Autistic brain dynamics and responsivity to multi-target pharmaco-challenge with Cannabidivarin (CBDV)

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Abstract

Autism is associated with differences in the functional connectivity of whole-brain networks but results vary across studies. One reason for this is that conventional analyses average functional connectivity across the scan, ignoring potential group differences in dynamic (time-varying) brain fluctuations which may be captured across an fMRI session. Another possibility is that what is different in autism is not functional connectivity at 'baseline' but how responsive brain networks are to neurochemical challenges. Here we selected the cannabis compound cannabidivarin (CBDV) as a neurochemical challenge because of its pleiotropic action across multiple receptors rather than have a more restricted challenge. We then compared both core network strength and dynamic features (dwell time and fractional occupancy), of whole-brain intrinsic connectivity networks (ICNs) from resting-state functional MRI in autistic (n = 14) and non-autistic (n = 17) male participants at baseline (placebo) and following administration of a 600 mg dose of CBDV. Autistic participants showed longer dwell time and fractional occupancy of a default-mode-network (DMN)-occipital-auditory transient state than non-autistic controls at baseline. The effect of CBDV on the dwell time of DMN-subcortical and DMN-occipital-auditory states differed between non-autistic and autistic participants: within the non-autistic group, the dwell time was significantly increased but shifts within the autistic group did not reach statistical significance. These differences in networks responsible for introspection and sensory and salience processing may reflect atypical (i.e., 'stickier') autistic brain dynamics and a pharmacologically atypical autistic brain.

Introduction

Autism is a neurodevelopmental condition characterised by differences in social communication and interaction, restricted or repetitive behaviours and interests, and altered sensory reactivities[1]. Autism is also associated with a higher likelihood of co-occurring mental (and physical) health problems[2,3]. Hence, a better understanding of the neurological basis of autism is essential for developing effective interventions to support autistic people and to improve their quality of life.

Previous studies have linked autism to altered brain intrinsic connectivity networks (ICNs)[4], the common patterns of functional connectivity also known as resting state networks (RSNs). Many studies have reported atypical ICNs in autism, but findings have been inconsistent across the literature, with substantial variability in the direction and extent of reported differences (for a review, see Hull et al.[5]). Some studies have found underconnectivity within the default mode network (DMN), salience network, and the language network[6,7]; while overconnectivity has been reported between the DMN and the rest of the brain and between cortical and subcortical systems[8]. One possible reason for these discrepancies is that previous studies largely compared groups "at rest". However, the brain works as a dynamic system constantly coping with external stimuli to maintain homeostasis. Emerging evidence suggests that the responsivity of neurochemical systems is atypical in autistic individuals[9–11]. Differences between autistic and non-autistic individuals may become more apparent when the brain is challenged through external stimuli, e.g. with a neurochemical drug[12]. In addition, most previous reports characterising

intrinsic connectivity networks in autism have not accounted for the dynamic nature of brain activity. Functional activity is not static. It fluctuates over time, and this temporal variation, i.e. dynamic activity, also embeds crucial information about behaviour[13], cognition[14,15], and emotional regulation[16–18]. Conventional approaches using average functional connectivity may therefore mask atypical fluctuations in brain activity and connectivity. Compared to non-autistic controls, autistic people are reported to exhibit fewer transitions between transient brain states, altered dwell time and fractional occupancy of transient states[19–22]. Those dynamic features were associated with autistic symptoms[23–25], though differential responsivity of ICN dynamics to neurochemical modulation in autistic and non-autistic individuals has not been explored yet.

In this study, we used cannabidivarin (CBDV), a multi-target phytocannabinoid constituent of *Cannabis sativa*, which has been shown to differentially modulate striatal-cortical functional connectivity in autistic and non-autistic adults[26]. CBDV is a non-psychoactive phytocannabinoid and has a broad pharmacological profile - acting across multiple targets involved in homeostatic regulation[27]. For example, CBDV exhibits agonism at the CB2 receptors[28,29] inhibits the activation of G protein-coupled receptor 55 (GPR55)[30], stimulates and desensitizes transient receptor potential channels (TRP Channels)[31,32], and partially activates dopamine D2-like receptors[33]. It has been found to potentially benefit individuals with neurodevelopmental conditions[27] and therefore, is well-suited for probing brain homeostasis in autistic and non-autistic individuals.

Although CBDV targets extend beyond striatal-cortical pathways, little is known of autistic whole-brain response to CBDV. Here, we applied a data-driven whole-brain approach to characterise ICNs and test whether there are differences in ICN core network strength and dynamic activity between autistic and non-autistic individuals at baseline and in response to CBDV.

Methods

Participants

We carried out a secondary analysis of a resting-state MRI dataset from a randomised, double-blind, cross-over study[26,34]. The study recruited 17 non-autistic and 17 autistic adult men. Potential participants with major mental disorders, genetic disorders associated with autism, intellectual disability, and/or any contra-indication for an MRI scan were excluded. All participants in the autism group had a clinical diagnosis of autism from a recognised assessment service. Participants attended two visits, at least 13 days apart to allow for drug wash-out. Participants received a 600 mg oral dose of CBDV (provided by GW Research Ltd, Cambridge, UK) at one visit and a matched placebo at the other, with the order randomized.

MRI Data Acquisition and Preprocessing

MRI images were acquired following a placebo or 600 mg CBDV administered using a 3T GE Excite II MRI scanner (GE Medical Systems, Milwaukee, WI, USA). Structural MRI images were acquired with a 3D inversion recovery prepared fast spoiled gradient recalled (IR-FSPGR) sequence (slice thickness = 1.1 mm, 124 slices, flip angle = 20°, field of view (FoV) = 280 × 280 mm, echo time (TE) = 2.820 ms, repetition time (TR) = 6.968 ms, inversion time (TI) = 450 ms, matrix = 256 × 256). Resting-state fMRI images were acquired using an echo-planar imaging (EPI) sequence (slice thickness = 3 mm, slice gap = 0.3 mm, 38 slices, flip angle = 75°, FOV = 240 × 240 mm, acquisition matrix = 64 × 64, TE = 30 ms, TR = 2000 ms). During each visit, we collected one session of resting-state fMRI images for 512 s, comprising 256 time points.

Resting-state fMRI data were preprocessed and denoised with AFNI 21.1.07[35]. First, the first 10 volumes as unstable volumes and spikes were truncated in each voxel's time series. fMRI images were then co-registered to the corresponding structural MRI images, and both were aligned to the standard Montreal Neurological Institute (MNI) template. Slice timing correction was applied, followed by segmentation of the brain into white matter, grey matter, and cerebrospinal fluid (CSF). Each volume was smoothed using a 6 mm full width at half maximum (FWHM) Gaussian kernel. Each session mean was scaled to 100. Finally, nuisance signals—including the average time series of six motion parameters, white matter, and CSF—were regressed out, and the data were band-pass filtered between 0.01 and 0.1 Hz. Resting-state fMRI sessions with a high level of motion (mean framewise displacement (FD) > 0.5 mm, as in Satterthwaite et al.[36] and Power et al.[37]) were excluded.

Data Analysis

We decomposed the fMRI data into voxel-wise components representing spatial patterns of coherent brain activity using group-level Independent Component Analysis (ICA)[38]. We extracted 30 components, representing a pragmatic balance between robustness and interpretability[39–41], from the resting-state fMRI data across all participants under placebo condition. The spatial maps of those components were regressed into original resting-state fMRI voxel-wise BOLD timeseries under both placebo and CBDV to retrieve the subject-specific spatial maps of ICNs and subject-specific timeseries of ICN activity at every timepoint via dual regression. We performed group ICA and dual regression with the MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) 3.0 toolkit[42] and the dual regression function in FSL 6.0.7.10[43].

The core network strength[39] of each ICN was computed to measure the total connectivity strength of ICNs, i.e., the average activation level of an ICN across voxels of each participant during the whole scanning period. We obtained binary thresholded ICN masks by including voxels of $Z > 3$ in the Z-transformed ICN spatial maps[39,44]. Subsequently, the core network strength was calculated as a mean beta value across voxels of a subject-specific spatial map within the binary ICN map with FSL 6.0.7.10.

To examine ICN dynamics, we employed *k*-medoid clustering to define transient brain states, i.e., the recurrent patterns of ICN dynamic activity. K-medoid clustering is a partitioning method to identify

clusters with minimal sums of dissimilarities between points in the data[45]. A medoid is an actual data point with the smallest total distances to other points in a cluster. We applied *k*-medoid clustering to the ICN activity matrix of 246 timepoints × 20 ICNs × 52 resting-state fMRI sessions (including placebo and CBDV conditions) including all participants. We used the Manhattan distance to compute the dissimilarity matrices for clustering. To determine the number of clusters, we used the Davies–Bouldin index and the Calinski–Harabasz index to select k = [6, 8, 10, 12] as potentially the optimal number of partitions (Supplementary Fig. 1). We then inspected the medoids at different k, and selected k = 10 as it clearly differentiated brain states of various ICN coactivation patterns.

The dynamics of ICN activity were then characterised by fractional occupancy and dwell time of identified brain states. Fractional occupancy is the probability of a brain state occurring during an fMRI session. It was calculated as the percentage of time points clustered into a certain brain state among all time points. Dwell time is the average duration of a certain brain state during an fMRI session. It was calculated as the average number of continuous time points clustered into a certain brain state for each session. Scripts of R codes available in the *stateR* package[46] were used to extract these features.

Statistical Analysis

We first performed multiple linear regressions to examine the baseline (placebo condition) differences in core network strength, fractional occupancy, and dwell time between autistic and non-autistic participants. We then compared the effects of CBDV on core network strength, fractional occupancy, and dwell time between non-autistic and autistic participants using linear mixed-effects models with group (autistic and non-autistic) × drug (placebo and CBDV conditions) interaction as a fixed effect and participant ID as a random effect. Where significant interaction effects were found ($p < 0.05$), we performed a stratification analysis with linear mixed-effects models in autistic and non-autistic participants separately to further examine the different effects of CBDV in the two groups.

We controlled in our analyses for age, IQ and mean FD. All continuous variables were standardised before being entered into equations. R 4.4.1 was used to perform *k*-medoid clustering, multiple linear regressions, and linear mixed models as well as computing fractional occupancy and dwell time. Given the exploratory nature of our analysis, we reported results with both uncorrected and corrected *p*-values for multiple comparisons of core network strength of 20 ICNs, and of fractional occupancy and dwell time of 10 brain states using the Benjamini-Hochberg false discovery rate (FDR)[47]. *p*-values of stratification analyses were corrected for two groups (autistic and non-autistic).

Results

Following the exclusion of sessions with excessive head motion (8 sessions), the final dataset comprised 52 sessions (31 placebo and 21 CBDV) from 31 participants (autistic: n = 14) (Table 1). All participants were scanned under placebo; and 21 (autistic: n = 8) were also scanned under CBDV. Demographic information for the participants is presented in Table 1. Paired t-tests indicated that there are no statistically significant differences in age and IQ between autistic and non-autistic participants.

Table 1
Demographic information of the Participants.

Characteristics	Mean (*SD*)		t-test	
	Non-autistic	Autistic	*t* (95% CI)	*p*
Placebo	*N* = 17	*N* = 14		
Age (years)	28.06 (6.37)	31.14 (10.19)	-0.99 (-9.59–3.42)	0.34
IQ	124.59 (12.71)	112.14 (20.22)	2.00 (-0.49–25.38)	0.06
CBDV	*N* = 13	*N* = 8		
Age (years)	28.08 (6.32)	31.13 (11.73)	-0.68 (-13.14–7.05)	0.51
IQ	127.00 (12.18)	111.25 (18.85)	2.11 (-0.76–32.26)	0.06

Notes. *SD*: Standard deviation. *CI*: Confidence interval.

Core Network Strength

We identified 20 ICNs, including the anterior and the posterior DMN, the executive control network, the praecuneus-posterior-cingulate-cortex (praecuneus-PCC) network, the primary visual network, and the auditory network (Fig. 1).

At baseline, the core network strength of the executive control network was stronger in autistic than in non-autistic participants, but this effect was not statistically significant after adjustment for multiple comparisons ($\beta = 0.962$, $p = 0.022$, $p_{FDR} = 0.442$, Supplementary Table 1, Fig. 2). There was no significant group × drug interaction effect.

Dynamic Activity of ICNs

To examine the effects of CBDV on brain dynamics, ICN activity (Fig. 1) at each timepoint (i.e. betas from dual regression stage 1) was clustered into 10 main brain states (Fig. 3). The 10 medoids are shown in Fig. 3B to present the typical ICN activity patterns of the 10 brain states identified. The ICN activity patterns of each transient brain states are described in Supplementary Table 2.

To examine the baseline differences in the dynamic features of ICNs, we compared the fractional occupancy and well time under the placebo condition between autistic and non-autistic participants. The fractional occupancy (Fig. 4A) and dwell time (Fig. 4B) of State 10 (active anterior DMN, cingulo-opercular network, executive control network, occipital pole, sensorimotor network, lateral sensorimotor network, auditory network) were higher in autistic adults compared to non-autistic controls at baseline (Fractional occupancy: $\beta = 1.083$, $p = 0.005$, $p_{FDR} = 0.046$. Dwell time: $\beta = 1.340$, $p = 0.005$, $p_{FDR} = 0.045$) (Supplementary Table 3).

Significant group × drug interaction effects on the dwell time of State 6 (active anterior and posterior DMNs, praecuneus-PCC network, vm PFC, auditory network, basal ganglia, thalamus) and State 10 (active anterior DMN, cingulo-opercular network, executive control network, occipital pole, sensorimotor network, lateral sensorimotor network, auditory network) were found, though not surviving multiple comparisons correction (State 6: β = -1.367, p = 0.015, p_{FDR} = 0.077; State 10: β = -1.430, p = 0.014, p_{FDR} = 0.077; Supplementary Table 4). Within groups, CBDV increased the dwell time of State 6 (β = 0.601, p = 0.039, p_{FDR} = 0.078)(Fig. 5A) and State 10 (β = 0.774, p = 0.004, p_{FDR} = 0.008)(Fig. 5B) in non-autistic participants while the effect elicited by CBDV in autistic participants did not reach statistical significance (State 6: β = -0.897, p = 0.126. State 10: β = -0.716, p = 0.253).

Discussion

In this study we first compared the baseline core network strength and dynamic features of ICNs between autistic and non-autistic men. At baseline, we showed differences in ICN core network strength and in brain dynamic features (dwell time and fractional occupancy) of transient brain states, though only differences in dynamic features survived multiple comparison correction. We also explored whether CBDV "shifted" ICN core network strength and dynamic features in the two groups. CBDV did not have a significant effect on core network strength in either autistic or non-autistic groups but altered the dynamic features (dwell time) of State 6 (high activity in the anterior and posterior DMNs, thalamus, basal ganglia, praecuneus-PCC network, ventromedial prefrontal cortex, and auditory network) and state 10 (primarily active anterior DMN, occipital pole, auditory network, executive control network, cingulo-opercular network, and sensorimotor networks) differentially in the autistic and non-autistic group (group × drug interaction). Overall, our findings provide new insights into patterns of whole-brain ICN dynamics at baseline and following CBDV modulation in autistic and non-autistic adults, suggesting potential atypical neurochemical regulation of autistic individuals.

Baseline differences in core network strength and dynamic ICN features

At baseline (placebo), we found higher core network strength of the executive control network in the autistic group compared to the control, though with limited effect size not surviving correction for multiple comparisons. The executive control network is part of the salience network identifying salient stimuli and guiding attention and cognitive processes[48,49]. Our finding is in line with previous studies suggesting alterations in the salience network in autism. Green et al.[50] and Uddin et al.[51] have shown hyperconnectivity within the salience network in autistic children, especially between the anterior insular and anterior cingulate cortex. We extend these findings to adults, suggesting a consistent pattern of atypical functional connectivity related to processing salient information in autism across the lifespan.

At baseline, we also found that autistic (compared to non-autistic) adults entered more frequently, and dwelled longer in, State 10 (displaying primarily active anterior DMN, occipital pole, auditory network,

executive control network, cingulo-opercular network, and sensorimotor networks). This result aligns with previous reports that autistic young people were more likely to enter a transient brain state — showing coactivated DMN, executive control network, auditory network, and visual network — and showed overconnectivity of DMN with the sensorimotor network and visual network in that state[52]. More frequent coactivation of those networks might imply increased functional integration across these systems, i.e. increased higher-order multisensory integration[53] and increased functional integration between the DMN and networks involved in sensory and salience processing and attention regulation in autism[54,55]. The longer dwell time suggests less transition out of this state and is potentially related to reduced reconfiguration flexibility in autism[56]. The altered higher-order multisensory and DMN integration and cognitive flexibility might underpin atypical social communication and restricted and repetitive behaviours in autistic individuals[54] and their differences in cognitive functions[57].

Differences in responsivity of dynamic ICN features to CBDV

Despite no effects of CBDV on ICN core network strengths, we have shown that the effect of CBDV on the dwell time of State 10 differed between non-autistic and autistic participants, though the group × drug interaction effect did not survive correction for multiple comparisons. After taking CBDV, non-autistic participants stayed longer in State 10, but this effect contrasts with autistic individuals, who already “stick” to this state more (increased fractional occupancy and dwell time of State 10) at baseline. The result might suggest that CBDV has increased functional integration across DMN, sensorimotor network, salience network, cingulo-opercular network, and visual network in non-autistic participants. We have previously found that the functional connectivity of DMN with visual network, salience network, and somatomotor network was increased in non-autistic, but decreased in autistic participants by μ-opioid, $GABA_A$, and $GABA_B$ receptor activation[58]. Our findings add to the growing evidence that neurochemical systems regulate functional connectivity differentially between autistic and non-autistic individuals, particularly between the DMN and networks responsible for sensory and salience processing and attention regulation.

We also found that CBDV distinctively modulated dwell time of State 6 (characterised by high activity in the anterior and posterior DMNs, thalamus, basal ganglia, praecuneus-PCC network, ventromedial prefrontal cortex, and auditory network) in autistic and non-autistic participants, though the group × drug interaction effect did not survive correction for multiple comparisons. After taking CBDV, non-autistic participants stayed longer in State 6 whilst autistic individuals tended to stay shorter in this state. The anterior DMN, posterior DMN, praecuneus-PCC network, and ventromedial prefrontal cortex are highly correlated with each other as a broader DMN[59]. The differential effects of CBDV on this state might indicate different response mechanisms of subcortical-cortical functional connectivity in non-autistic and autistic individuals. Our previous study has also demonstrated that CBDV differentially modulated the connectivity between the subcortical structure, striatum and cortical regions in autistic and non-autistic men[26]. CBDV targets—such as CB2, TRP channels, and GPR55—are abundant in subcortical

structures[60–63]. Together, these findings suggest that the differential modulation of CBDV might stem atypical subcortical neurochemical response mechanism in autism[64,65].

Post-hoc analysis of group × drug interaction showed increased dwell time of State 6 and 10 in the non-autistic group after CBDV, but a non-significant drug effect in autistic participants. These non-significant results may be partially explained by a small sample size and a relatively low dosage of CBDV administered. A low dose may have constrained the drug's efficacy, particularly given that our previous research has identified differential effects between low and high doses of other pharmacological challenges[58]. The considerable variability in CBDV-induced changes among autistic participants (Fig. 5) suggests heterogeneous responses in dynamic brain activity responding to neurochemical modulation. This variability underscores the importance of considering individual neurobiological profiles, rather than assuming uniform effects across the autistic population, in future development of pharmacological interventions.

Limitations

Several constraints should be considered when generalising our findings. First, as this is an early trial of the effect of CBDV in humans, our sample size is small and consists only of males, which limits the generalisability of our findings to broader population (e.g., females). Second, we acknowledge that some of the effect sizes of our results did not survive the *p*-value adjustment for multiple comparisons and should be interpreted with caution. We employed an exploratory method to examine potential group differences without strong a priori regional hypotheses; uncorrected results reported might inform future hypothesis-driven work. Third, our analysis only examined the temporal variability of ICN activity without comparing pairwise connectivity between ICNs as well as connectivity within each ICN; considering the sample size of our study, there is likely insufficient statistical power to examine pairwise coactivation between 20 ICNs, but based on activity fluctuation we have clustered into 10 well-separated brain states featuring key coactivation patterns of different ICNs.

Conclusions

Our study interrogates the differential response of ICN dynamic features to CBDV modulation in autistic and non-autistic individuals. Examining ICN dynamics might provide valuable insights into the impacts of pharmacological probes on brain functions beyond just characterising average functional connectivity. The heterogeneity observed in autistic response to CBDV underscores the potential of developing individualised intervention based on diverse neurochemical regulation in autism. Future research will likely explore the relationship between changes in functional connectivity and autistic profiles as well as the effects of CBDV on cognition and behaviours to understand the cascading effects of changes in brain organisation.

Overall, we have shown atypical brain dynamic features (fractional occupancy and dwell time) of a brain state featuring coactivation of the DMN and networks involved in salience and sensory processing and attention directing in autism. The impact of CBDV on ICN dynamics differed between autistic and non-

autistic participants, and our findings imply atypical integration of those networks in autism. The differential response of ICN dynamics to CBDV underscores the distinct neurochemical regulation of network integration in autistic and non-autistic males, highlighting the importance of accounting for individual neurobiological profiles in personalized interventions.

Declarations

Conflict of Interest

D.G.M.M. has consulted for Jaguar Gene Therapy LLC. G.M.M. has received funding for investigator-initiated studies from GW Pharmaceuticals and COMPASS Pathfinder Ltd.. G.M.M. has consulted for Greenwich Biosciences, Inc. Y.G., C.M.P., S.F., M.D., B.I.V., G.I., D.J.L., E.D., and D.B. have no conflicts to declare.

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Figures

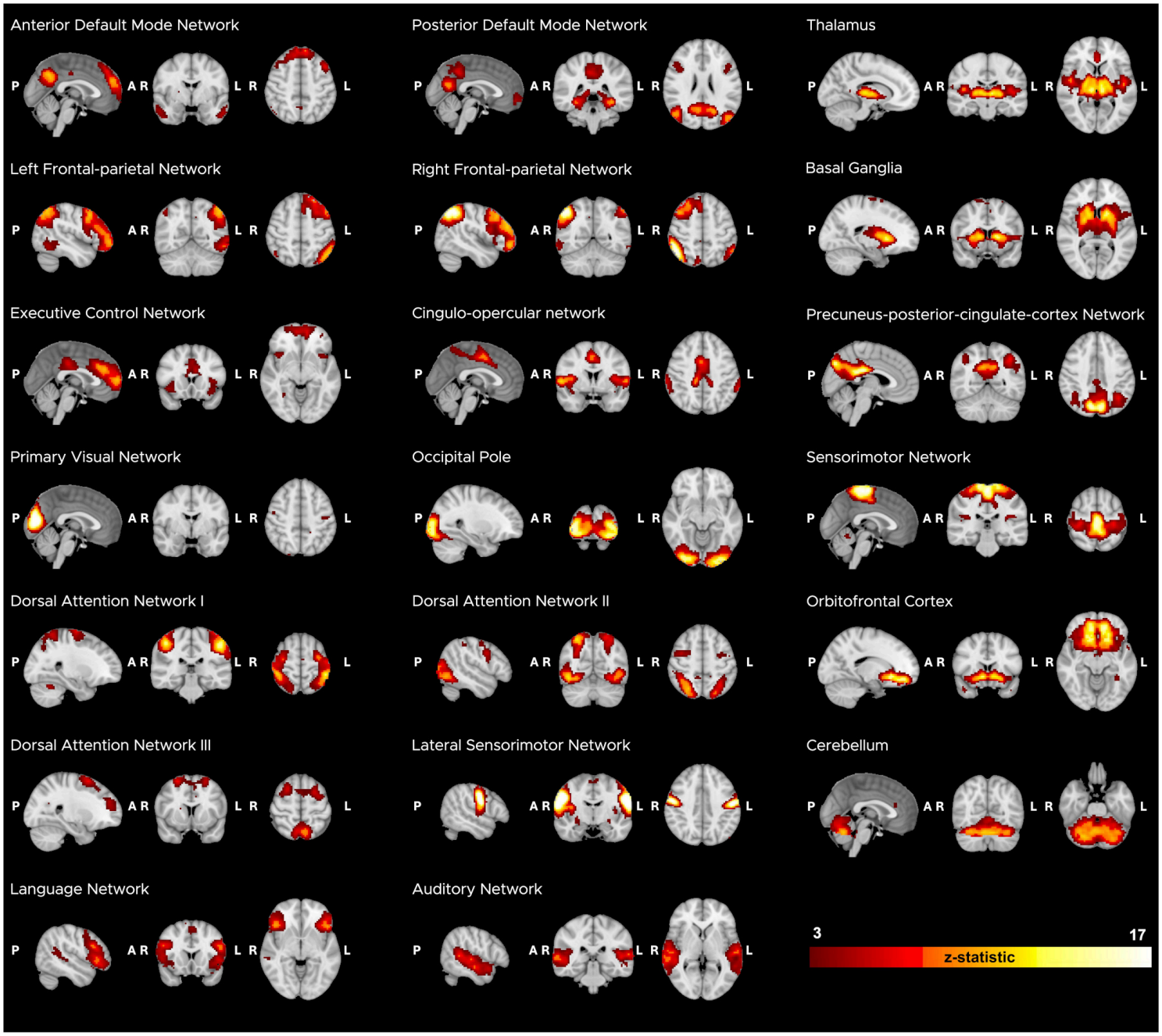

Figure 1

Intrinsic Connectivity Networks (ICNs) obtained with concatenated ICA from resting-state fMRI data under placebo condition including both autistic and non-autistic participants. z-statistics were thresholded at $z > 3$. L: left. R: right. P: posterior. A: anterior.

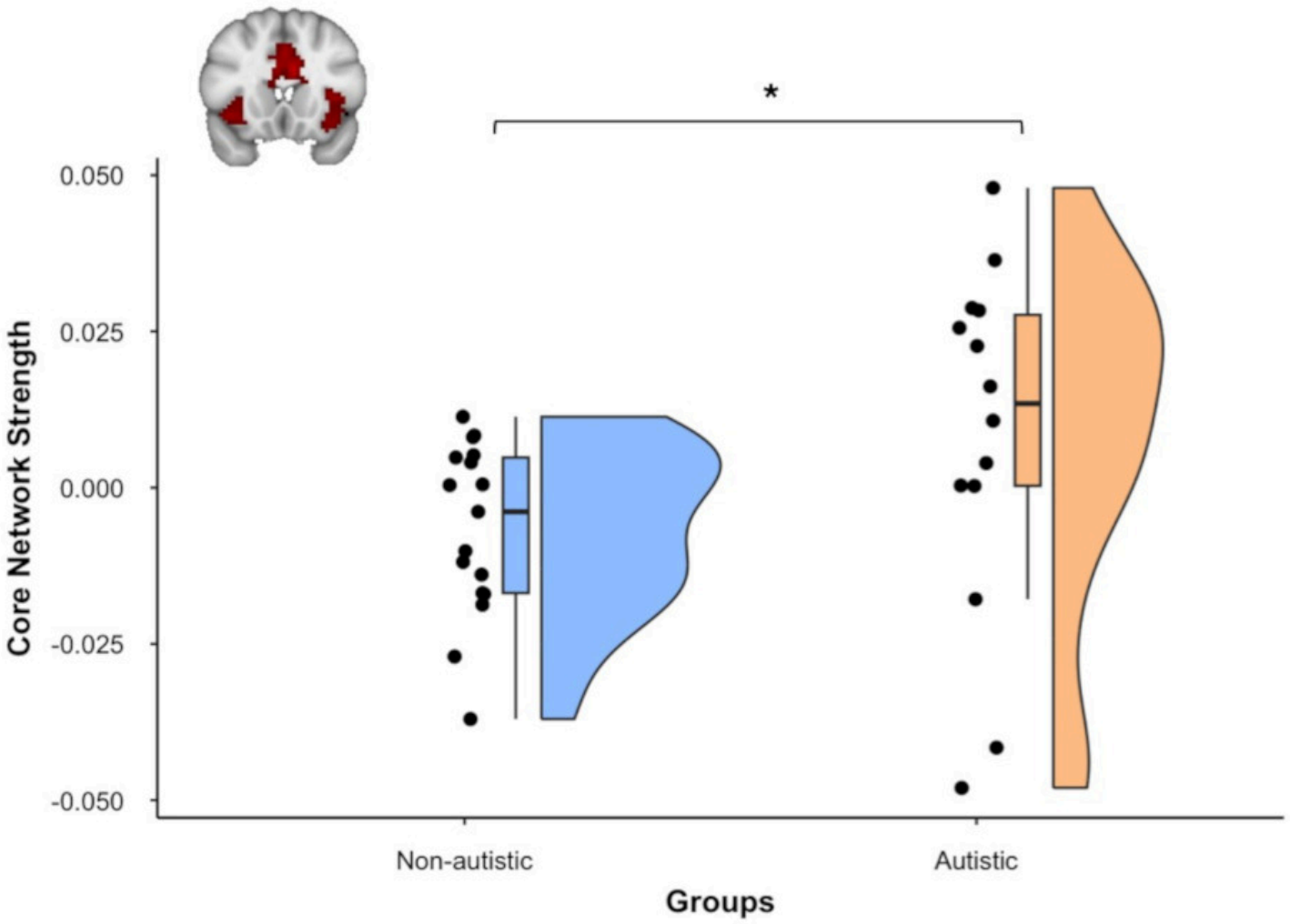

Figure 2

Baseline difference in the core network strength of the executive control network. The asterisk indicates that significant difference was found between the non-autistic and autistic groups ($p_{uncorrected} < 0.05$). The box bounds the interquartile range. Solid lines in the box indicate medians.

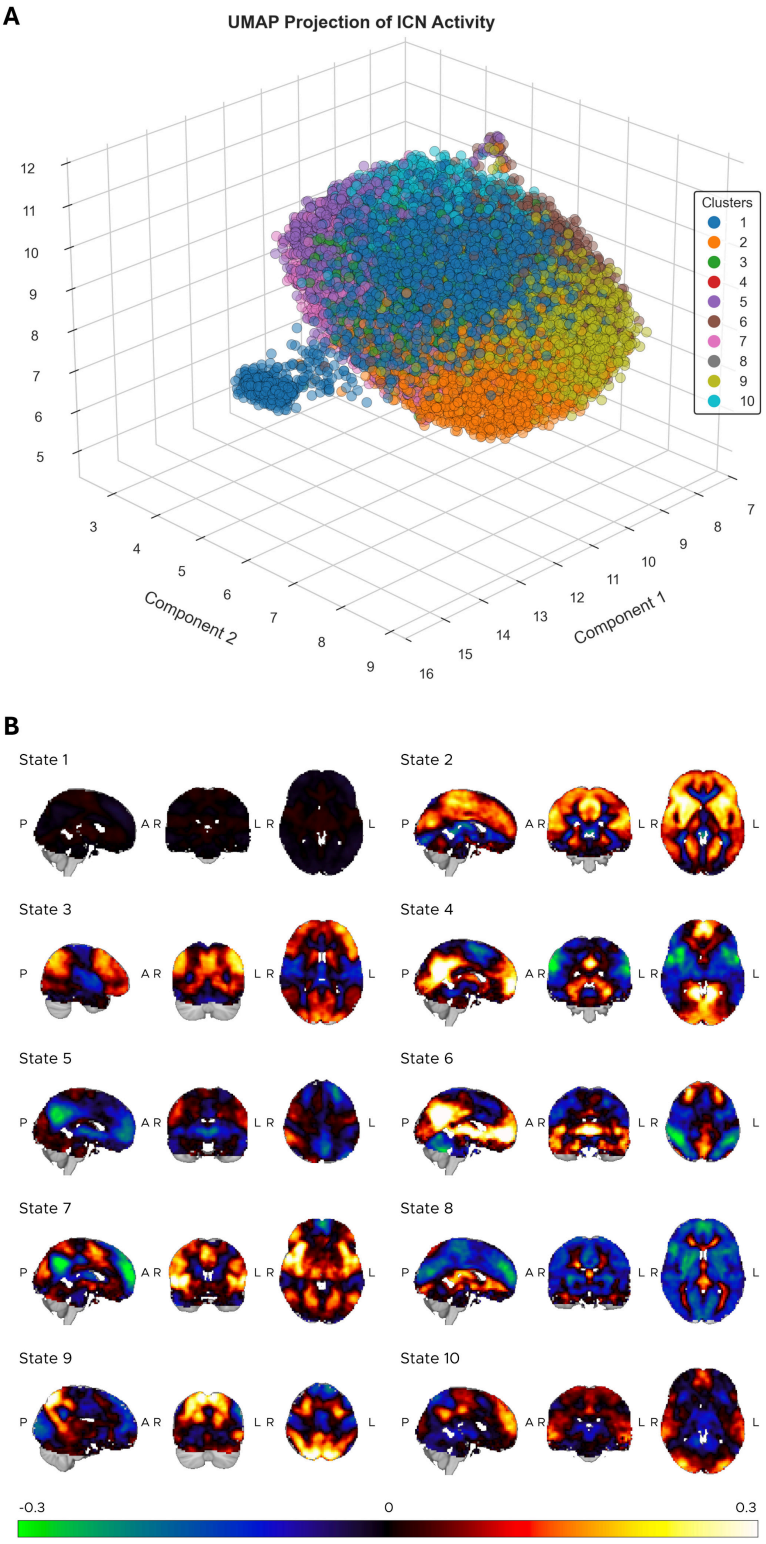

Figure 3

A) The ICNs activity at each timepoint was clustered into 10 transient brain states with K-medoid clustering. Uniform Manifold Approximation and Projection (UMAP) was used to reduce data dimensionality and project the data to a 3D space for visualisation purposes. B) The Medoids of the 10 Brain States. The medoid of each cluster represents the major spatial activity pattern of the brain state, being calculated as the weighted sum of ICN spatial maps ($\beta_{ICN\ 1,\ State\ n}$ ICN 1 spatial map + … + $\beta_{ICN\ 20,}$

State n ICN 20 spatial map). The red-yellow colour bar indicates brain regions with increased activity, while the blue-green colour bar indicates brain regions with decreased activity in that state. L: left. R: right. P: posterior. A: anterior.

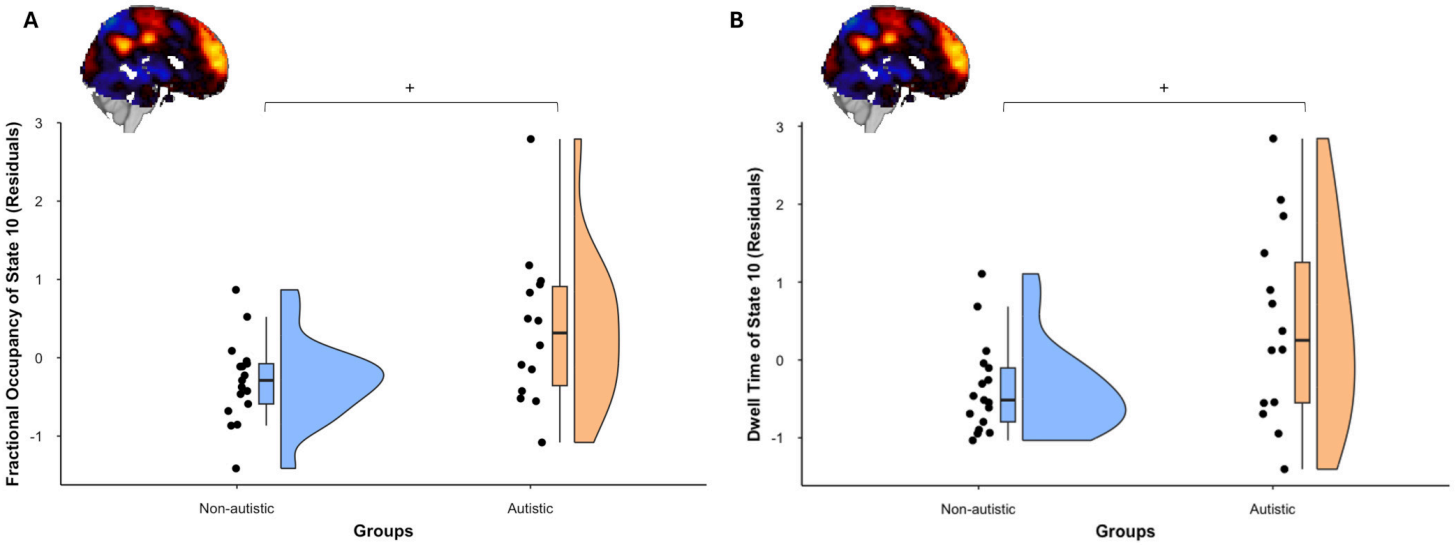

Figure 4

A) The fractional occupancy of State 10 (Active anterior DMN, cingulo-opercular network, executive control network, occipital pole, sensorimotor network, lateral sensorimotor network, auditory network) at baseline. B) The dwell time of State 10 at baseline. We plotted the residuals of fractional occupancy and dwell time after adjusting for age, IQ, and mean FD in multiple linear regression models. $^{+}$ indicates significant differences between the non-autistic and autistic groups ($p < 0.05$, FDR corrected). The box bounds the interquartile range. Solid lines in the box indicate medians.

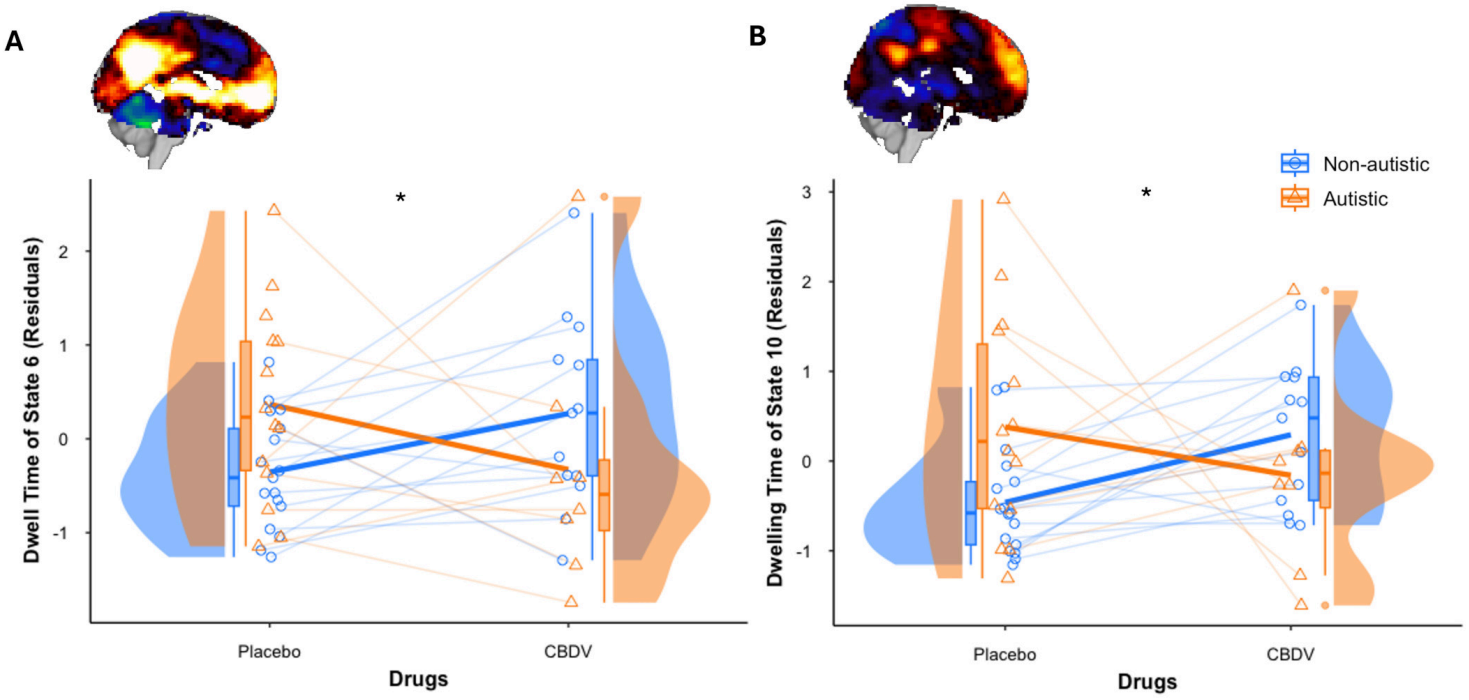

Figure 5

A) The dwell time of State 6 Under Placebo and CBDV conditions. B) The dwell time of State 10 under placebo and CBDV conditions. Residuals of dwell time were adjusted for age, IQ, and mean FD in a multiple linear regression model. * indicates significant group × drug interaction effect ($p_{uncorrected} < 0.05$). The box bounds the interquartile range. Solid lines in the box indicate medians.

Supplementary Files

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