McCormick, 2014; Huang et al., 2013; McKinnon, Yucel, Nazarov, & MacQueen, 2009; Neumeister et al., 2005), suggesting that hippocampal volume reduction is a trait characteristic of depression (Chan et al., 2016; Neumeister et al., 2005). However, despite the wealth of research supporting a volumetric reduction in the
hippocampus, there are also studies that have failed to replicate this finding (Hastings, Parsey, Oquendo, Arango, & Mann, 2004; Phillips, Batten, Tremblay, Aldosary, & Blier, 2015; Posener et al., 2003; Rusch, Abercrombie, Oakes, Schaefer, & Davidson, 2001; Vakili et al., 2000; Vythilingam et al., 2004), resulting in an inconclusive understanding of the specific role the hippocampus plays in depression.

A potential explanation for this discrepancy is offered by the neurotrophic hypothesis. Put briefly, the theory posits that a hypothalamic-pituitary-adrenal (HPA) axis that is dysregulated will produce a hyper-secretion of the stress hormone cortisol, leading to a decrease in hippocampal volume (Pruessner, Pruessner, Hellhammer, Pike, & Lupien, 2007). This decrease in volume might recover through the process of neurogenesis or growth in connections between neurons (Kempermann & Kronenberg, 2003). Recently, research has implicated antidepressant drugs as potentially acting to counter hippocampal volume loss by aiding neurotrophic effects subregionally (Malykhin, Carter, Seres, & Coupland, 2010), but the data to support such claims remain sparse in human studies. Moreover, it is established that the hippocampus is a cytoarchitectonically and functionally heterogeneous structure (Tamnes et al., 2014), but research investigating its specific subfields in relation to depression and antidepressant medication are scarce. As such, the main goal of the current thesis is to investigate hippocampal subfield volume differences between rMDD subjects and a control group. The motivation to do so can be explained in two parts; firstly, as a necessary replication of previous subfield analyses of the hippocampus, and secondly, to be the first study of its kind to investigate hippocampal subfield volume differences in a remitted depressed group using the new automated segmentation tool. A second goal of the current thesis is to in detail investigate the effects antidepressant drugs have on the hippocampus in a large adult depressed cohort. These effects will be investigated with a recently developed automated image analysis tool for subfield segmentation in an attempt to localise the effect of antidepressants to specific subregions of the hippocampus. As background, a review of the literature aims to describe the role of the hippocampus in depression and discuss how specific regions of the hippocampus are affected. Additionally, the literature examining the potential neurotrophic role of antidepressants on specific subregions of the hippocampus is reviewed.

1.1. The Role of the Hippocampus in Depression

The hippocampus is a sea-horse shaped structure residing in the medial temporal lobe (Ota et al., 2017) part of the limbic system of the brain. Its association to depression has been extensively
studied due to its proposed volume reduction in depressed cohorts (Schmaal et al., 2016b). Additionally, its role in learning, memory, emotion, and the regulation of the HPA axis (Malykhin et al., 2010; Philips et al., 2015) has made it a region of considerable interest for researchers studying depression. In terms of cognitive associations, human studies suggest that spatial learning is associated with the posterior subdivisions of the hippocampal structure (Woollett & Maguire, 2011), while verbal memory is associated with anterior subdivisions (Chen, Hamilton, & Gotlib, 2010). Due to the cognitive deficits experienced by sufferers of depression (Hubbard et al., 2016) and the observable atrophy associated with prolonged depression (Sapolsky, 2001), the hippocampus is seen as a core brain component deeply involved in the neurobiological basis of MDD (Han, Won, Sim, & Tae, 2016).

Although hippocampal volume loss is one of the most replicated findings of structured brain imaging studies on MDD (Han et al., 2016), why the hippocampus atrophies is less clear. No single mechanism has been identified as a clear casual factor, with most research implicating several factors as potentially involved in the observed atrophy; such as hyper-secretion of cortisol (Abercrombie et al., 2011), more severe symptoms of depression (Bernasconi et al., 2015), and episode frequency (MacQueen et al., 2003). Moreover, hippocampal volume loss is not exclusive to depression, with similar findings being reported in studies investigating childhood abuse (Vythilingam et al., 2002), genetic risk (Chen et al., 2010), schizophrenia (Ota et al., 2017), alcoholism, dementia, brain injury, Parkinson's, epilepsy, and Alzheimer’s disease (Geuze, Vermetten, & Bremner, 2005). Additionally, hyper-secretion of cortisol and elevated stress have been suggested as potential causal factors of atrophy in the hippocampus that may lead to volume reduction (Travis et al., 2016). Although the research on clarifying atrophy and volume loss causation remains wide-ranging, review of the literature still reveals a heavily weighted argument to suggest depression plays an important role, with numerous studies supporting this with reports of a smaller hippocampus in individuals with a history of depression (Bijanki et al., 2014; Bremner et al., 2000; Frodl et al., 2002; Han et al., 2016; Huang et al., 2013; Janssen et al., 2007; Lange & Irle, 2004; MacMaster & Kusumaker, 2004; MacQueen et al., 2003; Malykhin et al., 2010; Saylam, Üçerler, Kitiş, Ozand, & Gönül, 2006).

Bijanki et al. (2014) for example, investigated hippocampal atrophy in individuals with psychotic MDD. The results of the study revealed that the bilateral hippocampus was significantly smaller in individuals with psychotic depression compared to a control group. According to the findings, this reduction in the hippocampus, when averaged across the group, was 10.1% smaller in the depressed group. Chan et al. (2016) confirmed right hippocampal volume reduction in a
remitted depressed (rMDD) group, showing reductions of 12% compared to a control group with a low score of neuroticism. Moreover, a reduction of 26% was reported when remitted patients were compared to a control group with a high score of neuroticism. These findings support previous research carried out on rMDD subjects. Neumeister et al. (2005) reported a decrease in total hippocampal volume when comparing rMDD subjects with healthy controls, further supporting the notion that smaller hippocampal volume is a trait characteristic for MDD. Less recent work by Lange and Irle (2004), Bremner et al. (2000), and MacMaster and Kusumakar (2004) have reported even larger reductions, with a volume decrease of 12%, 19%, and 17% respectively. These findings are further supported by Cole et al. (2010), who reported reduction in volume in both left and right hemispheres in MDD patients, and MacQueen et al. (2003) who reported volume reduction in patients with multiple depressive episodes.

Despite the vast empirical support, there are also numerous studies that have found no significant hippocampal volume loss in depressed cohorts (Hastings et al., 2004; Phillips et al., 2015; Posener et al., 2003; Rusch et al., 2001; Vakili et al., 2000; Vythilingam et al., 2004). Namely, Rusch et al. (2001) investigated individuals with depression and control subjects by means of hippocampal morphometry. The findings revealed no significant differences in hippocampal volume. This finding could be countered with a suggestion that the study is confounded due to the sample being much younger than that of most research that does report significance, as younger adults usually have larger and healthier hippocampi (Iglesias et al., 2015). However, this does not invalidate the ambiguity in the field. Another study by Posener et al. (2003) found no difference in hippocampal volume, but did observe a difference in hippocampal shape. Vakili et al. (2000) reported no significant difference comparing hippocampal volume in MDD patients and control subjects. More recently, a study by Phillips et al. (2015) reported no differences in treatment resistant patients and healthy controls in terms of hippocampal volume or cortical thickness, however, the study did find that hippocampal volume increased over time in remitted patients while decreasing in non-remitters. The implication of this is that depressive symptoms may lead to shrinkage in the structure of the hippocampus or that no present differences exist due to the remission period possibly aiding a process of hippocampal recovery.

In addition to the inconsistencies in the reported results, notable limitations are highlighted in studies that have struggled to recruit adequate sample sizes (Bijanki et al., 2014; Chan et al., 2016; Janssen et al., 2007; Lange & Irle, 2004; MacMaster & Kusumakar, 2004; Phillips et al., 2015; Szymkowicz et al., 2016; Wisse et al., 2015) that produce robust power (McKinnon et al., 2009; Schmaal et al., 2016a; Schmaal et al., 2016b). Small sample sizes resulting in limited
statistical power therefore makes replication necessary (Wisse et al., 2015) and encourages conclusions made from the findings of previous research to be preliminary. A small sample size also deters reliable conclusions to be drawn due to the increased chance of type II error being made, as differences between groups might exist but not be found (Janssen et al., 2007; Szymkowicz et al., 2016). Lack of robust effect sizes in the covered literature also raises concerns, with some of the studies (Schmaal et al., 2016a) reporting a small effect size, \( d \approx -0.08 \) to \( -0.13 \), and numerous other studies not reporting effect sizes at all. Additional limitations emerge from failing to correct for multiple tests, or report on and/or control for effects of sex, intracranial volume (ICV), and age, as these factors also may have contributed to discrepancy in the research findings; for instance, individuals with larger ICV’s and those that are younger in age are likely to have larger hippocampi (Iglesias et al., 2015). In an attempt to better understand the discrepancy in previously reported findings, MRI research in recent years has turned towards investigation of the subfields of the hippocampus. The intention being to localise the association between the hippocampus and depression to specific subregions of the structure rather than discriminating between patient groups and healthy controls solely on judgement of the whole hippocampal structure.

1.2. Subregions of the Hippocampus

The hippocampal structure consists of distinct subfields, beginning with the dentate gyrus (and its granular cell layer) at the head of the structure, followed by the cornu ammonis areas comprising of the CA1, CA2, CA3, and CA4 (Tata & Anderson, 2010). The CA4 is also known as the hilar region and lies within the dentate gyrus (Iglesias et al., 2015). Just below the cornu ammonis area resides the subiculum, and together, these grey matter subregions form the hippocampal formation (Malykhin & Coupland, 2015). On the exterior side of the hippocampus white matter regions known as the fimbria and alveus surround the hippocampal head, and continuing ventrally of the hippocampal formation reside the presubiculum, the parasubiculum, and entorhinal cortex (Campbell & MacQueen, 2004). In the latest automated segmentation tool of the hippocampus implemented in the software suite FreeSurfer, there are also other structures of interest, such as the hippocampal fissure (sulcus between the dentate gyrus and subiculum), molecular layer (within the subiculum and CA1 fields), the hippocampal tail, and the HATA (hippocampus–amygdala-transition-area), with the latter forming the medial dorsal border of the hippocampus (Iglesias et al., 2015). See figure 1 for a visual representation of the hippocampal subregions.
The aforementioned subregions are likely to be differentially associated with depression with regard to their molecular mechanism (Wisse et al., 2015). In terms of their cognitive function, Bartsch and Wulff (2015) note the processing of information through the hippocampus as running through connected mossy fibres between the dentate gyrus, CA3, and CA1. Lavenex and Amaral (2000) state the cornu ammonis areas (CA1-3) as being particularly involved in learning and memory processing. Research has also identified the CA1 (Suthana, Ekstrom, Moshirvaziri, Knowlton, & Bookheimer, 2009) and subiculum (O’mara, Sanchez-Vives, Brotons-Mas, & O’hare, 2009) as being involved in the encoding of spatial information and memory.

Although research is yet to identify the specific functions of the subregions of the hippocampus, the evolution of neuroimaging protocols have advanced subfield segmentation from manual tracing of the hippocampus to the more refined and reproducible automated segmentation tools that exist today. Beginning with research that has used manual tracing methods to map surfaces of the subregions of the hippocampus, in a study by Cole et al. (2010), hippocampal volume in mid-life depression was examined in medication-naïve (never used antidepressants) patients and healthy controls. By means of manual tracing methods, the study reported deformations
localised to the subiculum, CA1, and CA2/3 subregions present in MDD patients. Huang et al. (2013) also used surface mapping to examine structural changes in the hippocampus in individuals with MDD. Researchers found that unmedicated MDD patients had smaller hippocampal volume in the CA1, CA2, and CA3 regions of the hippocampal body. The findings of the study note the differences in volume localised to these subfields may be due to a main effect of treatment (Huang et al., 2013). Malykhin et al. (2010) examined subregional volume in patients with moderate to severe depression. Following tracing procedures of the regions of interest, the study reported reductions in the volume of the right hippocampus, hippocampal tail bilaterally, and right hippocampal head. The implication of the results suggests that association to depression may be localised to specific subregions of the hippocampus.

There exists some dispute regarding the limitations of surface mapping and manual tracing of the hippocampus, with various studies reporting volumetric differences in varying subfields (Ballmaier et al., 2008; Cole et al., 2010; Huang et al., 2013; Malykhin et al., 2010; Neumeister et al., 2005; Posner et al., 2003). Furthermore, Wisse et al. (2015) notes that manual tracing cannot access the dentate gyrus located in the hippocampal head, and research conducting tracing procedures have turned over inconsistent results. Moreover, manual delineation is extremely labor-intensive and time consuming and thus tracing the subregions usually means relying on a smaller sample size, which in turns reduces statistical power (Iglesias et al., 2015). Despite these limitations, it is noteworthy to consider that the inconsistencies might stem from poorer MRI acquisition quality in previous years rather than the segmentation protocol. Older studies in particular (Bremner et al., 2000; Lange & Irle, 2004; MacMaster & Kusumakar, 2004) carried out magnetic resonance imaging using scanners that turn over poorer quality of images than that of more recent scanners that provide high field imaging strength (Wisse et al., 2015).

The shift in subfield analysis has most recently turned to automated procedures to carry out the process of segmenting the hippocampus. Szymkowicz et al. (2016) carried out a subfield analysis by means of the FreeSurfer image and analysis suite version 5.3, which segments seven subfields of the hippocampus in each hemisphere (CA1, CA2/3, CA4-dentate gyrus, subiculum, pre-subiculum, fimbria, and hippocampal fissure). In its examination of subfield differences in older adults, Szymkowicz et al. (2016) reported greater age effects on volume in those with lower depressive symptoms. Additionally, they found that depressive symptoms were associated with less age-related volume reduction in the right CA1 and subiculum, contradicting the hypothesis of the study and findings of previous research that implicate the CA1 subfield as a region of interest in depression (Huang et al., 2013). Recent criticism of the subfield segmentation procedure of
FreeSurfer 5.3 (de Flores et al., 2015; Wisse et al., 2014) claim the segmentation algorithm produces segmentations that are inconsistent with the actual brain anatomy (Whelan et al., 2016). It also uses a probabilistic atlas with a resolution only sufficient enough to produce a coarse segmentation of the subregions in standard resolution MRI (Iglesias et al., 2015). Further criticism points to the lack of labelling for the molecular layer, in addition to the tools’ inability to delineate the hippocampal tail and hippocampal head (Iglesias et al., 2015).

This criticism has lead to the emergence of a completely revised version of the FreeSurfer (FS) hippocampal subfield segmentation, that was implemented in version 6.0 and released in January 2017. This version produces better segmentation of the subregions and provides higher resolution quality (Iglesias et al., 2015). Whelan et al. (2016) tested the software package’s reliability in terms of withstanding the test-retest procedure as well as performing trans-platform tests to ensure subfield measures were consistent across different MRI scanners. The study describes twelve subfields in each hemisphere, including the; CA1, subiculum, hippocampal tail, fimbria, CA2/3, presubiculum, granule cells of the dentate gyrus (GC_DG), CA4, hippocampal fissure, molecular layer (ML), parasubiculum, and hippocampal-amygdaloid transition area (HATA). The findings of the study reported FS6.0 as having a good test-retest reliability, with segmentations produced at baseline and follow up having moderate-to-high spatial overlap. In terms of reproducibility, FS6.0 segmentations were strong and reliability was reported in eleven of the twelve subregions, with only the hippocampal fissure producing unreliable volume estimates. In contrast to inconsistent delineations of the hippocampal head and tail in FS5.3, the newly revised version reconstructs these subregions with great reproducibility, making it much more reliable and robust for genetic mapping due to better anatomical accuracy (Whelan et al., 2016).

One study that has used this segmentation protocol is Cao et al. (2017). In their investigation of hippocampal subfield volumes in mood disorders, Cao et al. (2017) compared individuals with bipolar disorder and MDD to healthy controls across eight of the subfields available in the developers version of FS6.0 (CA1, CA2/3, CA4, GC_DG, ML, presubiculum, subiculum, and hippocampal tail). The results of the study indicated no significant differences in volume between MDD patients and the healthy control group. However, patients with bipolar disorder had reduced volumes in the left GC_DG, ML, CA4 and both sides of the hippocampal tail, compared to both healthy controls and MDD patients. Cao et al. (2017) considered their own inability to control for some confounders, such as medication status due to the heterogeneous medication types used by patients. The supplementary information accompanying the study reveals that only 34 out of the 86 MDD patients were medication free at the time of the study. Furthermore, there is no information
provided on whether these medication free participants were medication-naïve or previous users that were just currently medication free. This confounding variable might not only account for the lack of volumetric differences between the groups in this study, but also a wide range of previous research (Huang et al., 2013; Phillips et al., 2015; Rusch et al., 2001; Vakili et al., 2000) that have not found hippocampal volume differences between groups using subjects currently or recently on medication. What is more, this is further supported by the list of studies that have found significant differences in medication-naïve groups (Chan et al., 2016; Frodl et al., 2002; MacMaster & Kusumakar, 2004; Steffens et al., 2000; Szymkowicz et al., 2016; Wisse et al., 2015; Zhou et al., 2016). One possible explanation for these results is that medication provides a neuroprotective effect from volume loss; this neuroprotective theory is well established in studies investigating the neurotrophic hypothesis of depression.

1.3. The Neurotrophic Hypothesis of Depression

The hypothalamic-pituitary-adrenal (HPA) axis is thought to be activated in times of stress, resulting in the release of glucocorticoids (expressed as cortisol in humans) in order for the body to adapt to the experienced stress (Pruessner et al., 2007). Research has shown the hippocampus to be largely affected by this process due to it being a primary site for glucocorticoids that is densely populated with glucocorticoid receptors (Abercrombie et al., 2011). Multiple feedback loops in the hippocampus aid regulation of the HPA axis and it is thought that depressed patients experience impaired feedback inhibition due to glucocorticoid receptor resistance (McKernan, Dinan, & Cryan, 2009). The neurotrophic hypothesis of depression posits that the hippocampus is affected by this process of increased stress/dysregulation of the HPA axis, which can lead to damage in associated structures of the brain when released in excess, thereby increasing risk of developing depression (Herbert, 2013). A dysregulation of cortisol levels may constitute as a vulnerability factor for major depressive disorder (Dedovic et al., 2010), indicating it plays a determining role in its pathogenesis (Mondelli et al., 2010) and the associated atrophy observed in the hippocampus.

Numerous studies have aimed to localise the volume loss in the hippocampus to specific subfields. Postmortem research carried out on 15 depressed individuals revealed low levels of cell death found in the subiculum, CA1, CA4, and dentate gyrus in the hippocampus of 11 of the 15 individuals (Lucassen et al., 2001). Animal studies have shown that elevated glucocorticoid levels lead to volume reduction, dendritic atrophy and neuronal loss in rats and primates (Sapolsky, Krey, & McEwen, 1985; Sapolsky, Uno, Rebert, & Finch, 1990). A review by Lucassen et al. (2006)
suggests that stress related atrophy to the hippocampus is largely localised to the CA1-4 subregions, with neuronal death being predominantly reported in the CA3 subregion in rats, (Czéh & Lucassen, 2007) with further impairment (Alfarez, Joëls, & Krugers, 2003) and synaptic plasticity suppression localised to the CA1 and dentate gyrus (Alfarez et al., 2003; Joëls et al., 2004). Studies investigating the effect of stress on the rat hippocampus have also shown shrinkage, reduction in cell proliferation, and reduced granule cell neurogenesis, with reduction being localised to the granule cell layer (Mirescu, Peters, & Gould, 2004; Pham, Nacher, Hof, & McEwen, 2003), CA1, CA3, dentate gyrus, and subiculum (Li et al., 2017). Research on rats has also reported reduction in postnatal neurogenesis from high-anxiety related behaviour (Lucassen et al., 2009). Reduction in cell proliferation is also seen at a prenatal level (Lemaire, Koehl, Le Moal, & Abrous, 2000). In terms of human trials, Travis et al. (2016) found that CA1-3 subfields are particularly vulnerable to hyperactivity of cortisol, reporting higher cortisol levels in MDD patients compared to healthy controls. However, human studies have been less conclusive. Gerritsen et al. (2011) for example, found smaller hippocampi in patients with depression but no association between this reduction in volume and HPA axis regulation, indicating no association between cortisol levels and hippocampal volume in MDD patients. This finding is also supported by previous research yielding similar results (Tessner, Walker, Dhruv, Hochman, & Hamann, 2007; Vythilingam et al., 2004). One possible explanation for the variation in results may derive from the neurotrophic effect of antidepressants drugs on the hippocampus.

1.4. The Effect of Antidepressant Drugs

Research has indicated antidepressants as potentially accounting for the lack of reduction in the hippocampus in MDD patients by acting as a neuroprotective agent, protecting hippocampal glucocorticoid receptors from the excess levels of cortisol (Campbell & MacQueen, 2004; Huang et al., 2013; Malykhin et al., 2010). A study by Anacker et al. (2011) has supported this theory, demonstrating the role of glucocorticoid receptors in the antidepressant-induced modulation of neurogenesis. Although speculative, this may explain the discrepancy in findings of studies that have investigated hippocampal volume reduction in the past, as a wealth of research has demonstrated differences in reduction found in patients that are active medication users compared to medication-naïve patients and healthy controls. For example, medicated MDD patients revealed a larger hippocampal body (Malykhin et al., 2010) and larger subiculum (Huang et al., 2013) compared to both unmedicated patients and healthy controls. In their high-field (4.7 Tesla scanner)
study of neuroplasticity, Huang et al. (2013) also found the CA1-3, dentate gyrus, and hippocampal tail subregions to be reduced in medication-naïve MDD patients, while differences in volume were not observed in those on long-term antidepressant treatment. Smaller volumes in medication-naïve groups have also been localised to the left hippocampus (Han et al., 2016; Saylam et al., 2006), CA4-dentate gyrus (Choi et al., 2017; Han et al., 2016) left CA2, CA3, and bilateral subiculum (Han et al., 2016).

Due to specific regions of the hippocampus being particularly sensitive to neurotrophic effects (Choi et al., 2017), the results indicate that medication status plays a fundamental role in hippocampal volume reduction. Research investigating antidepressant effects have also demonstrated that time spent untreated predicts hippocampal volume, whereas time treated with antidepressants does not correlate with hippocampal volume loss (Sheline, Gado, & Kraemer, 2003). Moreover, treatment for depression is associated with slower volume loss in both men and women (Elbejjani et al., 2015), with smaller hippocampal volume predicting lower remission rates post treatment (Colle et al., 2016). Opposing findings come from a study that administered antidepressants to depressed subjects and found no significant changes in hippocampal volume after an average treatment duration of 7 ± 3 months (Vythilingam et al., 2004). However, the study did not run a subregional analysis and so does not take into account the increases that might have been observed in the specific regions of interest (hippocampal formation). Furthermore, the average size of the hippocampal head did increase, as did performances in memory tasks. Therefore, trends in the data suggest antidepressants did positively influence hippocampal volume, just not significantly.

McKernan et al. (2009) reviewed a wealth of studies investigating the effects of antidepressants and found that selective serotonin reuptake inhibitors (SSRI’s) prevent cell death (Lee et al., 2001; Huang et al., 2007), and decreases apoptosis in the dentate gyrus of tree shrews (Czéh et al., 2001; Lucassen, Fuchs, & Czéh, 2004). Rat studies suggest antidepressants can induce neuronal sprouting and neurogenesis (Young, Bakish, & Beaulieu, 2002), in addition to increases in brain-derived neurotrophic factor (BDNF) in the dentate gyrus and CA3 regions (MacQueen et al., 2003). Primate studies suggest antidepressant therapy increases cell proliferation and neurogenesis (Perera et al., 2007). In terms of human application, postmortem studies reveal antidepressants increase neural progenitor cell numbers in the dentate gyrus (Boldrini et al., 2009). The collected findings suggest antidepressants either prevent volume loss by acting as a neuroprotective agent or help aid recovery by deterring suppression of hippocampal neurogenesis (Malykhin et al., 2010), with the latter being more in line with the neurogenic hypothesis of depression. As such, it is of interest to the present study to investigate the effect of medication status on rMDD subjects.
Antidepressant drugs acting as neuroprotective agents or countering neurogenesis suppression may explain the discrepancy in previous research. Understanding their effect on a subregional level can guide recommendations for more effective treatment strategies as well as more specific hypotheses in future research.

1.5. Summary

Neuroimaging studies investigating the association between MDD and the hippocampus postulate volume reduction as a trait characteristic of the disorder that is crucial to its pathophysiology (Baaré et al., 2010; Chen et al., 2010; Rao et al., 2010). However, it is not well understood whether this volume reduction emerges as a product of depression i.e. a scar effect (Chan et al., 2016), or whether it exists as a precursor (vulnerability marker) from genetic risk (Chen et al., 2010) or other abnormalities, such as dysregulation of the HPA axis; as suggested by the neurotrophic hypothesis of depression (Czéh & Lucassen, 2007; Lucassen et al., 2006; Travis et al., 2016). Further obscurity involves that of the effect of antidepressants, as several studies have demonstrated the neurotrophic effect of antidepressants on specific subregions of the hippocampus, particularly those localised to the subregions of the hippocampal formation (Huang et al., 2007; Perera et al., 2007). With a review of the literature, further support is drawn from research that has found no significant differences between medication-active MDD groups and healthy controls (Huang et al., 2013), indicating that medication use either protects the hippocampus or aids its recovery. If the use of antidepressant medication protects the hippocampal structure from atrophy or aids hippocampal neurogenesis/recovery then this may explain some of the variance in the reported findings that have been confounded by this potential interaction effect.

Further research is therefore necessary in order to investigate antidepressant effects on hippocampal volume in depressed cohorts. Further necessity emerges from the need to validate and replicate previous findings, in addition to addressing limitations of previous studies that have produced inconsistent results. A large sector of previous research has struggled with sample size, reported small power and effect sizes, as well as reported findings on the back of work carried out on lower resolution scanners, with use of outdated MRI analysis techniques. In light of this, it is of interest to the current study to address these limitations, specifically regarding poorer analysis techniques and less reliable segmentation protocols. A subfield analysis will therefore be conducted to test the newly available automated segmentation tool (FS6.0). With this segmentation protocol there are also more subfields available for analysis, helping better localise the potential association.
between subregions of the hippocampal structure and depression. By doing so, formulation of more specific hypotheses in subsequent research can eventually be made.

1.6. Research Aims and Hypotheses

The current study outlines two main research aims and their associated hypotheses:

1) The current study aims to investigate hippocampal subfield volume differences between adults with rMDD and healthy controls. Magnetic Resonance Imaging (MRI) data from a large dataset will be processed using a new automated segmentation tool released with FS 6.0, which necessitates the need for previous research to be validated through a more reliable analytical method (Iglesias et al., 2015; Whelan et al., 2016). The following project thus aims to fill this gap in research.

Hypothesis 1: Hippocampal subfield volume will be smaller in the rMDD group compared to the healthy control group, localised to specific subregions of the hippocampus. The largest differences are predicted to be found in the cornu ammonis (CA1-3) fields, molecular layer, and those associated with the dentate gyrus, such as the CA4 and granule cells of the dentate gyrus (one-tailed).

2) Due to the indicated neurotrophic effects of antidepressant drugs on hippocampal subfields in depression, the current thesis aims to explore group differences by discriminating between subjects in the rMDD group according to medication status. Differences in hippocampal subfield volumes are expected when comparing active-medication users, previous-medication users, and medication-naïve individuals. Based on the neurotrophic hypothesis, the largest differences are expected to be localised in subfields of the hippocampal formation, which include the CA fields and the dentate gyrus (CA1, CA2/3, CA4, GC_DG, and ML). Specifically, the current study has three medication-related hypotheses, forming the second, third, and fourth hypotheses.

Hypothesis 2: There will be no volumetric differences between the active-medication rMDD group and healthy control subjects. In line with previous research (Huang et al., 2013; Malykhin et al.,
2010), this two-tailed prediction derives from the proposed effect of antidepressant drugs acting as a neuroprotective agent.

**Hypothesis 3:** There will be no volumetric differences between the previous medication rMDD group and healthy control subjects. In line with previous research (Phillips et al., 2015), this two-tailed prediction derives from the potential neurotrophic effect antidepressant drugs may have in the recovery of previously experienced hippocampal volume reduction.

**Hypothesis 4:** There will be a difference between the medication-naïve rMDD group and healthy control subjects, with the medication-naïve rMDD group expected to have smaller subfields localised to specific subfields of the hippocampal formation (CA1, CA2/3, CA4, GC_DG, and ML). This one-tailed prediction derives from research that has shown medication-naïve MDD subjects to have smaller hippocampi than healthy control subjects (Choi et al., 2017; Han et al., 2016).
2. Methodology

2.1. Participants

Two hundred and sixty-eight participants (184 female, 84 male) were included in the final sample for the current cross sectional study. The age ranged from 18 to 71 years ($M = 40.16, SD = 13.35$). Participants were matched on age, education, and sex (table 1). Subjects in the rMDD group were recruited from two related and pre-registered clinical trials (Clin.gov ID: NCT0265862 and Clin.gov ID: NCT02931487) conducted by the Clinical Neuroscience Research Group at the Department of Psychology, University of Oslo. Participants in the healthy control group were recruited through social media. The rMDD group contained one hundred and ninety-one individuals (133 females, 58 males), whilst the healthy control group contained seventy-seven individuals (51 female, 26 male). Demographical characteristics are summarised in table 1.

Table 1. Participant Demographics and Clinical Data

<table>
<thead>
<tr>
<th></th>
<th>rMDD</th>
<th>Control</th>
<th>$p$</th>
<th>effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$M = 39.59$ (SD, 13.51)</td>
<td>$M = 41.56$ (SD, 12.92)</td>
<td>.28</td>
<td>$d = 0.15$</td>
</tr>
<tr>
<td>ISCED</td>
<td>$M = 5.86$, (SD, 1.28)</td>
<td>$M = 6.14$, (SD, .96)</td>
<td>.09</td>
<td>$d = 0.25$</td>
</tr>
<tr>
<td>Sex</td>
<td>Female = 133 (69.6%) Male = 58 (30.4%)</td>
<td>Female = 51 (66.2%) Male = 26 (33.8%)</td>
<td>.59</td>
<td>$\varphi = 0.03$</td>
</tr>
</tbody>
</table>

Note. ISCED = number of years in education. $M$ = mean. SD = Standard Deviation. Where $p < .05$ = a significant difference between groups. $d$ = Cohens $d$. $\varphi$ = Phi.

Antidepressant drug use was classified according to use of selective serotonin reuptake inhibitors (SSRI) or serotonin–norepinephrine reuptake inhibitors (SNRI), not antipsychotic drugs. A record of what specific SSRIs/SNRIs were taken include; Sertraline (Zoloft), Escitalopram, Lamotrigine (lamictal), Fluoxetine (prozac), Citalopram (cipramil), Venlafaxine (effexor), Duloxetine (cymbalta), Paroxetine (seroxat), and Fluvoxamine (fevarin). Market name is provided in brackets. Participants in the rMDD group who were currently using, had a history of using, or had never used antidepressant drugs thus formed three subgroups; active medication rMDD, previous medication rMDD, and medication naïve rMDD. Demographic and clinical data for these rMDD subgroups are found in table 2.
Written informed consent was obtained from all participants. All participants included in the study were required to speak Norwegian. Inclusion criteria for the patient group involved meeting the diagnostic criteria for previously diagnosed depression or having had at least one episode of depression if participants were comorbid or diagnosed with another mood disorder. Inclusion criteria for the control group required participants to have no history of mental health issues. The exclusion criteria for both patient and control group subjects were as follows: neurological disorders, abnormal structures of the brain such as unusually large ventricles (as determined by radiologists consulted at Rikshospitalet), contraindications for MRI, medical disorders that are known to affect central nervous system structure or function, and being pregnant at the agreed time of scanning. Patients with comorbidity status were not excluded from the final sample. All scans were manually checked for movement artifacts or noise.

2.2. Ethics

Potential participants were provided with written information and were informed the study was investigating attention and its influence on mood over time in relation to depressive symptoms (Appendix 1). All participants provided oral and written informed consent (Appendix 2) prior to taking part in the study. All participants were given code numbers instead of the use of names for anonymity purposes. Protection of participant data was ensured and no individuals other than those in the Clinical Neuroscience Research Group had access to participant information. The study was approved by the Regional Committee for Medical and Health Research Ethics (project number 2014/17), and was conducted in accordance with ethical standards specified in the 1964 Declaration...
of Helsinki. The main project was funded by the Research Council of Norway, whilst the MRI data collection was financed by Health South-East and the Department of Psychology.

2.3. Procedure

The study was carried out in two separate locations; the interview stage was carried out at the Department of Psychology, University of Oslo (Forskningsveien 3A), whilst the MRI acquisition was carried out at the Oslo University hospital (Rikshospitalet). During the interview stage, participants were assessed to determine their inclusion in the study using the M.I.N.I International Neuropsychiatric Interview (MINI 6.0) in line with DSM-IV and the ‘International Classification of Diseases’ (ICD-10) psychiatric disorders. During the MRI stage of the study, participants were asked to fill out a safety screening form regarding the presence of metal/implants in the body in order to identify anything that may prohibit them from inclusion in this part of the study. Following safety regulations, participants were scanned for an average of fifty minutes. This scanning time did not vary across groups. In case of the need to withdraw, participants were provided with an emergency button in addition to being told they could communicate this orally via an intercom system.

2.4. MRI Acquisition and Processing

MRI data were collected using a 32-channel head coil on a 3 T Philips Ingenia scanner (Philips Healthcare) at Rikshospitalet, Oslo University Hospital. The pulse sequence used for morphometric analyses was a T1-weighted 3D turbo field echo (TFE) scan with the following parameters: repetition time (TR) = 3000 ms, echo time (TE) = 3.61 ms, flip angle = 8°; acquisition duration = 3 min 16 s. Each volume consisted of 184 transverse slices with voxel sizes of 1 x 1 x 1 mm. Raw datasets were transferred to Linux workstations for processing and analysis at the fMRI-lab, University of Oslo. Each patient scan was visually inspected and only scans deemed to have no brain abnormalities and minimal movement artifacts were included in the analyses. Based on this inspection and in line with the exclusion criteria of the study, four participants were removed from the sample prior to the reporting of the final sample size. Prior to exclusion, two of these participants would have been in the rMDD group whilst the other two would have been in the healthy control group.
2.5. Volumetric Analysis

Image processing was performed on the Abel Cluster Supercomputer, owned by the University of Oslo and the Norwegian metacentre for High Performance Computing (NOTUR), and operated by the Department for Research Computing at USIT, the University of Oslo IT-department (http://www.hpc.uio.no/). The high-resolution T1-weighted MRI data were preprocessed using FreeSurfer 5.3 and a fully automated reconstruction of the T1-weighted input; optimised using FLAIR (http://surfer.nmr.mgh.harvard.edu/). This pipeline includes motion correction, removal of non-brain tissue, automated Talairach transformation, segmentation of subcortical volumetric structures, intensity normalisation, tessellation of surfaces, automated topology correction, and surface deformation to optimally place tissue borders (see https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all for complete pipeline).

The process of hippocampal subfield segmentation involved use of the new developer version of FreeSurfer 6.0 that generates an automated segmentation of the hippocampal subfields using bayesian inference based on a statistical atlas built primarily upon ultra-high resolution (~0.1 mm isotropic) ex vivo MRI data. Twelve subfields in each hemisphere are calculated (CA1, subiculum, hippocampal tail, fimbria, CA2/3, presubiculum, GC_DG, CA4, hippocampal fissure, ML, parasubiculum, and HATA). For the purpose of the current study, nine of these subfields (CA1, subiculum, hippocampal tail, CA2/3, presubiculum, GC_DG, CA4, ML, and parasubiculum) were used for volumetric analysis. Figures 2, 3, and 4 demonstrate the output of a hippocampal segmentation conducted on one of the participants.
Figure 2. Sagittal view of the brain and the hippocampus: showing the location of the left (1) and right (2) hemisphere of the hippocampus and its subregions.

Figure 3. Coronal view of the brain and the hippocampus: moving from anterior to posterior (1-4).

Figure 4. Axial view of the brain and the hippocampus: moving from ventral to dorsal (1-4).
2.6. Statistical Analysis

2.6.1. Preliminary Analysis

All statistical analyses were performed using IBM SPSS Statistics version 24. Demographic data were examined using descriptive statistics, independent samples t-tests, and a chi-square to demonstrate that there were no significant differences between the rMDD group and the healthy controls in terms of age, sex, and education (table 1). This was repeated for rMDD medication status groups against healthy controls (table 2). Preliminary analyses were executed by means of a repeated measures General Linear Model (GLM) in order to test for effects of age, ICV, hemisphere, and sex on hippocampal subfield volumes. Correction for total intracranial volume (ICV) was carried out in order to control for structure scaling with general head size (Malone et al., 2015). FreeSurfer determines this estimated ICV using an atlas scaling factor (Buckner et al., 2004). All continuous variables were normally distributed. Power estimations were performed with G*power 3.1.

2.6.2. Demographic and Clinical Data

In terms of differences between the rMDD group and healthy controls, an independent samples t-test revealed that on average, participants in the rMDD group were slightly younger ($M = 39.59, SE = 0.98$), than those in the healthy control group ($M = 41.56, SE = 1.47$). This difference, -1.97, BCa 95% CI [-5.51, 1.58], was not significant $t(266) = -1.09, p = .28, d = 0.15$. On average, participants in the rMDD group had fewer years of education ($M = 5.86, SE = 0.09$), than those in the healthy control group ($M = 6.14, SE = 0.11$). This difference, -0.28, BCa 95% CI [-0.597, 0.039], was not significant $t(266) = -1.73, p = .09; d = 0.25$. A chi-square test of goodness-of-fit was performed to ensure gender split was not significantly different in the rMDD group compared to healthy controls. Gender was found to be equally distributed in the population, $\chi^2 (1, N = 268) = .295, p = .59, \phi = 0.03$ (see table 1 for summary). This procedure was repeated for each medication status subgroup (active, previous, naïve) compared to healthy controls for sex, age, and education (results are summarised in table 2).
2.6.3. Analysis on the Effect of Diagnosis

A multivariate analysis of covariance (MANCOVA) was carried out in order to test the first hypothesis; exploring differences between the rMDD group and healthy controls. Diagnosis group (rMDD and healthy control) was set as a between-subjects factor, acting as the independent variable, with hippocampal subfield volumes entered in as dependent variables. To account for inter-individual variability in head size, ICV was entered as a covariate in addition to age, but not sex, as preliminary analysis revealed no effect of gender. This is consistent across all statistical analysis performed on the data. To localise potential differences between groups, follow-up univariate analysis of covariance (ANCOVAs) were carried out for each dependent variable, with age and ICV as covariates.

2.6.4. Analysis on the Effect of Medication

Three separate MANCOVAs were carried out to test the second, third, and fourth hypothesis. Revised briefly, these hypotheses predict: that there will be no volumetric difference between the active-medication rMDD group and the control group, that there will be no volumetric difference between the previous medication rMDD group and the control group, and finally, that there will be a difference between the medication-naïve rMDD group and the control group. Hippocampal subfield volumes were included as dependent variables, with age and ICV as covariates. Medication status (active-user, previous-user, naïve) was set as a between-subjects factor and formed the independent variable, whereby each MANCOVA tested a medication status subgroup against the control group separately using the select cases function in SPSS. Follow-up ANCOVAs were carried out and reported for MANCOVAs reporting significant differences only. MANCOVAs not obtaining significance did not warrant follow-up ANCOVAs. Hippocampal subfield volumes were entered as the dependent variables, with age and ICV as covariates. Medication status (active-user, previous-user, naïve) was set as a between-subjects factor and formed the independent variable.

2.6.5. Statistical Significance Threshold

The statistical significance threshold was set at $p < 0.05$. Correction for multiple comparisons to control for potentially inflated type 1 error was not carried out due to the exploratory nature of the ANCOVAs reported; which were handled as follow-up analysis to explore specific effects across hippocampal subfields. Moreover, where MANCOVAs were statistically significant, this correction
was deemed unnecessary, and follow-up ANCOVAs were carried out to localise and describe where the previously found effects were seen. Where MANCOVAs were not significant, ANCOVAs were carried out exclusively as exploratory rather than follow-up, with representative tables available only as supplementary information in the appendices, and values not reported in the results. The logic for not correcting for multiple comparisons was preferred to the alternative method of correcting values by a factor of 18, which would have yielded a new threshold for significance set at \( p < .002 \), following a Bonferroni correction. Further justification is taken from previous research that has analysed data identically; and as this study aims to partly represent a replication and succession of previous research in the field, similar statistical analysis procedures are preferred. A final remark on this logic is that a Bonferroni correction requires variables to be orthogonal i.e. independent of each other (Perneger, 1998), and this assumption is violated in the current study; as the subfields are highly correlated and not independent. Additionally, given the associated increased rate of making a type II error (false negative), a Bonferroni overcorrects for type I error and thus the correction would be too conservative (Field, 2013). Given that type I errors cannot decrease without inflating type II errors, this issue essentially became a trade-off for the most statistically appropriate method of analysing the data, and thus no correction for multiple comparisons was carried out, and ANCOVAs following non-significant MANCOVAs were deemed inappropriate. Effect sizes were reported as partial eta-squared (\( \eta^2 \)), and described as specified by Cohen (1992).
3. Results

3.1. Preliminary Analysis

Preliminary investigation by means of a repeated measures General Linear Model (GLM) was carried out to test interaction effects of age, ICV, hemisphere, and sex on hippocampal subfields. Mauchly’s test indicated that the assumptions of sphericity had been violated $\chi^2(35) = 1537.06, p = .000$, therefore Greenhouse-Geisser corrected tests are reported ($\epsilon = .52$). The results show that hippocampal subfield volumes were significantly affected by age, $(F(4.18, 1098.92) = 7.08, p = .000, \eta^2_p = .026)$, and ICV, $(F(4.18, 1098.92) = 23.57, p = .000, \eta^2_p = .082)$ but not by sex, $(F(4.18, 1098.92) = .87, p = .48, \eta^2_p = .003)$ or hemisphere, $(F(4.46, 1172.18) = 2.03, p = .08, \eta^2_p = .008)$. As such, only age and ICV are used as covariates in the forthcoming analysis.

3.2. Hippocampal Subfield Volumes: rMDD vs Healthy Controls

A MANCOVA was carried out to test the effect of diagnosis on the hippocampal subfield volumes, with age and ICV as covariates. Using Pillai’s trace, a MANCOVA investigating the first hypothesis revealed a significant main effect of diagnosis on hippocampal subfield volumes, $(V = 0.12, F(18, 247) = 1.90, p = .008, \eta^2_p = 0.12)$, with smaller hippocampal subfield volumes present in the rMDD group compared to healthy controls. Follow-up ANCOVAs investigating the effect of diagnosis (rMDD vs control subjects) on each individual hippocampal subfield, with age and ICV as covariates were carried out (see table 3). ANCOVAs on the outcome variables revealed significant effects localised to the left hemisphere in the presubiculum, $(F(1, 266) = 3.44, p = .033, \eta^2_p = .013)$, parasubiculum, $(F(1, 266) = 4.02, p = .023, \eta^2_p = .015)$, molecular layer, $(F(1, 266) = 4.09, p = .022, \eta^2_p = .015)$, granule cells of the dentate gyrus, $(F(1, 266) = 3.09, p = .040, \eta^2_p = .012)$, and CA4, $(F(1, 266) = 2.80, p = .048, \eta^2_p = .010)$. Significant effects localised to the right hemisphere were present in the presubiculum, $(F(1, 266) = 3.46, p = .032, \eta^2_p = .013)$, and molecular layer, $(F(1, 266) = 2.79, p = .048, \eta^2_p = .010)$. All reported subfields were smaller in the rMDD group compared to healthy controls, except for the presubiculum bilaterally. There were no main effects of diagnosis on the remaining hippocampal subfields. Effect sizes can be interpreted as small for ANCOVAs and large for the MANCOVA. Results are summarised in table 3.
Table 3. Mean Hippocampal Volume (mm³) in rMDD Patients and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>rMDD $(n = 191)$</th>
<th>Healthy Control $(n = 77)$</th>
<th>Diagnosis $F(1,266)$</th>
<th>$P$</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>549.71 ± 66.21</td>
<td>544.36 ± 54.38</td>
<td>.319</td>
<td>.286</td>
<td>.001</td>
</tr>
<tr>
<td>Subiculum</td>
<td>449.63 ± 56.18</td>
<td>450.28 ± 49.41</td>
<td>.163</td>
<td>.343</td>
<td>.001</td>
</tr>
<tr>
<td>CA1</td>
<td>680.80 ± 83.94</td>
<td>684.61 ± 67.98</td>
<td>.566</td>
<td>.227</td>
<td>.002</td>
</tr>
<tr>
<td>PreSubiculum</td>
<td>336.81 ± 41.40</td>
<td>326.45 ± 39.00</td>
<td>3.44</td>
<td>.033</td>
<td>.013</td>
</tr>
<tr>
<td>ParaSubiculum</td>
<td>69.59 ± 11.77</td>
<td>71.85 ± 9.64</td>
<td>4.02</td>
<td>.023</td>
<td>.015</td>
</tr>
<tr>
<td>Molecular Layer</td>
<td>549.83 ± 57.74</td>
<td>562.68 ± 61.19</td>
<td>4.09</td>
<td>.022</td>
<td>.015</td>
</tr>
<tr>
<td>GC_DG</td>
<td>329.34 ± 40.11</td>
<td>335.29 ± 33.40</td>
<td>3.09</td>
<td>.040</td>
<td>.012</td>
</tr>
<tr>
<td>CA2/3</td>
<td>247.20 ± 34.00</td>
<td>246.97 ± 36.13</td>
<td>.000</td>
<td>.494</td>
<td>.000</td>
</tr>
<tr>
<td>CA4</td>
<td>270.96 ± 33.08</td>
<td>276.01 ± 27.46</td>
<td>2.80</td>
<td>.048</td>
<td>.010</td>
</tr>
<tr>
<td><strong>Right Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>555.09 ± 74.04</td>
<td>549.84 ± 62.06</td>
<td>.336</td>
<td>.282</td>
<td>.001</td>
</tr>
<tr>
<td>Subiculum</td>
<td>445.83 ± 57.15</td>
<td>444.79 ± 46.96</td>
<td>.001</td>
<td>.485</td>
<td>.000</td>
</tr>
<tr>
<td>CA1</td>
<td>711.55 ± 88.60</td>
<td>704.57 ± 81.41</td>
<td>.195</td>
<td>.330</td>
<td>.001</td>
</tr>
<tr>
<td>PreSubiculum</td>
<td>318.04 ± 39.11</td>
<td>308.76 ± 35.90</td>
<td>3.46</td>
<td>.032</td>
<td>.013</td>
</tr>
<tr>
<td>ParaSubiculum</td>
<td>67.15 ± 11.28</td>
<td>68.59 ± 9.60</td>
<td>1.55</td>
<td>.108</td>
<td>.006</td>
</tr>
<tr>
<td>Molecular Layer</td>
<td>564.99 ± 63.69</td>
<td>575.67 ± 56.85</td>
<td>2.79</td>
<td>.048</td>
<td>.010</td>
</tr>
<tr>
<td>GC_DG</td>
<td>344.65 ± 40.22</td>
<td>347.58 ± 38.97</td>
<td>1.13</td>
<td>.145</td>
<td>.004</td>
</tr>
<tr>
<td>CA2/3</td>
<td>272.29 ± 33.47</td>
<td>269.11 ± 32.81</td>
<td>.525</td>
<td>.235</td>
<td>.002</td>
</tr>
<tr>
<td>CA4</td>
<td>287.99 ± 32.83</td>
<td>290.62 ± 31.24</td>
<td>1.06</td>
<td>.152</td>
<td>.004</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. $\eta^2$ = Partial eta squared. n = number of participants. Nominaly significant values ($p < .05$) in bold.
3.3. Medication Status on Hippocampal Subfield Volumes

3.3.1. Active Medication vs Healthy Control

A MANCOVA was carried out to test the effect of active medication use on hippocampal subfield volumes, with age and ICV as covariates. Using Pillai’s trace, a MANCOVA revealed no statistically significant effect of medication on hippocampal subfield volumes, \((V = .192, F(18, 119) = 1.57, p = .078, \eta^2_p = 0.19)\). In other words, hippocampal subfield volumes in the rMDD active medication group were similar to that of the control group. Exploratory ANCOVAs exploring the effect of active medication use across each individual hippocampal subfield are provided as supplementary information (Appendix 3).

3.3.2. Previous Medication vs Healthy Control

A MANCOVA was carried out to test the effect of previous medication use on hippocampal subfield volumes, with age and ICV as covariates. Using Pillai’s trace, a MANCOVA revealed a significant effect of previous medication use on hippocampal subfield volumes, \((V = .233, F(18, 112) = 1.89, p = .024, \eta^2_p = 0.23)\), with previously medicated rMDD subjects possessing, on average, larger hippocampal subfield volumes than healthy controls. Follow-up ANCOVAs were carried out to localise the effect of previous medication use across each individual hippocampal subfield. ANCOVAs on the outcome variables revealed no statistically significant effects across any of the subfields. However, trend level effects were present in the left presubiculum, \((F(1, 112) = 3.40, p = .067, \eta^2_p = .026)\), right presubiculum, \((F(1, 112) = 3.51, p = .063, \eta^2_p = .026)\), left CA4, \((F(1, 112) = 3.08, p = .082, \eta^2_p = .023)\), and right CA2/3, \((F(1, 112) = 3.34, p = .070, \eta^2_p = .025)\), with smaller hippocampal subfield volumes present in the control group, bar the left CA4. Effect size can be interpreted as large for the MANCOVA. Results are summarised in table 4.

3.3.3. Medication Naïve vs Healthy Control

A MANCOVA was carried out to test the effect of medication naïvety on hippocampal subfield volumes, with age and ICV as covariates. Using Pillai’s trace, a MANCOVA yielded no statistically significant effect of medication naïvety on hippocampal subfield volumes, \((V = .168, F(18, 128) = 1.43, p = .064, \eta^2_p = 0.17)\). Exploratory ANCOVAs exploring the effect of medication naïvety across each hippocampal subfield are provided as supplementary information (Appendix 4).
Table 4. Mean Hippocampal Volume (mm$^3$) in Previous Medication Users and Healthy Controls

<table>
<thead>
<tr>
<th>Subfield</th>
<th>Previous Users $(n = 56)$</th>
<th>Healthy Control $(n = 77)$</th>
<th>$F(1,129)$</th>
<th>$P$</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>548.80 ± 66.83</td>
<td>544.36 ± 54.38</td>
<td>.012</td>
<td>.914</td>
<td>.000</td>
</tr>
<tr>
<td>Subiculum</td>
<td>458.37 ± 58.59</td>
<td>450.28 ± 49.41</td>
<td>.103</td>
<td>.748</td>
<td>.001</td>
</tr>
<tr>
<td>CA1</td>
<td>687.07 ± 81.78</td>
<td>684.61 ± 67.98</td>
<td>.263</td>
<td>.609</td>
<td>.002</td>
</tr>
<tr>
<td>PreSubiculum</td>
<td>342.64 ± 45.65</td>
<td>326.45 ± 39.00</td>
<td>3.40</td>
<td>.067</td>
<td>.026</td>
</tr>
<tr>
<td>ParaSubiculum</td>
<td>70.75 ± 11.21</td>
<td>71.85 ± 9.64</td>
<td>1.59</td>
<td>.210</td>
<td>.012</td>
</tr>
<tr>
<td>Molecular Layer</td>
<td>556.35 ± 61.68</td>
<td>562.68 ± 61.19</td>
<td>1.68</td>
<td>.198</td>
<td>.013</td>
</tr>
<tr>
<td>GC_DG</td>
<td>331.31 ± 41.65</td>
<td>335.29 ± 33.40</td>
<td>2.68</td>
<td>.104</td>
<td>.020</td>
</tr>
<tr>
<td>CA2/3</td>
<td>251.38 ± 36.34</td>
<td>246.97 ± 36.13</td>
<td>.070</td>
<td>.792</td>
<td>.001</td>
</tr>
<tr>
<td>CA4</td>
<td>271.76 ± 33.12</td>
<td>276.01 ± 27.46</td>
<td>3.08</td>
<td>.082</td>
<td>.023</td>
</tr>
<tr>
<td><strong>Right Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>561.17 ± 77.20</td>
<td>549.84 ± 62.06</td>
<td>.264</td>
<td>.609</td>
<td>.002</td>
</tr>
<tr>
<td>Subiculum</td>
<td>456.31 ± 59.23</td>
<td>444.79 ± 46.96</td>
<td>.618</td>
<td>.433</td>
<td>.005</td>
</tr>
<tr>
<td>CA1</td>
<td>729.66 ± 94.94</td>
<td>704.57 ± 81.41</td>
<td>1.46</td>
<td>.229</td>
<td>.011</td>
</tr>
<tr>
<td>PreSubiculum</td>
<td>323.51 ± 40.16</td>
<td>308.76 ± 35.90</td>
<td>3.51</td>
<td>.063</td>
<td>.026</td>
</tr>
<tr>
<td>ParaSubiculum</td>
<td>68.50 ± 11.17</td>
<td>68.59 ± 9.60</td>
<td>.283</td>
<td>.596</td>
<td>.002</td>
</tr>
<tr>
<td>Molecular Layer</td>
<td>577.47 ± 62.66</td>
<td>575.67 ± 56.85</td>
<td>.164</td>
<td>.687</td>
<td>.001</td>
</tr>
<tr>
<td>GC_DG</td>
<td>353.96 ± 43.49</td>
<td>347.58 ± 38.97</td>
<td>.045</td>
<td>.832</td>
<td>.000</td>
</tr>
<tr>
<td>CA2/3</td>
<td>281.63 ± 35.00</td>
<td>269.11 ± 32.81</td>
<td>3.34</td>
<td>.070</td>
<td>.025</td>
</tr>
<tr>
<td>CA4</td>
<td>295.38 ± 34.95</td>
<td>290.62 ± 31.24</td>
<td>.040</td>
<td>.842</td>
<td>.000</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. $\eta^2$ = Partial eta squared. $n$ = number of participants. Nominally significant values ($p < .05$) in bold.
Figure 5. Hippocampal subfield volumes in active medication, previous medication, medication naïve and healthy control subjects, respectively. Tail, hippocampal tail; Sub, subiculum; CA, cornu ammonis 1-4; Pre, presubiculum; Para, parasubiculum; ML, molecular layer; GC DG, granule cells of the dentate gyrus.
4. Discussion

4.1. Main findings

The current hippocampal subfield analysis was the first using the novel segmentation tool to investigate hippocampal subfield volume differences in rMDD subjects and healthy controls. Moreover, it was the first to investigate the effect of antidepressant drugs on hippocampal subfield volumes in rMDD subjects. The current study replicated findings of hippocampal volume reduction in an rMDD group. By means of a MANCOVA, the results of the study revealed a significant main effect of diagnosis on hippocampal subfield volumes. Furthermore, observable differences were followed up at a subregional level with the aim of localising potential differences in volume to specific subfields of the hippocampus. Smaller subfield volumes were found in the parasubiculum, ML, and GC_DG, localised to the left hemisphere, and ML, localised to the right hemisphere, with the direction of the results revealing smaller subfield volumes in the rMDD group compared to healthy controls. However, the presubiculum was larger in the rMDD group in both hemispheres. Findings of the effect of diagnosis are in line with the first hypothesis of the study, predicting hippocampal subfield volume reduction in the rMDD group. Furthermore, predictions made regarding the localisation of these differences is supported, with smaller hippocampal subfield volume found in the ML bilaterally, and GC_DG and CA4 of the left hemisphere.

In terms of the effect of medication, beginning with active medication use, the findings of the study revealed no significant hippocampal subfield volume differences between active medication rMDD subjects and healthy controls by means of a MANCOVA. The findings are in line with the second hypothesis of the study, which predicted no differences between the active medication users and healthy controls due to a potential neurotrophic factor involved in protecting specific subfields of the hippocampus and/or aiding the process of neurogenesis. More interestingly, observations of the means revealed active medication users had larger hippocampal subfield volume than control subjects in ten of the subfields (Appendix 3). This is also visible in figure 5. Interpretation of the data suggests antidepressant drugs have an observable effect on hippocampal subfield volume.

The current study also hypothesised no difference in hippocampal volume when comparing previous medication users and healthy controls. However, results of the MANCOVA revealed a statistically significant difference in hippocampal subfield volume, and thus the third hypothesis was rejected. Although the results failed to support the hypothesis, examination of the unexpected difference between the groups revealed that the overall increase in volume was in fact present in the
previously medicated rMDD subgroup; not the control group. Moreover, no subfield revealed statistically significant differences between groups with follow-up ANCOVAs, supporting the direction of the hypothesis. The implications of this may suggest the role of antidepressants as a neurogenesis promoting factor in the hippocampus that has lead to volume enhancements beyond the average measure of healthy control subjects. Thus, although the hypothesis is rejected, the direction of the data supports the predictions made regarding the potential neurotrophic effect of antidepressant drugs. Effect sizes can be interpreted as large for the MANCOVA.

Lastly, medication naïve subjects were compared with healthy controls. The results showed no significant effect of medication naïvety on hippocampal subfield volume by means of a MANCOVA. Exploratory ANCOVAs however, revealed significant differences in the GC_DG and CA4, localised to the left hemisphere, with trend level effects present in the left subiculum and ML, and right parasubiculum, ML, GC_DG, and CA4 (Appendix 4). The subfields were all observed as smaller in the medication naïve rMDD group compared to healthy controls. The results indicate that although no main effect of medication naïvety on hippocampal subfield volumes exist, effects of medication naïvety may exist on a subregional level, but as the ANCOVAs are exploratory, interpretations should be made with caution. Nevertheless, the fourth hypothesis was rejected, as previous predictions anticipated the medication naïve rMDD group would have significantly smaller hippocampal subfield volumes than healthy controls. However, despite this, it is important to note it was hypothesised that the largest differences would be localised to the hippocampal formation, and the findings of the exploratory ANCOVAs support this with smaller hippocampal subfield volume reported in the GC_DG and CA4.

Observation of the differences in means (Appendix 4/figure 5) reveal that all subfields except the left HC tail and presubiculum, localised to both hemispheres, were smaller in volume in the medication naïve rMDD group compared to healthy controls. This supports the direction of the prediction the study made with regard to the potential effect of depression on hippocampal subfields. However, as this group was unprotected from the effects of medication and expected to be the most vulnerable, larger differences were expected. The findings may be explained by a possible recovery in volume that emerged from the varying remission periods subjects underwent.

4.2. Hippocampal Subfield Volume

The current study joins a sizeable sector of research that has reported differences in individuals with a history of depression and healthy controls (Bijanki et al., 2014; Bremner et al., 2000; Frodl et al.,
A closer look at the results reveals that the left parasubiculum, ML, GC_DG, CA4, and right ML were all smaller in volume in the rMDD group compared to healthy controls. The only subfield larger in the control group was present in the presubiculum bilaterally. Previous high-field MRI studies of hippocampal subfields (Malykhin & Coupland, 2015) have commonly localised differences between MDD and control subjects to the dentate gyrus (Cao et al., 2017; Huang et al., 2013; Travis et al., 2016), supporting current findings of lower volume found in the GC_DG and CA4, subregions that are directly associated with the dentate gyrus. This is consistent with volume reduction in the dentate gyrus reported by Ballmaier et al., (2008) and Treadway et al., (2015).

The current study also highlights the molecular layer subfield as smaller in both hemispheres but as this structure is one that is new to automated segmentations of the hippocampus, and any comparison to previous research should be drawn with caution, particularly when it is noted as a label consisting of two parts; the subiculum and CA fields (Iglesias et al., 2015). Further research is necessary to deem this subfield a region of interest in association to depression. Apart from the ML association to CA fields, the CA1-3 subfields revealed no differences between groups, a finding inconsistent with many studies that have found reductions in the hippocampus most commonly localised to these regions (Ballmaier et al., 2008; Cole et al., 2010; Huang et al., 2013; Malykhin & Coupland, 2015). These findings may be explained by a population in remission of depression or the fact that 119 out of the 191 subjects in the rMDD sample were either currently taking antidepressants or have a history of previous medication use. A history of antidepressant use has been demonstrated to have a substantial impact on increase in hippocampal subfield volumes, both in previous research (Huang et al., 2013; Philips et al., 2015) and potentially in the current study. If this is the case, it may have acted as a confounding variable that could explain why no differences were found in the CA1-3 subfields or why larger differences were not found in general.

4.3. Medication Status

Previous research investigating medication naïve MDD groups reveal reductions in volume localised to the CA1-4 and dentate gyrus (Choi et al., 2017; Han et al., 2016; Huang et al., 2013) compared with healthy subjects. Han et al. (2016) also localised reductions to the subiculum bilaterally, with further research reporting reductions in total hippocampal volume (Saylam et al., 2002; Han et al., 2016; Huang et al., 2013; Janssen et al., 2007; Lange & Irle, 2004; MacMaster & Kusumaker, 2004; MacQueen et al., 2003; Malykhin et al., 2010; Saylam et al., 2006).
2006; Choi et al., 2017) in medication naïve subjects. Cole et al. (2010) reported deformations localised to the subiculum, CA1, and CA2/3 subregions present in medication naïve MDD patients. With numerous subfields being implicated across both hemispheres of the hippocampus, it may not be a surprising finding that the current study observed all medication naïve subfields, bar the parasubiculum, to be smaller in volume in comparison to their active medication counterparts. Medication naïve MDD groups may be more vulnerable to volume loss than those with a history of medication use, and this vulnerability seems to be more localised to the hippocampal formation; specifically the CA fields and dentate gyrus. This is also evident in the current study, however, the ANCOVAs supporting this were exploratory in nature. Additionally, the MANCOVA demonstrated no significant difference between the medication naïve rMDD group and healthy controls, so any interpretations made remain speculative. A possible explanation for the MANCOVA yielding no significance may be down to a large majority of the individuals included in the experimental group being remitted patients of MDD as opposed to currently depressed ones. The implication of this is that a period of remission may have taken place, aiding hippocampal recovery in previously depressed individuals. This is supported by previous research that has observed an increase in hippocampal volume over time in remitted patients and a decrease in volume in non-remitters (Phillips et al., 2015).

The major novel finding emerged from a MANCOVA that revealed previous medication users as having significantly larger hippocampal subfield volumes than that of healthy control subjects. Observation of the means (table 4/figure 5) revealed that 13 out of the 18 subfields were larger volume in the previously medicated rMDD group compared to healthy controls. The follow-up ANCOVAs revealed no subfield as statistically different between previous medication users and healthy controls, supporting the direction of the predictions made regarding the third hypothesis. Findings from the medication active group revealed 10 out of 18 subfields as possessing larger subfield volume compared to healthy controls. The collective data reveals very limited subfield volume reduction in both previous and active medication users compared to healthy controls, and this is supported by previous research. Huang et al. (2013) found both the subfield and posterior hippocampal volume reductions were limited to unmedicated MDD subjects; they were not present in MDD subjects on long-term antidepressants. Malykhin et al. (2010) reported medicated MDD patients as showing increased hippocampal body volume compared with both healthy controls and unmedicated MDD patients, a finding consistent with the findings of the current study.

Of course opposing findings exist, as Vythilingam et al. (2004) reported antidepressant drugs did not change hippocampal volume. This is in contrast to cases where the hippocampus has
increased in volume or seen a reversal, albeit partial, in tissue loss. Bremner (2002), for example, found that post traumatic stress disorder (PTSD) patients had increased hippocampal volume following a year of treatment. Yucel et al. (2007) found that long term lithium treatment increased the volume of the hippocampus in those with bipolar disorder. The results provoke an important question with regard to why there is a significant difference between previous users and healthy controls but not between active medication users and healthy controls. One possible explanation for this is the clinical time delay involved (Sairanen, Lucas, Ernfors, Castrén, & Castrén, 2005), which may take antidepressant drugs weeks before positive effects are experienced by MDD patients (van Praag et al., 2002). In such a case, currently active medication users may be experiencing the neuroprotective effect of antidepressants but they are yet to reach the potential next stage of neurogenesis flourishing as a continuation of the former stage, which previous antidepressant users may have had the advantage of reaching due to previous long term exposure to antidepressants. This interpretation is speculative and future research is needed to investigate active vs previous medication groups, tracking duration of treatment and changes in hippocampal subfield volume over time.

If research is to be conclusive about the effect of antidepressants on hippocampal subfields, then better efforts have to be made to understand what this effect entails. Research demonstrating no differences between medicated patient groups and healthy controls suggest antidepressants either act as a neuroprotective agent or that there has been a recovery in volume following previous volume loss in the hippocampus. Due to the cross-sectional design of the study, the findings can only imply protective effects (Huang et al., 2013), not causality. Thus, more conclusive results require longitudinal studies that compare pre- and post treatment measures to determine whether a neuroprotective argument is appropriate or whether the observed effect of antidepressants may be better explained by the process of neurogenesis, or a combination of both, as previously speculated. The difference between previous and active medication users may suggest neurogenesis is the more likely explanation, with 14 out of 18 subfields being larger in previous users (figure 5). The new born neurons that migrate into the granule cell layer follows a mossy fibre pathway toward the CA3 region (Bartsch & Wulff, 2015) before they can contribute to the function of the hippocampus (Sairanen et al., 2005). This not only supports a neurogenic approach, but is also in line with the time delay of clinical effects of antidepressants (Sairanen et al., 2005) as the process may take weeks (van Praag et al., 2002).
4.4. The Neurogenic Hypothesis of Depression

The volumetric decrease observed in the medication naïve group is particularly interesting given the increase in volume present in subjects with current or previous use of antidepressants. These observations in the data go beyond the neuroprotective theory and are more consistent with research that has demonstrated hippocampal volume loss as reversible, particularly in the remission phase of depression (Frodl et al., 2002). Most intriguing are the volumetric differences observed in this study regarding the GC_DG and CA4, as these findings are consistent with previous research using the FS6.0 segmentation approach (Cao et al., 2017) as well as the neurogenic hypothesis of depression (Kempermann & Kronenberg, 2003), whereby neurogenesis is restricted to granular zones of the dentate gyrus (Sapolsky, 2000; Sapolsky, 2001). The neurogenic hypothesis can be understood in two parts; chronic stress directly suppressing generation of new neurons (Czéh et al., 2001; Czéh et al., 2002; Pham et al., 2003) and treatment for depression enhancing hippocampal neurogenesis (Kempermann & Kronenberg, 2003) through stimulation of neural progenitor cell numbers in the dentate gyrus. This has been demonstrated postmortem using antidepressants as a form of treatment (Boldrini et al., 2009). If stimulation of hippocampal progenitor cells through antidepressant treatment (Sairanen et al., 2005) is characteristic of early stages of hippocampal neurogenesis (Kempermann & Kronenberg, 2003), then the volumetric changes observed in the current study may be characteristic of later stages of neurogenesis, although this is speculative without a measure of the rate of neurogenesis.

Previous research in the field validate interpretations made regarding the findings of the current study, with postmortem research demonstrating low levels of cell death found in the CA4 and dentate gyrus (Lucassen et al., 2001) and a wide range of studies reporting the granule cell layer and dentate gyrus as regions of interest regarding reduced granule cell neurogenesis, hippocampal shrinkage, and synaptic plasticity suppression (Alfarez et al., 2003; Joëls et al., 2004; Li et al., 2017; Mirescu et al., 2004; Pham et al., 2003). However, cell death is also reported in the subiculum, CA1 (Lucassen et al., 2001), and CA3 subregion in rats (Czéh & Lucassen, 2007), findings that are not in line with the data of the medication naïve rMDD group. Nonetheless, these subfields (CA1, CA2/3, subiculum) are all smaller in the medication naïve rMDD group compared to the active rMDD, previous rMDD, and control groups; across both hemispheres. The volume reduction trends in the data across the medication naïve group potentially support the first stages of both the neurogenic and neurotrophic hypotheses of depression, whereby dysregulation of cortisol released by the HPA axis, and subsequent reduction in growth factors, such as Brain Derived
Neurotrophic Factor (BDNF) lead to volume reduction in the hippocampus (MacQueen et al., 2003), however, cortisol measures are needed to validate this claim.

Similarly, both hypotheses also rely on recovery of the hippocampus and remission of MDD subjects as dependent upon the reversal of volume loss and neurogenic suppression. The current study provides evidence for this with larger subfield volume measures reported across the subfields of interest when comparing current and previous medication users to both medication naïve subjects and healthy controls. This demonstrates a neurotrophic effect that is also present in a wealth of previous work, where antidepressants have been shown to prevent cell death (Huang et al., 2007; Lee et al., 2001), decrease apoptosis in tree shrews localised to the dentate gyrus (Czéh et al., 2001; Lucassen et al., 2004), induce neuronal sprouting and neurogenesis (Young, Bakish, & Beaulieu, 2002), increase cell proliferation (Perera et al., 2007), and increase BDNF in the dentate gyrus and CA3 subregions (MacQueen et al., 2003). However, despite the clear trends in the data, the interpretations made are limited because a cross-sectional design cannot conclude differences between groups are directly associated with the effect of antidepressant drugs, nor can the study conclude the difference in volume localised to specific subfields such as the GC_DG and CA4 provide direct evidence of antidepressants promoting neurogenesis, leading to hippocampal volume recovery. Longitudinal studies tracking the effect of antidepressant treatment prior to and post depressive episodes are needed in future research. Tracking volumetric changes over time within subjects may help evaluate the rate of neurotrophic effects. Another factor that may explain differences in volume is individual differences in resilience. Those that take antidepressants may be more resilient than those that do not take antidepressants.

4.5. Matters of Contention

The interpretations made from the results of the study, specifically the ANCOVAs, should be made with caution. The current study classed ANCOVAs carried out following statistically significant MANCOVAs as follow-up analyses, carried out for the purpose of describing and localising significant differences between groups. However, ANCOVAs were also carried out for exploratory purposes for hypothesis 2 and 4, following no significant differences reported in the initial MANCOVAs. Justification for this comes in two parts. Firstly, although the second hypothesis investigating active medication users vs healthy controls reported no statistical significance, the results of the MANCOVA supported the hypothesis of the study, and exploratory ANCOVAs were thus deemed relevant for supplementary evidence. Secondly, the latest subfield
segmentation tool (FS 6.0) is a new method of analysis, and so exploratory ANCOVAs were deemed of interest even for MANCOVAs that did not attain a statistical significant result. Nevertheless, these ANVOCAs were not reported in the results, they were interpreted with caution, and the representative tables were moved to the appendices as supplementary information.

Caution should also be taken regarding any interpretations made from the findings of the study due to the numerous variables that have been implicated in previous research investigating hippocampal volume reduction in depression. In addition to focus on medication, variables such as episode frequency, symptom severity, age of onset, genetic risk, and comorbidity, have all demonstrated an influence on reported hippocampal volume, (Choi et al., 2017; Elbejjani et al., 2015; Elvsåshagen et al., 2016; Ota et al., 2017; Phillips et al., 2015; Zhou et al., 2016) making it difficult to form conclusive interpretations regarding direct causes of volume loss. Beginning with episode frequency, research shows that frequency of depressive episodes is correlated with reduction in volume, with studies reporting episodes as a predictor of decreased total brain volume (Posener et al., 2003), reduced volume in the hippocampus (MacQueen et al., 2003), and reduced volume in the left dentate gyrus-CA4 (Elvsåshagen et al., 2016). MacQueen et al. (2003) investigated both first and multiple episode MDD groups and found that hippocampal volume reduction was only present in the multiple-episode patient group. The implication of this is that research attributes volume loss as a product of having recurrent depression, which may suggest volume reduction is a scarring factor, as suggested by Chan et al. (2016), rather than a pre-existing vulnerability that makes you more susceptible to depression. Although, existing scar effects of previous episodes may constitute as vulnerability markers, increasing the risk of recurrence, but this is speculative.

Regarding symptom severity, research has demonstrated that more severe depressive symptoms at the time of scanning are correlated with a greater reported reduction in volume (Bernasconi et al., 2015), with higher self-reported depressive symptoms being associated with reduction in the volume of the hippocampus and parahippocampus (Zhou et al., 2016), and more depressive symptoms being associated with accelerated hippocampal volume loss (Elbejjani et al., 2015). Moreover, research has claimed that the reduction in volume and general atrophy to the hippocampus are not as present in remitted groups of depression (Caetano et al., 2004). Reduced hippocampal volume is associated with both early and late onset depression (Choi et al., 2017; Hickie et al., 2005; MacMaster & Kusumaker, 2004). Studies that have investigated volume deficit exclusively in early onset groups have found a smaller left hippocampal volume (up to 17%) in adolescent patients of depression (MacMaster & Kusumaker, 2004). However, the same study
reported that hippocampal volume negatively correlated with age of onset, in that volume reduces as age of onset increases. This relationship is heavily supported, with numerous studies reporting age of onset as being negatively related to hippocampal volume (Hickie et al., 2005; Lloyd et al., 2004; Sivakumar et al., 2015; Steffens et al., 2000), with localisation of the reduction in late onset groups being present in the subregions of the subiculum and CA1 subfields of the left hemisphere (Ballmaier et al., 2008), and CA1, CA3, and dentate gyrus (Choi et al., 2017).

Another factor to consider is genetic risk. Chen et al. (2010) reported findings on the volume of the hippocampus in a sample of females at risk of depression. The results of the study show that daughters of mothers with recurrent episodes of depression had significantly less gray matter density in the bilateral hippocampus. When compared to the low-risk group, this reduction in size was 6.3% smaller in the left hippocampus and 2.2% smaller in the right hippocampus. This is supported by Baaré et al. (2010) and Rao et al. (2010), who found a reduction in hippocampal volume in at-risk groups. The implication of these findings is that a reduction in the hippocampus may exist prior to the onset of depression, and as such, hippocampal volume reduction may serve as a marker of vulnerability to the disease instead of being a product of having depression (Baaré et al., 2010; Rao et al., 2010), a notion not supported by Chan et al. (2016) and MacQueen et al. (2003), who suggest that this reduction in volume is a neural marker and scar effect of depression.

The problem of comorbidity in relation to this study derives from the inclusion of participants that were comorbid. These were individuals that had been diagnosed with other mental health illnesses such as anxiety related disorders that were still included in the sample due to meeting the inclusion criteria of having a previously diagnosed episode of depression. This is a confounding variable as studies have demonstrated hippocampal volume reduction in patients of schizophrenia, bipolar disorder, post-traumatic-stress-disorder, and individuals with a history of childhood trauma (Cao et al., 2017; Gurvits et al., 1996; Heckers, 2001; Ota et al., 2017; Swayze et al., 1992; Vythilingam et al., 2002). Recently, individuals with bipolar disorder and MDD were compared to healthy controls by means of the new developer version of FS6.0. Cao et al. (2017) found significant differences between subfields of patients with bipolar disorder compared to controls but no significant difference between the MDD and the healthy control group.

These findings indicate that depression may not be directly linked to hippocampal volume loss, but rather, a commonality in a variety of mental disorders may be linked to the reduction in volume. One proposition is the associated dysregulation of the HPA axis and hyper secretion of cortisol that may contribute to hippocampal volume loss (Hinkelmann et al., 2009; Travis et al., 2016), which may explain why volume loss is observed across a variety of mental disorders. With
studies reporting the hippocampus as a whole shrinks after severe stress persists (Lucassen et al., 2006) and elevated glucocorticoids being shown to lead to neuronal death, specifically killing CA3 neurons in rats (Sapolsky, 2000), it is vital for future research to include cortisol measures when investigating hippocampal volume loss. Moreover, investigating the effect of other forms of treatment, such as cognitive behavioural therapy, or cognitive training tasks such as ABM (Browning, Holmes, Charles, Cowen, & Harmer, 2012) may help separate any effect these may have on hippocampal volume measurements.

4.6. Strengths and Limitations

There are several methodological aspects of the current study that need to be addressed. The study did not take note of the duration of antidepressant administration in active or previous medication users. Neither did it collect data with regard to when antidepressant treatment was stopped in the previous user rMDD group. Future research could control for this during the initial screening procedure, although this may be difficult to do as it may require getting detailed patient journals, and even then, these might not include the required detail of information. Furthermore, the specific antidepressants used were not taken into account for the analysis, having grouped all medication together on the account that they were SSRI’s/SNRI’s. However, the study did keep a record of the SSRI’s/SNRI’s that were taken, in addition to controlling for the exclusion of antipsychotics and other antidepressants that were not SSRIs/SNRIs (e.g., benzodiazapenes). Given that the subjects were on varying antidepressants, the results cannot rule out a potential medication effect.

Methodologically, MRI scanning predominantly occurred within a month of possible confounding protocols, carried out in line with the wider research project by the Clinical Neuroscience Research Group. Specifically, half the rMDD sample, or thereabout, received Attentional Bias Modification (ABM) training (Browning et al., 2012) for two weeks. Although unlikely, whether or not this has an effect on hippocampal subfield volume is unknown. In terms of the first research aim, investigating differences between the rMDD group and healthy controls, contrasts between groups at a subregional level may have been confounded by the use of medication and thus potential neurotrophic effects present in a substantial population of the patient sample. This was why it was important to test groups discriminated by medication status separately against the healthy control group rather than against each other.

With regard to statistical concerns, a major strength of the study came from the large sample size for tests investigating differences between the rMDD and control group, as this increases the
power to detect for differences. However, rMDD subgroups discriminated by medication status were under-sampled; not reaching their required sample size target for adequate power to detect associations in these subgroups, inflating type II (false negative) error rates. In terms of effect size, although multivariate tests revealed large effect sizes, the partial eta squared reported for each subfield was small for ANCOVA tests, suggesting the quantifiable effect of the difference between groups on a subfield level suffered from lack of power. It is important to note the mean age in the medication naïve rMDD group (36) compared to the other rMDD groups and the control group (40-42). The medication naïve rMDD group is the youngest, and reduced hippocampal volume may therefore not be as present or at least as prominent as expected due to younger individuals usually possessing larger hippocampi (Iglesias et al., 2015).

In terms of volumetric analysis and reproducibility, Whelan et al. (2016) noted FS6.0 segmentations as strong and highly reliable in eleven of the twelve subregions, with only the hippocampal fissure producing unreliable volume estimates. As such, the current study chose to exclude this subfield. Additionally, the fimbria was excluded due to it being white matter that extends to form the fornix, and the HATA was excluded due to it forming the medial and slightly dorsal border of the hippocampus (Iglesias et al., 2015), making it a transitional area to the amygdala. Although the remaining nine subfields for each hemisphere scored high in reliability, the current study was constrained for time and thus segmentations were performed on the developer version of the new automated segmentation tool released with FreeSurfer 6.0, prior to the official release in January 2017. Additionally, due to it being new, the FS6.0 segmentation tool is not a method that has been consistently used under the same conditions, so replication is key for reliability. Nevertheless, the use of a state-of-the-art segmentation approach is a major strength of the study, as well as the high-field MRI; providing higher quality spatial resolution than previous (1.5 Tesla) scanners not sufficient enough for hippocampal subfields in vivo (Malykhin & Coupland, 2015).

4.7. Future Directions and Conclusion

In conclusion, the current hippocampal subfield analysis was the first using the novel segmentation tool to investigate hippocampal subfield volume differences in rMDD subjects and healthy controls. Additionally, it was the first to investigate potential effects of antidepressant drugs on hippocampal subfield volumes in rMDD subjects. The findings demonstrate that differences between rMDD subjects and healthy controls exist on a multivariate level. An additional MANCOVA revealed
differences between previous medication rMDD subjects and healthy controls. Interpretation of the findings suggest that history of antidepressant administration neurotrophically enhances hippocampal subfield volume, potentially by reversing neurogenic suppression in the hippocampus. The exploratory follow-up ANCOVAs and trends in the data suggest this is a more plausible explanation than antidepressants acting as neuroprotective agents, given that patient groups revealed larger subfield volumes than even the controls in some subfields; although it may be the case that the two are interrelated and part of a continual process of hippocampal recovery. If the effect was solely neuroprotective, then the patient and control hippocampi are expected to be of similar size. Speculation regarding the potential cause of hippocampal subfield volume reduction propose volume loss is a result of decreased neurotrophic support and HPA axis dysregulation, leading to neurotoxic effects on hippocampal neurons and suppression of neurogenesis. This is in line with the neurogenic hypothesis of depression. One might thus speculate that recovery of hippocampal volume may be achieved by targeting neuronal growth factors such as BDNF to counter the inhibition of neurogenesis. Future research should investigate this claim.

The current study argues that although hippocampal volume reduction is seen as a trait characteristic of depression (Chan et al., 2016; Neumeister et al., 2005), volume loss is not directly caused by the disorder. However, hippocampal volume loss may increase risk of depression, and the recurrence and duration of the disorder may leave scarring effects (Chan et al., 2016) on the hippocampus. One clinical implication of the present study proposes earlier treatment may be an effective treatment strategy for prevention of hippocampal subfield volume reduction and subsequent memory/learning impairment that occurs in individuals with depression. The current study joins an early sector of research investigating hippocampal subfields, and so findings of the study should be used to formulate more specific hypotheses in subsequent research. As the design was cross-sectional, longitudinal neuroimaging studies are needed to determine any direct effects of antidepressant drugs on hippocampal subfields. Longitudinal studies are also needed to clear up the role of stress and dysregulation of the HPA axis. Tracking hippocampal subfield volume changes over time may elucidate this matter. Understanding the role of neurogenesis on hippocampal function and MDD etiology is essential to help further treatment recommendations. Finally, the study adds to the literature that suggests hippocampal volume reductions are present in individuals with a history of depression. Moreover, the findings suggest there is a neurotrophic effect of antidepressant drugs on specific subfields of the hippocampus. This adds to the evolving research informing our fractional understanding of the neuroanatomical foundation for depression and guides future research investigating the antidepressant mechanisms involved.
5. References


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Czéh, B., Michaelis, T., Watanabe, T., Frahm, J., de Biurrun, G., van Kampen, M., ... & Fuchs, E. (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proceedings of the National Academy of Sciences, 98(22), 12796-12801. doi: 10.1073/pnas.211427898


Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Archives of general psychiatry, 57(10), 925-935. doi:10.1001/archpsyc.57.10.925


38-43. doi: 10.1016/j.ajp.2014.11.005


6. Appendices

Appendix 1)

Information sheets.

VEELCOMEN TIL FORSKNINGSPROJEKTET
FOR OPTRÆKKONIBET OG FUNNBE

Vi har vedtægt 2 møtre der ………………. ve…………

Adresse: Psychologisk Institut, U.O.3

Kontaktinformation: Jan Funcken (tlf.: 60-30 66 72)

Gir os gerne at vlæmme de derlomb, så ikke der er ting: Tel.: 40-58 66 72

Denne overskrift vil se de på en 4-sider af forskningsrapporten til lærerne.

Ansvarlig er (1. fotografi) til (8. fotografi), (1. fotografi) til (8. fotografi).

Denne konference laver vi mere i kontekst med vores forskning ved et uddannelsesprojekt.

Vedtager denne konference vil skelne os fra det, som der er gaavet på en (2. fotografi) til (8. fotografi).

Denne delen bør bruges som et afsnitt af rapporten.

Vedtager denne delen bør bruges som et afsnitt af rapporten.

Hvis du gerne vil bli med på dagens møde, så er det et af de gode ting: Tel.: 60-32 62 72 eller

Valid projekt.

Information om ditt projekt for opptrekkonibet og funnbe

Kapitel A. Udviklingsforløb og beskæftigelse

Oversigt over udviklingsforløbet og beskæftigelse

Sommeren 2006

Information om projektet

Selskabsforløb

Kapitel B. Personværdi, bistand og forhindring

Personværdi

Oversigt over personværdi

Selskabsforløb

Kapitel C. Kapitalplaner og -forløb

Kapitalplaner

Oversigt over kapitalplaner

Selskabsforløb

Kapitel D. Personværdi, bistand og forhindring

Personværdi

Oversigt over personværdi

Selskabsforløb

Kapitel E. Kapitalplaner og -forløb

Kapitalplaner

Oversigt over kapitalplaner

Selskabsforløb

Kapitel F. Personværdi, bistand og forhindring

Personværdi

Oversigt over personværdi

Selskabsforløb

Kapitel G. Kapitalplaner og -forløb

Kapitalplaner

Oversigt over kapitalplaner

Selskabsforløb

Kapitel H. Personværdi, bistand og forhindring

Personværdi

Oversigt over personværdi

Selskabsforløb

Kapitel I. Kapitalplaner og -forløb

Kapitalplaner

Oversigt over kapitalplaner

Selskabsforløb

Kapitel J. Personværdi, bistand og forhindring

Personværdi

Oversigt over personværdi

Selskabsforløb

Kapitel K. Kapitalplaner og -forløb

Kapitalplaner

Oversigt over kapitalplaner

Selskabsforløb
Consent form.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Stedfortrodende samtykke når berettiget, enten i tillegg til personen selv eller istedenfor

(Signert av nærstående, dato)

Jeg bekrerter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)
Appendix 3)

**Supplementary Table 1.**

Supplementary Table 1. Mean Hippocampal Volume (mm³) in Medication Active Patients and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Medication Active</th>
<th>Healthy Control</th>
<th>Diagnosis</th>
</tr>
</thead>
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<tr>
<td></td>
<td>( (n = 63) )</td>
<td>( (n = 77) )</td>
<td>( F(1,136) )</td>
</tr>
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<td><strong>Left Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>550.98 ± 66.62</td>
<td>544.36 ± 54.38</td>
<td>.388</td>
</tr>
<tr>
<td>Subiculum</td>
<td>453.71 ± 51.73</td>
<td>450.28 ± 49.41</td>
<td>.143</td>
</tr>
<tr>
<td>CA1</td>
<td>685.95 ± 77.27</td>
<td>684.61 ± 67.98</td>
<td>.000</td>
</tr>
<tr>
<td>PreSubiculum</td>
<td>337.17 ± 39.63</td>
<td>326.45 ± 39.00</td>
<td>.03</td>
</tr>
<tr>
<td>ParaSubiculum</td>
<td>68.04 ± 11.69</td>
<td>71.85 ± 9.64</td>
<td>5.73</td>
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<tr>
<td>Molecular Layer</td>
<td>551.05 ± 49.92</td>
<td>562.68 ± 61.19</td>
<td>2.25</td>
</tr>
<tr>
<td>GC_DG</td>
<td>332.92 ± 41.62</td>
<td>335.29 ± 33.40</td>
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<td>CA2/3</td>
<td>251.70 ± 34.29</td>
<td>246.97 ± 36.13</td>
<td>.553</td>
</tr>
<tr>
<td>CA4</td>
<td>275.20 ± 34.93</td>
<td>276.01 ± 27.46</td>
<td>.117</td>
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<tr>
<td><strong>Right Hippocampus</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>556.98 ± 68.62</td>
<td>549.84 ± 62.06</td>
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<td>447.01 ± 46.49</td>
<td>444.79 ± 46.96</td>
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<td>709.55 ± 72.51</td>
<td>704.57 ± 81.41</td>
<td>.104</td>
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<td>PreSubiculum</td>
<td>318.44 ± 34.48</td>
<td>308.76 ± 35.90</td>
<td>3.03</td>
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<td>ParaSubiculum</td>
<td>66.63 ± 10.74</td>
<td>68.59 ± 9.60</td>
<td>1.52</td>
</tr>
<tr>
<td>Molecular Layer</td>
<td>561.29 ± 55.20</td>
<td>575.67 ± 56.85</td>
<td>3.57</td>
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<td>GC_DG</td>
<td>344.08 ± 34.90</td>
<td>347.58 ± 38.97</td>
<td>.650</td>
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<td>CA2/3</td>
<td>270.79 ± 29.84</td>
<td>269.11 ± 32.81</td>
<td>.040</td>
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<tr>
<td>CA4</td>
<td>287.60 ± 28.84</td>
<td>290.62 ± 31.24</td>
<td>.804</td>
</tr>
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</table>

Data are mean ± standard deviation. \( \eta^2 \) = Partial eta squared. \( n \) = number of participants. Nominally significant values (\( p < .05 \)) in bold.
Supplementary Table 2. Mean Hippocampal Volume (mm³) in Medication Naïve Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Subfield</th>
<th>Medication Naïve (n = 72)</th>
<th>Healthy Control (n = 77)</th>
<th>F(1,145)</th>
<th>P</th>
<th>ηp²</th>
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<tr>
<td><strong>Left Hippocampus</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hippocampal Tail</td>
<td>549.29 ± 66.29</td>
<td>544.36 ± 54.38</td>
<td>1.01</td>
<td>.159</td>
<td>.007</td>
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<tr>
<td>Subiculum</td>
<td>439.25 ± 57.15</td>
<td>450.28 ± 49.41</td>
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<td>CA1</td>
<td>671.42 ± 91.19</td>
<td>684.61 ± 67.98</td>
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<td>.154</td>
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<td>326.45 ± 39.00</td>
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<td>70.04 ± 12.28</td>
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<td>Molecular Layer</td>
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<td>562.68 ± 61.19</td>
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<td>.018</td>
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<td>324.67 ± 37.57</td>
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<td><strong>.030</strong></td>
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<td>266.63 ± 31.26</td>
<td>276.01 ± 27.46</td>
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<td>.023</td>
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<tr>
<td>Hippocampal Tail</td>
<td>548.70 ± 76.61</td>
<td>549.84 ± 62.06</td>
<td>.355</td>
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<td>.395</td>
<td>.000</td>
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<td>66.55 ± 11.87</td>
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<td>558.53 ± 70.55</td>
<td>575.67 ± 56.85</td>
<td>2.09</td>
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<td>GC_DG</td>
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<td>347.58 ± 38.97</td>
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<td>269.11 ± 32.81</td>
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<td>CA4</td>
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Data are mean ± standard deviation. ηp² = Partial eta squared. n = number of participants. Nominally significant values (p < .05) in bold.