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DOI:

[10.1177/0269881119858306](https://doi.org/10.1177/0269881119858306)

Document Version

Peer reviewed version

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Citation for published version (APA):

Pretzsch, C. M., Voinescu, B., Mendez, M. A., Wichers, R., Ajram, L., Ivin, G., ... McAlonan, G. M. (2019). The effect of cannabidiol (CBD) on low-frequency activity and functional connectivity in the brain of adults with and without autism spectrum disorder (ASD). *Journal of Psychopharmacology*.
<https://doi.org/10.1177/0269881119858306>

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The effect of Cannabidiol (CBD) on low frequency activity and functional connectivity in the brain of adults with and without Autism Spectrum Disorder (ASD).

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Abstract

Background: The potential benefits of cannabis and its major non-intoxicating component cannabidiol (CBD) are attracting attention, including as a potential treatment in neurodevelopmental disorders such as autism spectrum disorder (ASD). However, the neural action of CBD, and its relevance to ASD, remains unclear. We and others have previously shown that response to drug challenge can be measured using functional magnetic resonance imaging (fMRI); but that pharmacological responsivity is atypical in ASD.

Aims: Therefore, we hypothesized that there would be a (different) fMRI response to CBD in ASD.

Methods: To test this, task-free fMRI was acquired in 34 healthy men (half with ASD) following oral administration of 600 mg CBD or matched placebo (random order; double blind administration). The ‘fractional Amplitude of Low Frequency Fluctuations’ (fALFF) was measured across whole brain; and, where CBD significantly altered fALFF, we tested if functional connectivity (FC) of those regions was also affected by CBD.

Results: CBD significantly increased fALFF in the cerebellar vermis and the right fusiform gyrus. However, post-hoc within-group analyses revealed that this effect was primarily driven by the ASD group, with no significant change in controls. Within the ASD group only, CBD also significantly altered vermal FC with several of its subcortical (striatal) and cortical targets; but did not affect fusiform FC with other regions in either group.

Conclusion: Our results suggest that, especially in ASD, CBD alters regional fALFF and FC in/between regions consistently implicated in ASD. Future studies should examine if this affects the complex behaviours these regions modulate.

Introduction

Autism spectrum disorder (ASD) is a common (Baio et al., 2018) and complex neurodevelopmental psychiatric condition, which shortens life expectancy by up to 20 years (Hirvikoski et al., 2016). Moreover, 70% of autistic individuals are estimated to have co-occurring conditions, e.g. epilepsy (Tuchman and Rapin, 2002), mood and anxiety disorders (Joshi et al., 2013). However, there are no effective pharmacological treatments for the core symptoms of ASD, and individuals often respond poorly to conventional treatments of complicating mental or physical illnesses. Alternative treatment approaches, such as cannabis and its major non-intoxicating component cannabidiol (CBD) (Fetterman and Turner, 1972), are therefore increasingly explored.

There is accumulating evidence for the efficacy of CBD in several conditions, such as spasticity in multiple sclerosis (Zajicek et al., 2003) and schizophrenia (Bhattacharyya et al., 2018); and in conditions associated with ASD, including social phobia (Bergamaschi et al., 2011) and epilepsy (Devinsky et al., 2016). Moreover, there are preliminary reports of beneficial effects of medical marijuana in idiopathic ASD itself (Campbell et al., 2017; Aran et al., 2018). For instance, a recent study has demonstrated that CBD-rich cannabis reduced behavioural outbreaks in children with ASD and severe behavioural problems (Aran et al., 2018). Thus, a role for CBD in ASD management warrants further attention. Consequently, there are now at least two clinical trials of CBD in ASD (clinicaltrials.gov; identifiers NCT03900923 and NCT02956226). Thus, a role for CBD in ASD management warrants further attention. However, we still have only limited understanding of how the typical human brain, let alone the autistic brain, responds to CBD. Hence, a fuller understanding of the mechanism of action

of CBD on brain, and its relevance to ASD, is desirable before investing in large scale clinical trials.

Brain responsivity to pharmacological challenges can, for example, be measured using functional magnetic resonance imaging (fMRI) (Bhattacharyya et al., 2015; Fusar-Poli et al., 2010; Grimm et al., 2018). Evidence that such approaches are sensitive to CBD effects comes from studies in both neurotypicals and individuals with schizophrenia. For instance, CBD reduced fronto-striatal – and decreased mediotemporal-prefrontal – FC during a visual oddball salience task; and enhanced salience processing (Bhattacharyya et al., 2015). CBD also disrupted prefrontal-subcortical connectivity during the processing of fearful faces (Fusar-Poli et al., 2010), and increased fronto-striatal activity at rest (Grimm et al., 2018). In schizophrenia, an acute dose of CBD was reported to ‘normalize’ brain activity in regions during a verbal learning paradigm (Bhattacharyya et al., 2018). Similarly, fMRI has been used as a marker for other drug challenges in ASD, such as riluzole, propranolol or oxytocin (Narayanan et al., 2010; Gordon et al., 2016; Ajram et al., 2017). However, the vast majority of these previous studies acquired fMRI during cognitive tasks.

Although this approach provides valuable task-specific information, it constrains the analysis of drug response to task-relevant brain regions (and so misses the potential impact of a drug on whole brain function). Moreover, in a condition like ASD, where the performance of such higher cognitive tasks can be compromised (e.g. (Ashwin et al., 2007; Daly et al., 2012)), disentangling the effects of a drug from the demands of the task can be challenging. Thus, to examine whole-brain impact of CBD in individuals with and without ASD, we elected to use a resting state design.

Therefore, in this randomized, placebo-controlled, double-blind, cross-over study we adopted a whole-brain, task-free (resting state) design to compare brain response to CBD in individuals with and without ASD. We used resting state fMRI to examine the fractional amplitude of low frequency fluctuations (fALFF) (0.01-0.1Hz) as a measure of spontaneous regional brain activity (Cordes et al., 2001). These low frequency oscillations are thought to subsequently support the synchronisation of activity between spatially distinct regions (i.e. functional connectivity, FC) (Friston et al., 1993). Therefore, in regions where a significant shift in fALFF was observed, we also conducted a secondary seed-based analysis of FC of that region with the rest of the brain. Data were acquired following a single oral dose of 600 mg CBD or a matched placebo (at least 13 days apart). We predicted that CBD would alter regional fALFF; and subsequently shift FC of those regions which responded. Moreover, based on our previous findings that the autistic brain responds atypically to pharmacological challenge (Ajram et al., 2017), we expected that responsivity to CBD would be different in autistic compared to neurotypical individuals.

Materials and Methods

Procedure

This research was conducted in accordance with the Declaration of Helsinki, at the Institute of Psychiatry, Psychology, and Neuroscience (IoPPN) at De Crespigny Park, SE5 8AF, London, UK (August 2016 to February 2017). Ethical approval for this study was provided by the King's College London Research Ethics Committee, study reference HR15/162744. All participants provided written informed consent. Every participant took part in all aspects of this case-

control observational study. The Medicines and Health Research Authority (MHRA) in the UK confirmed that our study design is not a Clinical Trial; however, in the interests of transparency this observational study was registered on clinicaltrials.gov (identifier: NCT03537950, entry name: HR15-162744).

This was a placebo-controlled, randomised, double-blind, repeated-measures, cross-over study conducted as part of a larger investigation into the role of phytocannabinoids in ASD. Drugs were allocated in a pseudo-randomised order, so that each participant received each drug (PLC, CBD) once, and that approximately half of our participants attended a placebo visit before CBD; and half received CBD before placebo. This randomisation was implemented by Prof McAlonan using <https://www.random.org/>. Participants and those assessing outcomes were blinded to the assignment. Participants attended for two visits. To allow for drug wash-out, visits were separated each by a minimum of 13 days, with all attempts made to keep between-visit time consistent across all visits and participants. Moreover, time of data acquisition from both groups was largely overlapping. On each visit, urine samples were taken to screen for illicit substances (a full list is included below). Subsequently, participants underwent a brief health check and received a liquid oral dose of the pharmacological probe (600 mg of CBD or a matched placebo, both provided by GW Research Ltd, Cambridge, UK). The dose of 600 mg of CBD has previously been demonstrated to be sufficient to elicit an effect on brain in adults, e.g. (Bhattacharyya et al., 2015); while being very well tolerated (GW Pharmaceuticals Investigator Brochure 2015). After a second brief health check to test for potential acute adverse reactions/side effects, participants underwent scanning timed to coincide with peak plasma (2 h) concentration. Following the scan, participants received a

third health check to ensure they had experienced no ill-effects and were fit to leave the department.

Participants

Potential participants were excluded if they had a comorbid major psychiatric or medical disorder affecting brain development (e.g. schizophrenia or epilepsy), a history of head/brain injury, a genetic disorder associated with ASD (e.g. tuberous sclerosis or Fragile X syndrome), an IQ below 70, or were reliant on receiving regular medication known to modulate directly the neurotransmitters glutamate and GABA. However, we included participants on other medications which are frequently prescribed in this condition. Thus, one autistic participant received a single dose of Ritalin on the morning of each test day, and another autistic participant took a single dose of sertraline on the morning of each visit. All participants were asked to abstain from using cannabis and/or other illicit substances in the month prior to scanning, and from drinking alcohol on the day prior to testing. Moreover, data from individuals who screened positive for illicit substances in the urine drug screening were excluded. Thus, we retained data from 34 subjects (17 neurotypicals, 17 ASD) (see Table 1 for demographics). All participants in the ASD group had a clinical diagnosis of ASD made according to ICD10 research criteria, supported by the use of standardised research diagnostic instruments (Autism Diagnostic Observation Schedule, ADOS; and Autism Diagnostic Interview-Revised, ADI-R) (World Health Organisation, 2016; Lord et al., 1994; Lord, 1989).

Imaging data acquisition

We acquired all imaging data on a 3T GE Excite II magnetic resonance imaging (MRI) scanner (GE Medical Systems, Milwaukee, WI, USA). Our scanning protocol included a structural MRI scan acquired using a 3D inversion recovery prepared fast spoiled gradient recalled (IR-FSPGR) sequence (slice thickness = 1.1 mm, spatial positions = 124, flip angle = 20°, field of view (FoV) = 280 mm, echo time (TE) = 2.844 ms, repetition time (TR) = 7.068 ms, inversion time (TI) = 450 ms, matrix = 256x256). This structural MRI scan was used for co-registration of the functional volumes. The scanning protocol further included a resting state MRI scan. This scan was acquired using an echo-planar imaging (EPI) sequence (slice thickness = 3 mm, slice gap = 3.3 mm, flip angle = 75°, FoV = 240 mm, TE = 30 ms, TR = 2000 ms, TI = 0 ms). We collected data for 256 time points, i.e. the resting state scan lasted 512 s.

Urine test

We performed liquid chromatography-mass spectrometry (LC-MS) analysis on urine samples provided by each participant before the drug administration to test for the presence or absence of illicit substances that could confound potential effects of the pharmacological probes tested here. Participants showing positive results for any of the drugs tested, including Amphetamines (Amphetamine, Methamphetamine, MDMA/Ecstasy), Benzodiazepines, Cannabis, Cocaine (as benzoylecgonine), Methadone and its metabolite EDDP, and Opioids (6-Monoacetylmorphine, Morphine, Codeine, Dihydrocodeine), were excluded from the analysis. This resulted in the exclusion of four subjects (two neurotypicals, two ASD) from the original sample.

Data processing

Structural data processing

T1-weighted structural MRI volumes were inspected manually to ensure adequate data quality and signal-to-noise ratio. Next, structural volumes were normalized to Montreal Neurological Institute (MNI) space, and segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF), to enable the removal of WM and CSF confounds using linear regression.

Resting state data processing

We processed our resting state data using in-house software, CONN v.17c (Whitfield-Gabrieli and Nieto-Castanon, 2012), DPABI v.2.1 (Yan et al., 2016), and MATLAB R2017a (The MathWorks, Inc., MA, USREF). To ensure adequate data quality, all data were inspected manually for artefacts such as blurring, distortions, ghosting, or warping. The first 5 functional volumes were discarded to allow for magnetization equilibrium.

Using CONN, all remaining functional volumes were slice-time corrected (sinc-interpolation), realigned (within runs to first volume, then all volumes to first volume of first run), and normalized to MNI space, using the segmented (into GM, WM, and CSF) structural scans.

Head motion is thought to affect measures of FC (Power et al., 2012), and could potentially affect fALFF. To account for head motion, for each subject and run, we computed the frame-to-frame displacement (> 1.5 mm/° translation/rotation classified as motion outlier). Following previous studies (e.g. (Gordon et al., 2016)), we used a joint threshold, where subjects with movement in any dimension ≥ 2 mm and/or $\geq 15\%$ of volumes identified as

motion outliers were excluded from the analysis. This resulted in the exclusion of four runs from four subjects (all ASD). Thus, we retained a sample of 17 neurotypicals and 13 individuals with ASD.

fALFF data processing

Using DPABI, the functional data were detrended (linear trend removal), and denoised (WM, CSF, and movement confounds removed through linear regression). Subsequently, the time series of each voxel was transformed into the frequency domain using a Fast Fourier transform (as described in (Song et al., 2011)). Next, we obtained the power spectrum and calculated the square root (amplitude) at each frequency of the power spectrum. The sum of amplitudes across the low frequency spectrum ($0.01 < f < 0.1$ Hz) was divided by that across the entire frequency range ($0 < f < 0.25$ Hz). Individual fALFF maps were then standardized (Z-value; subtraction of global mean, and division by standard deviation) within a brain mask (average GM mask across all subjects) and smoothed (using a Gaussian filter with a 6 mm full width at half maximum (FWHM) kernel). In summary, our analysis of low frequency fluctuations proceeded on a voxel by voxel basis.

FC data processing

Using CONN, data were smoothed (using a 6 mm FWHM Gaussian kernel), detrended (linear trend removal), denoised (WM, CSF, realignment, and movement confounds removed through linear regression), and band-pass filtered.

In contrast to our fALFF analysis, our analysis of interregional FC used the average activity within predefined regions of interest (ROIs), based on standard whole brain atlases provided by CONN). These FC measures reflect the correlations between pairs of discrete ROIs. We

tested for experimental effects on the FC between key regions – identified in the voxel-based analysis of low frequency fluctuations, using standard analysis of variance.

Statistical analysis

Demographic measures (age, IQ) were compared using a one-way ANOVA (significance level $p < 0.05$).

We first compared baseline levels of fALFF within GM regions between groups using a two-sample t-test with non-parametric inference (TFCE, $p_{FWE} < 0.05$) with 5000 permutations.

To test our primary hypothesis that CBD modulates fALFF within GM regions, we used a 2 x 2 factorial design with group (neurotypicals, ASD) as between-subject factor, drug (PLC, CBD) as within-subject factor, and regional fALFF as the dependent variable. We used non-parametric inference at a familywise error rate (FWE) $q = 0.05$ (5000 permutations) and Threshold Free Cluster Enhancement (TFCE). As outlined in the introduction, we also wanted to examine the consistency of this response across groups and to establish if a CBD effect was indeed present within each group. Hence, in regions where we discovered a significant effect of drug, we performed planned post-hoc tests of these drug effects within each group separately using non-parametric inference (TFCE, $p_{FWE} < 0.05$) with 5000 permutations.

Our secondary hypothesis was that CBD modulates FC between the regions identified in our fALFF analysis and the rest of the brain separately in ASD and neurotypicals. To this aim we conducted planned post-hoc tests within each group to examine potential effects of CBD on FC of those regions with the rest of the brain, using a seed-based repeated-measures design (connection-level threshold: $p_{\text{uncorr}} = .05$; seed-level threshold, and False Discovery Rate (FDR): $p_{\text{FDR}} = .05$).

All analyses were performed using SPSS 24.00 software (SPSS, Chicago, IL, USA), DPABI v.2.1 (Yan et al., 2016) and Conn v.17c (Whitfield-Gabrieli and Nieto-Castanon, 2012).

Results

Demographics

Groups did not differ significantly in age ($F(1) = 0.634$, $p = .443$) or in full-scale IQ ($F(1) = 3.230$, $p = .083$). We observed no subjective or objective ill-effects/harm following administration of the study drug in any of our participants. Table 1

Table 1 Participant demographics for all subjects including standard deviations. Abbreviations: autism spectrum disorder, ASD; female, F; F statistic and degrees of freedom, F(dof); full scale intelligence quotient, FSIQ; male, M.

Variable (SD)	Neurotypicals	ASD	F(dof)	p
N (M/F)	17 (17/0)	13 (13/0)	--	--
Age (y)	28.47 (6.55)	30.85 (9.79)	$F(1) = .634$	$p = .443$
FSIQ	124.59 (12.71)	114.46 (18.18)	$F(1) = 3.230$	$p = .083$

Fractional Amplitude of low frequency fluctuations (fALFF)

There were no baseline group differences in fALFF within GM regions (TFCE, $p_{\text{FWE}} > .05$); however, there was a main effect of drug. Thus, across both groups, CBD (compared to PLC) increased fALFF in the cerebellar vermis VI (TFCE, $p_{\text{FWE}} = .048$, $k = 4$, centre of gravity in mm

(CoG): $x = -1$, $y = -65$, $z = -6$) and in the right fusiform gyrus (TFCE, $p_{FWE} = .041$, $k = 14$, CoG: $x = 28.9$, $y = -48.7$, $z = -6.86$), as depicted in Figure 1. There was no main effect of group, nor a drug \times group interaction. [insert Figure 1.]

Post-hoc within-group analyses confirmed a significant effect of drug in the ASD group (vermis VI: TFCE, $p_{FWE} = .045$, $k = 7$, CoG: $x = 21.1$, $y = -55.7$, $z = -14$; fusiform: TFCE, $p_{FWE} = .029$, $k = 19$, CoG: $x = 28.3$, $y = -51.8$, $z = -9.58$), but not in neurotypicals, as shown in Figure 2. [insert Figure 2.]

Functional connectivity (FC)

There were no baseline group differences in vermal or fusiform FC. In the ASD group, CBD significantly increased vermal FC with the left ($T(11) = 2.57$, $p = .026$) and right caudate ($T(11) = 2.26$, $p = .045$); and decreased vermal FC with the temporo-occipital part of the left middle temporal gyrus ($T(11) = -2.81$, $p = .017$), the right anterior supramarginal gyrus ($T(11) = -2.73$, $p = .02$), the left superior parietal lobe ($T(11) = -2.54$, $p = .027$), and the left superior frontal gyrus ($T(11) = -2.29$, $p = .043$). In contrast, CBD had no significant effect on vermal or fusiform FC with any other regions in the neurotypicals; but this between-group difference in responsivity was not significant. These findings are summarised in Table 2 and Figure 3. [insert Figure 3.]

Table 2 Drug effects on functional connectivity in the autism spectrum disorder (ASD) group. Abbreviations: cannabidiol, CBD; left, L; not significant, n.s.; placebo, PLC; right, R.

Seed region	Contrast	Target region	Statistic
<i>Drug effect in ASD</i>			

Vermis VI	CBD > PLC	R caudate	T(11) = 2.57, p = .026
		L caudate	T(11) = 2.26, p = .045
	CBD < PLC	L temporo-occipital middle temporal gyrus	T(11) = -2.81, p = .017
		R anterior supramarginal gyrus	T(11) = -2.73, p = .020
		L superior parietal lobe	T(11) = -2.56, p = .027
		R superior parietal lobe	T(11) = -2.54, p = .027
		L superior frontal gyrus	T(11) = -2.29, p = .043
Right Fusiform	CBD > PLC	--	n.s.
	CBD < PLC	--	n.s.

Discussion

Here we report, for the first time, that CBD ‘shifts’ fALFF and FC in the adult human brain. Specifically, we found that CBD significantly increased fALFF in the cerebellar vermis VI and the right fusiform gyrus. However, post-hoc within-group testing indicated that this shift was most prominent in ASD, and not significant in controls (please note that we did not identify a significant group by drug interaction). Moreover, in ASD, but not controls, the shift in fALFF in the cerebellum (but not fusiform gyrus) was accompanied by widespread changes in vermal FC with several of its subcortical and cortical targets.

Cerebellar vermis

In the typical brain, the cerebellar vermis and its cerebellar-subcortical-cortical circuitry are increasingly understood to subserve a critical role in movement, language, and social

processing (D'Mello and Stoodley, 2015). In ASD, however, there have been reports of functional anomalies in the cerebellum, which are thought to contribute to the disruption of these processes observed in ASD; but the results have been inconsistent.

For example, vermal hypoactivation during simple motor tasks (finger tapping) (Muller et al., 2001) and during auditory perception (listening to tones) has been observed in adults (Muller et al., 1999) with ASD. In children and adolescents with ASD, FC of the vermis with sensorimotor regions has been reported to be higher; whereas vermal FC with prefrontal and motor regions has been recorded as lower (Khan et al., 2015) compared to neurotypicals. Hence, the results of functional imaging studies in ASD may depend on the task under investigation. Here we used a non-task design and observed no baseline differences in cerebellar fALFF and FC measures (with other regions) in participants with and without ASD. Instead, we found that cerebellar activity and connectivity was modifiable, and especially so in ASD. Specifically, we found that the CBD-induced increase in vermal fALFF in ASD was accompanied by an increase in cerebellar-subcortical (striatal) FC. However, CBD also decreased cerebellar-cortical FC. This suggests that, in autistic adults, rather than inducing a general and unidirectional shift in FC, CBD appears to 'tune' FC in a region- or connection-specific manner. It was beyond the scope of the present study to examine the cognitive or behavioural implications of this response.

Right fusiform gyrus

In the neurotypical brain, the right fusiform is commonly associated with the visual processing of words (McCandliss et al., 2003) and parts of the body (vs objects), e.g. faces (Morris et al., 2007). In ASD, face processing is overwhelmingly reported to be impaired (Ashwin et al., 2007;

Kleinhans et al., 2008); and this is consistent with a wealth of evidence for functional fusiform anomalies in this condition. For instance, neuroimaging studies have reported hypoactivation (Perlman et al., 2011) and impaired FC (Kleinhans et al., 2008) during face processing. Again, using a task-free design we found no baseline differences in the activity of this region; but our results indicate that CBD modulates fusiform activity, and the ASD group was particularly responsive. Our study design does not speak to whether face processing in ASD would also be altered by CBD, but it does indicate that the functional dynamics of this region in ASD differ from controls, in that activity can be modulated by CBD.

Although CBD shifted fALFF in both the vermis 6 and right fusiform gyrus, it only altered FC between the vermis (and other regions) and not between the fusiform (and other regions) of individuals with ASD. The reason for this is not known. One explanation is that the connections of the fusiform with other regions are relatively limited, certainly compared to the cerebellum. Fusiform FC (with other regions) in ASD specifically may be further restricted by the anatomy of the fusiform region in this condition. For example, the grey-white matter boundary of the fusiform gyrus has been observed to be disrupted in ASD (Andrews et al., 2017), as has the integrity of white matter tracts in this location (Barnea-Goraly et al., 2004). Abnormalities in white matter connections could restrict the impact of CBD on wider FC from this seed. In contrast, previous studies of the vermis indicate that the microstructural integrity of white matter connections in this region is intact or even higher in ASD (Noriuchi et al., 2010; Ben Bashat et al., 2007); and thus there may be more 'capacity' for FC of the cerebellum with other regions to change in response to pharmacological challenge in ASD

Neurobiological basis of CBD effects on fALFF and FC

The neurobiological underpinnings of these effects are unclear. However, previous studies indicate that CBD can influence excitatory glutamate and inhibitory GABA pathways, which play a crucial role in the regulation of LFF and FC (Farrant and Nusser, 2005).

Preclinical studies suggest that CBD has a range of actions which may, for example, converge in a modulation of brain excitation and inhibition (Musella et al., 2009; Kaplan et al., 2017; Santana et al., 2004). This is important because the balance of excitation and inhibition is postulated to help establish and maintain LFF and FC. Inhibitory phasic GABAergic interneurons, for instance, are key modulators of temporo-spatial signal integration and propagation. Through feed-forward inhibition, these neurons can synchronise large numbers of pyramidal cells and thus provide the basis for coordinated firing across distinct brain regions (Farrant and Nusser, 2005). Similarly, tonic GABAergic neurons can shape LFF by tuning cell conductance and thus controlling the amount and duration of voltage response to excitatory input (Farrant and Nusser, 2005). Several targets of CBD in the brain, e.g. TRPV1, GPR55, and 5-HT receptors, have been linked to brain glutamate and/or GABA signalling (Musella et al., 2009; Kaplan et al., 2017; Santana et al., 2004) and so may impact upon this neural activity. For instance, TRPV1-activation through CBD has been reported to increase glutamatergic excitation (Musella et al., 2009). In contrast, CBD antagonism on GPR55 has been observed to increase the firing of GABAergic interneurons (Kaplan et al., 2017). Finally, CBD is an agonist on 5-HT_{1A} and 5-HT_{2A} receptors, which can be found on both excitatory and on inhibitory neurons (Santana et al., 2004); thus, CBD action through these receptors may both enhance and reduce excitatory and inhibitory transmission. In ASD, however, these targets of CBD have been reported to be altered, e.g. (Veenstra-VanderWeele et al., 2012;

Kerr et al., 2013). This may partly explain the differential findings in ASD compared to neurotypicals observed here.

An alternate explanation for the sensitivity of ASD and not controls to a drug challenge targeting glutamate-GABA systems, may be the abnormalities in these systems – and their responsivity - in ASD. For instance, there is reduced expression of GABA receptors in the fusiform in ASD (Oblak et al., 2011). Similarly, decreased levels both of glutamic acid decarboxylase (the enzyme responsible for converting glutamate to GABA) and of inhibitory Purkinje cells in the vermis in ASD (Murakami et al., 1989; Yip et al., 2007) suggest inhibition deficits in this condition. Thus, our results open the possibility that in ASD, CBD boosts LFF (and FC) especially in regions where GABA systems are impaired. This proposed link between the glutamate-GABA system and functional circuitry is supported by previous findings of a significant correlation between cerebellar excitation-inhibition levels and cerebro-cerebellar FC in adolescents and adults with ASD (Hegarty et al., 2018).

Limitations

We acknowledge that our study has important limitations.

First, our study sample size was modest because: i) we applied strict recruitment criteria (e.g. exclusion of candidates with co-morbid psychiatric or medical conditions, which can be challenging in ASD); ii) participants were asked to adhere to time-intensive repeated testing over several weeks, involving drug administration - this commitment was not always practical; and iii) we performed rigorous data quality control, and excluded some datasets. Nonetheless, our repeated-measures design mitigated these constraints to some extent by

reducing inter-subject variability (each participant had two scans and thus acted as their own 'control') and thus increasing statistical power. In addition, our findings survived rigorous statistical thresholding

Second, fALFF analysis is focused on a single frequency range (0.01-0.1 Hz). However, in the human brain, a range of frequency bands are thought to interact and to possess hierarchical structure. For instance, EEG approaches have shown that delta (1-4 Hz) phase modulates theta (4-10 Hz) amplitude, and that theta phase modulates gamma (30-50 Hz) amplitude (Lakatos et al., 2005). Future studies are required to investigate the impact of CBD across different frequency bands to obtain a more comprehensive view of CBD effects in the neurotypical and autistic brain.

Third, in the present study we investigated the impact of acute CBD administration. However, the effects of longer periods of treatment with CBD on fALFF and FC (and eventually on clinically relevant indices) are difficult to predict and may vary between individuals. Therefore, future studies are required to examine the effects of chronic CBD administration on brain, and whether the acute response to CBD may help predict the impact of sustained treatment.

Fourth, there are two qualifications that need to be considered when interpreting our results. These qualifications pertain to most studies of resting state functional connectivity. The first pertains to the nature of resting state fMRI: by its nature, this task free paradigm relinquishes experimental control over neuronal processing or attentional set. It is therefore possible that the differences we have observed reflected group differences in the way that subjects

responded to the scanning experience. The second issue is specific to the way in which we quantified functional connectivity. By using the correlation coefficient as a measure of functional connectivity, we acknowledge that a significant effect on a correlation coefficient can be produced either by a change in the linear coupling between two regions, or by a difference in noise. In other words, an increase (or decrease) in the correlation coefficient can be explained by a decrease (or increase) in noise or random effects due to mental or other (e.g., motion) effects. In short, a difference in correlation can be explained either in terms of a difference in variance explained in one region by another region – or by a difference in the variance not explained (i.e., noise). A more direct way to test for differences in linear coupling would have been to use a form of psychophysiological interaction in which the Fisher transformed correlation coefficients are replaced by the regression coefficients (obtained by regressing the activity of a target region on a source region). We will consider this in future work.

Conclusions

In summary, we report the first evidence that CBD ‘shifts’ fALFF and associated FC in the adult autistic brain. Thus, in ASD, CBD can alter a crucial property of brain function, and targets key regions commonly implicated in the condition. Future studies are required to i) investigate if CBD-induced alterations of fALFF and FC in ASD impact on the cognitive processes and behaviours these regions modulate; and ii) examine whether brain response to an acute dose of CBD may help predict response to sustained treatment in ASD.

Funding and Disclosure

This study was an Investigator Initiated Study (G.M.) which received funding from GW Pharma (G.M., S.W., D.M.). GW Pharma had no role in the data collection or analysis of results, nor in the decision to publish. The authors also acknowledge infrastructure and training support from the National Institute for Health Research (NIHR) Mental Health Biomedical Research Centre (BRC) for Mental Health at South London and Maudsley NHS Foundation Trust and Institute of Psychiatry, Psychology and Neuroscience, King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health, U.K. Additional sources of support included the Sackler Institute for Translational Neurodevelopment at King's College London (G.M., C.M.P., D.G.M.), Autistica (C.M.P.), the Medical Research Council (MRC) Centre grant (MR/N026063/1) (G.M.) and EU-AIMS (European Autism Interventions)/EU AIMS-2-TRIALS, a European Innovative Medicines Initiative Joint Undertaking under Grant Agreements No. 115300 and 777394, the resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (Grant FP7/2007–2013) (G.M., D.M., E.D.). Finally, the authors sincerely thank all the participants.

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Figure 1 Drug effects on the fractional amplitude of low frequency fluctuations across the grey matter (cannabidiol > placebo). Numbers above the slices indicate location in z-direction (in mm). Scans are oriented in neurological convention, where right (R) equals right, and left (L) equals left. P values (P), as indicated by the colorbar, are corrected for multiple comparisons (TFCE, FWE).

Figure 2 Post-hoc test of drug effects on the fractional amplitude of low frequency fluctuations within each region of interest (cannabidiol > placebo) in autism spectrum disorder. Top row (A): drug effects within the cerebellar vermis IV; Bottom row (B): drug effects within the right fusiform gyrus. Numbers above the slices indicate location in z-direction (in mm). Scans are oriented in neurological convention, where right (R) equals right, and left (L) equals left. P values (P), as indicated by the colorbar, are corrected for multiple comparisons (TFCE, FWE).

Figure 3 Drug effects on functional connectivity of cerebellar vermis VI in the autism spectrum disorder (ASD) group (cannabidiol > placebo). T-values (T) of edges, as indicated by colorbar, are corrected for multiple comparisons at connection- and seed-level ($p = .05$ and $p_{FDR} = .05$). Abbreviations: autism spectrum disorder, ASD; cannabidiol, CBD; left caudate, L.Caud; left superior frontal gyrus, L.SFG; left superior parietal lobe, L.SPL; left middle temporal gyrus (temporo-occipital part), L.toMTG; right anterior supramarginal gyrus, R.aSMG; right caudate, R.Caud; right superior parietal lobe, R.SPL; placebo, PLC; cerebellar vermis VI, Verm6.

