Non-invasive Brain Stimulation Modulates GABAergic Activity in Neurofibromatosis 1

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Abstract

Neurofibromatosis 1 (NF1) is a single-gene disorder associated with cognitive phenotypes common to neurodevelopmental conditions such as Autism Spectrum Disorder (ASD) & Attention Deficit Hyperactivity Disorder (ADHD). GABAergic dysregulation underlies working memory impairments seen in NF1. This mechanistic experimental study investigates whether application of anodal transcranial direct current stimulation (atDCS) can modulate GABA and working memory in NF1. 31 adolescents with NF1 were recruited to this single-blind sham-controlled cross-over randomized trial. Active or sham tDCS was applied to the left Dorsolateral Prefrontal Cortex (DLPFC) and Magnetic Resonance Spectroscopy was collected before and after intervention in the left DLPFC and occipital cortex. Higher baseline GABA in the left DLPFC was associated with faster response times (RT) on baseline working memory measures. AtDCS was seen to significantly reduced GABA as compared to sham stimulation in the left DLPFC. There was no effect of atDCS on Glutamate/glutamine (Glx) in the left DLPFC or on GABA/Glx in the occipital cortex. This first such study in adolescents with NF1, showed that atDCS modulates inhibitory activity in the DLPFC. Given the strong evidence linking GABA abnormalities to cognitive deficits across neurodevelopmental conditions such as ASD, modulation of GABA using atDCS offers a promising therapeutic approach.

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Background

Perturbations in gamma-aminobutyric acid (GABA) inhibitory neurotransmission have been postulated to underlie common psychiatric disorders such as schizophrenia and neurodevelopmental conditions such as Autism Spectrum Disorders (ASD)(1, 2). GABAergic neurotransmission plays a central role in homeostatic plasticity mechanisms to maintain a fine balance between excitation/inhibition and promote network stability(3). Indeed, based on findings of preclinical studies, numerous pharmacological therapies (for instance bumetanide(4), memantine (5), arbaclofen(6)) hypothesized to target GABAergic neurotransmission have been tried in neuropsychiatric conditions, with the goal of restoring inhibitory/excitatory balance, particularly in the prefrontal cortex. However, human clinical trials of GABAergic drugs have shown limited therapeutic success, highlighting both the fundamental gap in mechanistic translation and need for objective clinical measures linked to the known mechanism of disease(7). Mechanistic studies in humans are the next important step to fully understand the role of GABAergic signaling and for the development of biologically-targeted therapies for neuropsychiatric disorders.

Syndromic versions of common mental disorders, although rare, can be used to provide important clues about underlying molecular pathogenesis. An example of this is Neurofibromatosis 1 (NF1), a rare single-gene disorder with birth incidence of 1: 2700(8), that is known to be associated with GABAergic dysfunction. Although NF1 is commonly recognised for its cutaneous manifestations and increased

tumour predisposition(9), significant morbidity is caused by cognitive, social and behavioural difficulties(10). The cognitive-behavioural phenotype in NF1 has been well-described and the disorder presents with many phenotypes seen in common mental illnesses. For instance, up to 70% of individuals with NF1 have impairments in working memory, 25% may have comorbid Autism Spectrum Disorder (ASD) and upto 50% Attention Deficit Hyperactivity Disorder (ADHD)(11, 12). As a monogenic disorder, the underlying neurobiology of NF1 is well-understood through the use of animal models. Mutation of the *Nf1* gene causes disinhibition of the RasMAPK and other downstream signalling pathways resulting in changes of synaptic proteins causing GABAergic overactivity and impairments in synaptic plasticity(13). More specifically, animal studies have demonstrated that GABAergic dysfunction underlies working memory impairments in NF1 by disrupting corticostriatal activity(14). Studies of GABA function *in vivo* are limited, with only two previous reports suggesting GABA dysfunction in children and adults with NF1(15, 16) associated with impairments in cognition, motor skills(17) and impulse control(18).

More recently, interventions such as Non-Invasive Brain Stimulation (NIBS) that can be used to modulate cortical plasticity have generated much interest as putative therapeutic treatments for learning impairments such as working memory deficits(19, 20). In this context, anodal transcranial Direct Current Stimulation (atDCS), which involves passing a small electric current through the scalp via scalp electrodes, has been of particular interest. AtDCS has facilitatory effects on the underlying neural tissue and has been shown, using Magnetic Resonance Spectroscopy (MRS), to reduce GABA in the stimulated cortex in healthy populations. This represents a putative mechanism to explain its known local ability both to induce temporary long-term potentiation like effects and to increase network-level functional connectivity(21). NIBS techniques therefore provide a distinct advantage over pharmacological agents in being able to induce local cortical changes.

In this study, we use NF1 as a model to study how inter-individual differences in GABA function relate to working memory performance- a phenotype shared across several neurodevelopmental disorders. We test the responsiveness of the GABAergic system to atDCS applied to the dorsolateral prefrontal cortex (DLPFC), a region chosen for its critical role in working memory in humans(22). We hypothesized that application of atDCS to the left dorsolateral prefrontal cortex (DLPFC) would reduce GABA and improve performance on working memory tasks.

Results

Thirty-one participants participated in the experimental sessions over two separate days. Twenty-nine participants completed both study visits, and two participants only completed one visit (further visits suspended due to COVID-19 related lockdown). The clinical characteristics of the sample are described in Table 1.

Table 1
Clinical characteristics of the study sample

Males	15/31
Age (mean)	14.7 yrs (11.4-18.3)
Pre-existing diagnoses(n)	
<i>ADHD</i>	8
<i>ASD</i>	3
Medications	
<i>Stimulants</i>	6
<i>Atomoxetine</i>	1
Vineland Adaptive Behavior Composite(mean)	68.4 (13.0)
Conners (mean)	
<i>Inattention T score</i>	78.7 (13.0)
<i>Hyperactivity T score</i>	69.1(18.2)

1. Higher baseline DLPFC GABA associated with shorter response times

We first tested the hypothesis that higher baseline GABA concentration (measured at the first visit) would be related to better working memory performance. No significant correlation between baseline GABA and working memory accuracy was observed (Verbal working memory $r(27)=0.24$, $p=0.21$, Visuospatial working memory $r(27)=0.11$, $p=0.58$) but there was a significant correlation between GABA and RTs on both verbal and visuospatial working memory, such that patients with higher GABA showed faster RTs (Verbal working memory $r(27)=-0.44$, $p=0.02$, Visuospatial working memory $r(27)=-0.65$, $p<0.001$, figure 1)). This result was neurotransmitter specific: no significant relationship was observed between Glx and RT (Verbal RT $r(27)=0.09$, $p=0.63$, Visuospatial RT $r(27)=0.14$, $p=0.46$) and there was a significant difference between the GABA/Glx correlation coefficients for working memory RT (verbal RT $z=-2.07$, $p=0.04$, visuospatial RT $z=-3.43$, $p<0.001$). This finding was also anatomically specific: there was no relationship between OCC GABA and RT (Verbal RT $r(27)=-0.01$, $p=0.95$, Visuospatial RT $r(27)=-0.03$, $p=0.86$) and there was a significant difference between the DLPFC/ OCC GABA and RT correlation coefficients (verbal RT $z=-1.73$, $p=0.04$, Visuospatial RT $z=-2.79$, $p<0.001$).

There was no relationship between parent rated measures and DLPFC GABA including baseline adaptive functioning ($r(27)=0.18$, $p=0.34$), inattention ($r(27)=-0.07$, $p=0.73$) or hyperactivity ($r(27)=-0.19$, $p=0.32$).

Similarly, no relationship was observed between DLPFC Glx and baseline adaptive function ($r(27)=-0.10$, $p=0.60$), inattention ($r(27)=-0.09$, $p=0.66$) or hyperactivity ($r(27)=-0.14$, $p=0.48$). No relationship was observed between any of the neurotransmitters in the occipital cortex and parent reported metrics.

2. Anodal tDCS is associated with greater reduction in GABA relative to sham but there is no effect on behavioural outcomes

We then wanted to investigate whether atDCS induced the previously reported decrease in GABA in DLPFC. AtDCS led to a greater decrease in DLPFC GABA relative to sham ($F_{1,46}=4.12$, $p=0.05$, $\eta_p^2=0.08$). There was a 26.77% (SD 4.05%) change in GABA in atDCS group as compared to 11.45% (SD 4.07%) change in the sham group. There was no significant effect of atDCS on Glx ($F_{1,46}=0.69$, $p=0.41$, $\eta_p^2=0.02$). In the control occipital voxel, there was no significant effect of atDCS on GABA ($F_{1,49}=0.94$, $p=0.34$, $\eta_p^2=0.02$) or Glx ($F_{1,49}=0.01$, $p=0.94$, $\eta_p^2=0.00$, figure 2).

There was no effect of atDCS on Corsi block memory span after accounting for baseline performance ($F_{1,57}=0.84$, $p=0.36$, $\eta_p^2=0.01$) or Corsi block RT ($F_{1,57}=0.02$, $p=0.88$, $\eta_p^2=0.00$). On the task performed inside the scanner, there was no effect of atDCS on task accuracy (2-back task accuracy; $F_{1,44}=0.05$, $p=0.82$, $\eta_p^2=0.00$) or RT (2-back task RT $F_{1,44}=0.71$, $p=0.41$, $\eta_p^2=0.02$, figure 3).

3. Adaptive function predicts GABA response to atDCS

Given previous reports that atDCS may be more effective in those with lower functioning, we tested whether there was any correlation between GABA change following atDCS and baseline adaptive functioning (parent reported Vineland ABC) and working memory. In the atDCS group, there was a significant inverse correlation between baseline adaptive functioning and the change in GABA in DLPFC ($r(22)=-0.43$, $p=0.04$, figure 4) suggesting that the lower the adaptive functioning, the greater the GABA change in response to atDCS. In the sham group, there was no significant association between GABA change and adaptive functioning ($r(23)=-0.09$, $p=0.67$). However, there was no difference between the active/sham GABA-ABC correlation coefficients ($z=-1.21$, $p=0.22$).

4. Side effects associated with stimulation

We administered a standard side-effect rating scale with rating of 1-3 (none, mild, severe) after each experimental session. All side-effects are listed in Table 2. More participants in the active group reported tingling, warmth, sleepiness and trouble concentrating. There were no mood changes reported by any participants.

Table 2
Number of individuals reporting side effects associated with stimulation in both groups

	Active		Sham	
	Frequency of reported symptoms (n)			
	mild	severe	mild	severe
Tingling	12	1	5	0
Itching	7	1	8	0
Warmth	5	2	4	1
Burning	1	1	0	0
Pain	0	1	3	0
Redness	0	0	0	0
Sleepiness	12	3	9	4
Trouble concentrating	11	1	9	1
Mood changes	0	0	0	0

[INSERT Table 2 HERE]

Discussion

Using a rare genetic disorder known to be associated with GABAergic dysregulation, the goal of this study was to examine how inter-individual differences in GABA relate to working memory and to test the responsiveness of the GABAergic system to the application of atDCS to DLPFC. This is the first such study in adolescents with NF1, showing that atDCS modulates inhibitory activity in the DLPFC. Baseline GABA function did not relate with working memory accuracy, but higher GABA levels were associated with faster response times on the tasks. Furthermore, individuals with lower adaptive function showed a greater GABA response to atDCS. Overall, this study suggests that atDCS has an excitatory effect on the prefrontal cortex.

GABAergic interneurons within the prefrontal cortex have a well-established role in supporting working memory function. Converging evidence from animal and post-mortem studies suggests GABAergic interneuron dysfunction may underlie cognitive impairment seen in many neuropsychiatric diseases(23). We found that higher DLPFC GABA in NF1 was associated with faster RTs but did not show a significant association with working memory accuracy or general adaptive function. This relationship showed regional specificity and neurochemical specificity – as it was not observed in the occipital cortex, and specific to inhibitory (GABA) neurotransmission, suggesting frontal inhibitory neurotransmission as an

important neural correlate of the NF1 cognitive profile. Our findings are in line with a previous report showing that higher frontal GABA in NF1 was associated with faster responses on inhibitory-control tasks(18). Although our study was limited by not including a control population, studies in healthy populations suggest that higher frontal GABA is associated with superior cognitive abilities but a more cautious response style(24), rather than faster response times as observed in this study. Our findings in the NF1 cohort may therefore indicate a disruption of the relationship between DLPFC GABA and working memory performance and are in line with patterns seen in disorders like schizophrenia(25). It is possible to speculate that this disrupted GABA-working-memory relationship reflects increased GABAergic inter-neuronal inhibition and alterations in GABA dynamics in the prefrontal cortex as previously reported in animal models of NF1(14).

Performance of a cognitive task and the resultant changes in neural activity may be associated with a variety of neurometabolic effects including changes in GABA, Glx, aspartate, glucose and lactate(26). Application of atDCS, particularly when combined with a cognitive task has been shown to modulate executive functioning both in healthy subjects and those with underlying psychiatric disorders. We observed significantly greater reduction in DLPFC GABA in response to atDCS as compared to the sham stimulation. AtDCS had no differential modulatory effect on GABA in the occipital cortex showing regional specificity for its effects. We did not see a change in Glx in response to left DLPFC stimulation as reported by two previous studies(27). This discrepancy may be explained by inclusion of patient cohort known to be associated with GABAergic dysregulation rather than a healthy population and due to methodological differences between these studies such as electrical montage and participants being engaged in a cognitive task whilst the stimulation was being delivered. Our results demonstrate that atDCS modulates neuronal excitability of the DLPFC and merits further investigation in disorders like NF1, where GABAergic overactivity has been shown to underlie cognitive(28) and social deficits(13). This study did not examine dynamic functional changes in neurometabolite concentrations in response to cognitive tasks but rather measured changes at baseline and following task completion. Further research should seek to apply functional MRS techniques to study GABA dynamics during the application of atDCS and further our understanding of neurometabolite responses to neural activation.

The mechanism of action of atDCS is known to be mediated via effects on GABAergic neurotransmission but with possible effects on dopaminergic neurotransmission. Preclinical studies suggest that stimulation leads to a rapid change in glutamic acid decarboxylase (GAD65 and GAD67), resulting in reduced conversion of glutamate to GABA (29). The prefrontal cortex is heavily regulated by dopamine and stimulation of frontal cortex may lead to an increase in extracellular dopamine concentrations(30). A recently published study demonstrated that stimulation of left DLPFC in healthy populations is associated with reduction of GABA in left DLPFC but also modulation of both GABA and dopamine in the striatum, demonstrating the effect of atDCS beyond the targeted cortical structures(31). Given the importance of corticostriatal activity in NF1 and other neuropsychiatric conditions, it will be important to determine in future studies whether stimulation of DLPFC has a modulatory effect on the neurotransmitters in the striatum. Non-invasive brain stimulation may therefore be particularly relevant to the NF1 population, which is associated with GABAergic overactivity downstream to RasMAPK

overactivity but also reduced dopaminergic neurotransmission mediated via the cyclic AMP pathway(32-34).

Changes in GABA post stimulation were not mirrored by changes in working memory behavioural metrics. These results are perhaps not surprising as previous studies in healthy populations have demonstrated no behavioural effects of a single session of atDCS over DLPFC(35, 36). It is possible that behavioural changes develop over a longer period or that repeated sessions of atDCS are required for a measurable impact on behavioural outcomes. We used 1mA current based on previous data in paediatric cohorts, but it is possible that higher currents may be needed for behavioural effects. Performance fatigue/drowsiness due to a long period of time inside the scanner could also have contributed to the lack of observed behavioural effects. Decreased performance and increased RT due to fatigue could potentially negate any learning/stimulation related behavioural effects. We found that GABA response to atDCS was significantly higher in those with lower baseline adaptive functioning. There is well recognised inter- and intra-individual variability in response to atDCS and baseline functioning has been shown to contribute to this(37, 38). Previous work has shown that atDCS applied to the motor cortex reduced GABA and the extent of this reduction correlated to the degree of motor learning(39). Better understanding of how inter-individual factors may affect response to atDCS is therefore crucial for successful clinical translation for therapeutic purposes.

Limitations

Limitations of our work include use of brief behavioural outcome measures post intervention(chosen to reduce participant burden) and the lack of a healthy control group. It is important to note that GABA levels measured in this study will contain contributions from co-edited macromolecule signal (so-called GABA+), but the relative contribution of these macromolecular signals are thought to be constant and hence unlikely to account for within participant/session GABA changes. Strengths of this study included application of intrascanner atDCS allowing us to probe its effects on GABA function pre- and post-application without moving the participant out of the scanner. Further studies are needed to characterise the neurometabolic effects of atDCS particularly the effects of repeated sessions of atDCS. It will be important to clarify whether there is an effect of atDCS on subcortical structures particularly the striatum, given the important role of cortico-striatal circuitry for complex cognitive functions.

Working memory is of central importance for effective human behavior and an important predictor of academic success (40). Working memory and other learning impairments are commonly associated with NF1 and other neurodevelopmental and mental health conditions with significant impact on trajectories of academic achievement and overall quality of life. Cognitive remediation techniques may offer some amelioration but there are no effective pharmacological therapies available. Given the strong evidence linking GABA abnormalities to cognitive deficits across neurodevelopmental conditions such as ASD, modulation of GABA using atDCS offers a promising novel therapeutic approach. In summary, this is the first study in an adolescent population combining atDCS and MRS online demonstrating direct

modulation of neurometabolites with atDCS in real time. Further studies are needed to probe the mechanistic effects of atDCS including individualization of stimulation parameters.

Methods

Subjects

Thirty-one adolescents aged 11-17 years were recruited via the Northern UK NF-clinical research network. Inclusion criteria included (i) Clinical diagnosis made using the National Institute of Health diagnostic criteria(41) and/or molecular diagnosis of NF1; (ii) No history of intracranial pathology other than asymptomatic optic pathway or other asymptomatic and untreated NF1-associated white matter lesion or glioma; (iii) No history of epilepsy or any major mental illness; (iv) No MRI contraindications. Participants on pre-existing medications such as stimulants, melatonin or selective serotonin re-uptake inhibitors were not excluded from participation. The study was conducted in accordance with local ethics committee approval (Ethics reference:18/NW/0762, ClinicalTrials.gov Identifier: NCT0499142. Registered 05/08/2021; retrospectively registered, <https://clinicaltrials.gov/ct2/show/NCT04991428>).

Experimental Procedure - cross over intervention design

The effect of atDCS on GABA and working memory was tested using a two parallel-arm, single(participant)-blinded, sham-controlled cross-over design. Each participant had two study visits at least one week apart- one with atDCS intervention and with sham as placebo control. The order of these sessions was randomized and counter-balanced. Baseline assessments (as described below) were conducted at the first visit. Subjects were positioned comfortably in the scanner and a high-resolution T1-weighted image was acquired (see figure 5). The T1-weighted image was used to place a voxel of interest (VOI) by hand - over the DLPFC and another VOI in the occipital cortex. MRS was acquired from both voxels and participants were asked to perform a working memory task for 24 minutes (4 blocks of 6-minutes each) during which fMRI data were acquired(the results of which are not reported here). AtDCS or sham stimulation was started after the first block of working memory task and continued for 15 minutes during which the participant engaged in 2 more blocks of working memory tasks. Between each working memory block, participants were asked if they were comfortable and instructions were repeated again. Following tDCS, participants performed the final block of the working memory task. Finally, MRS was acquired again from both voxels. T2-weighted images were acquired at the first visit (after the T1 image) and reviewed by a paediatric neuroradiologist (SS) to rule out NF1 associated tumours. The sample size of 30 participants in this study, powered on the expected change of 20% in GABA following tDCS based on our previous work(42).

tDCS stimulation

AtDCS was delivered via a NeuroConn DC-STIMULATOR MR with the anode placed over F3 position in the international 10–20 system and the cathode over the Cz position. Scalp was cleaned with Nuprep gel and Ten20-paste was used as a conductive medium between the scalp and the electrodes. For anodal stimulation, the current was ramped up over 15 s, held at 1 mA for 15 min and then ramped down over 15 s. For sham stimulation, the current was ramped up over 15 s and then immediately turned off. The current parameters were chosen based on our previous experience from a pilot clinical trial of safety in this cohort (clinical trials identifier: NCT03310996). The atDCS induced electrical fields are simulated in figure 6. SimNIBS 3.2 (<https://simnibs.github.io/simnibs/build/html/index.html>) was used to estimate the electric field induced by tDCS (43, 44). The headreco pipeline (45) was used to segment the different tissue types and create a finite element mesh corresponding to an example T1 image from an open source dataset (46). The anode and cathode were placed at F3 and Cz respectively, and the standard SimNIBS conductivity values were used.

Structural and MRS data acquisition and analysis

Scanning was performed on a Philips Achieva 3T scanner (Best, NL) using a 32-channel head coil. 3D T1-weighted magnetic resonance images were acquired sagittally with a magnetization prepared rapid acquisition gradient-echo sequence (repetition time = 8.4 ms; echo time = 3.77 ms; flip angle = 8°, inversion time = 1150 ms, 0.94 mm in-plane resolution and 150 slices of 1mm). Single voxel ¹H MRS data were acquired before and after stimulation from two volumes of interest (VOI) in each participant. One VOI (40 x 20 x 24 mm) was placed in the left DLPFC and a control VOI (20 x 50 x 20mm) was placed within the posterior occipital lobe, centred on the mid-sagittal plane to cover both hemispheres (figure 5). For detection of GABA, GABA-edited MEGA-PRESS spectra (47, 48) were acquired with a repetition time of 2000 ms, echo time of 68ms, 1024 sample points collected at a spectral width of 2 kHz, as previously described (49). The DLPFC MRS took approximately 7 min to acquire, with 96 averages and OCC voxel took 3 min to acquire with 32 averages. The number of averages were chosen to approximately match spectral quality between DLPFC and OCC.

Quantification was conducted using the Advanced Magnetic Resonance (AMARES)(50) routine in the Java-based magnetic resonance user's interface (jMRUI5.1, EU project)(51). To improve the display of the spectra, line broadening of 6Hz was used. No time-domain filtering was performed on the data before analysis by AMARES. Metabolite resonances including GABA, glutamate + glutamine (Glx) and N-acetylaspartate (NAA) were calculated relative to the unsuppressed water signal from the same voxel. To examine partial volume effects on MRS voxels of interests, the T1-weighted anatomical images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). Voxel registration was performed using custom-made scripts developed in MATLAB by Dr. Nia Goulden, which can be accessed at <http://biu.bangor.ac.uk/projects.php.en>. The scripts generated a mask for voxel location by combining location information from the Philips SPAR file with orientation and location information contained within the T1 image.

In the aTDCS group, 28/29 DLPFC pre-intervention spectra and 24/29 post-intervention spectra were included for analyses (1 pre-intervention and 3 post intervention spectra rejected due to movement artefacts and 2 rejected due to >3SD difference in pre-post intervention NAA line width) and 29/29 pre-intervention OCC spectra and 28/29 post-intervention OCC spectra were included (1 post-intervention spectra not acquired due to technical difficulties). In the sham group, 31/ 31 DLPFC pre- intervention spectra and 25/31 DLPFC post-intervention spectra were included for analyses (5 rejected due to movement artefacts and 1 rejected due to >3SD difference in pre-post intervention NAA line width) and 29/31 pre-intervention OCC spectra and 25/31 post intervention OCC spectra were included for analyses(2 pre and 3 post-intervention spectra not acquired due to technical difficulties, 2 post-intervention spectra rejected due to movement artefacts and 1 due to >3SD change in NAA LW). The calculation of partial volume within the VOIs provided the percentage of each tissue type within the relevant voxels. The DLPFC VOI consisted of 39% ($\pm 17\%$) of GM, 24% (± 23) of WM, and 37% ($\pm 31\%$) of CSF and the OCC VOI, 50% ($\pm 10\%$) of GM, 37% ($\pm 15\%$) of WM, and 13%($\pm 9\%$) of CSF. The tissue fraction was used as a covariate in the baseline analyses of the relationship between GABA and behavioural measures.

Baseline assessments

Detailed cognitive assessments were carried out to assess working memory at baseline, at the first visit of the participant. Both verbal and visuospatial working memory were assessed using the n-back task. The task was programmed in-house using E-Prime software. Each participant completed verbal and visuospatial tasks at four levels of complexity- 0-back, 1-back, 2-back and 3-back tasks. For the verbal task, random letters were presented one at a time and the participant was asked to respond with a key-press if the letter corresponded to the letter one (1-back), two (2-back) or 3 (3-back) letters before. For the 0-back verbal task, participants were asked to press the key to the occurrence of the letter 'X'. For the visuospatial n-back task, blue squares were presented sequentially on a black 2 x 2 grid. Participants were instructed to respond with a key press if the position of the square matched the position one (1-back), two (2-back) or 3 (3-back) positions before. For the 0-back visuospatial task, participants were asked to respond with a key press to the occurrence of an orange square. Each participant was presented with three blocks of each n-back task (24 blocks in total). All stimuli were presented for 500 ms and the inter-stimulus interval was set to 1,500 ms. Accuracy was calculated as the proportion of correctly identified hits + correct omissions within each block (correct hits + correct omissions/ total responses) averaged across each n-back condition as presented in Table 1. Response times (RT) were calculated only for time to correct response to target stimuli, averaged across each n-back condition.

Parent-rated Vineland Adaptive Behaviour Scale - third edition(52) was administered to the parents to assess child adaptive behaviour with overall functioning computed as standardized age equivalent and expressed as an Adaptive Behaviour Composite (ABC). Conners 3 rating scale(53) was used as a standardized measure for parent reported ADHD symptoms. It consists of 27 items each rated on a 4-point Likert scale (0 = not true at all to 3 = very much true) in five subscales: attention, hyperactivity,

learning problems, oppositionality and peer problems. The inattention and hyperactivity subscales are reported below.

Behavioural outcome measures

At the start and end of each scanning session, while outside the scanner, participants were asked to complete the computerised Corsi block task on the Psychology Experiment Building Language (PEBL) (54). In this task, 9 identical blue blocks are presented on the screen. These blocks light up on the screen in a sequence, which starts off as a simple sequence of two blocks and increases in complexity based on participant performance. The participant is asked to mimic the sequence observed on the screen. A measure of the memory span and mean RT is reported.

Within the scanner, participants performed 4 runs of working memory tasks- one run each before and after stimulation and two during the atDCS/sham stimulation. Each run consisted of 6 blocks each of 0-back and 2-back verbal working memory task as described above. Each block was 30 s long and consisted of 9 target stimuli. Accuracy was calculated separately for 0-back and 2-back tasks (correct hits + correct omissions/ total responses). RT were calculated only for time to correct response to target stimuli.

Statistical analysis

Statistical analyses were performed in SPSS version 25 and R version 1.2. Partial Pearson's correlations were used to investigate the relationship between GABA in DLPFC and OCC and the behavioural outcomes using tissue fraction as a covariate in the analyses. The Fishers Z transformation was used to compare the correlation coefficients. Comparison of correlation coefficients was undertaken using Fisher's transformation. Group differences in metabolites post intervention were analysed using linear regression models adjusting for baseline values of the relevant outcome as a linear covariate. A p value < 0.05 was considered significant.

Declarations

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Author contributions

SG designed the study, was responsible for data collection and the overall conduct of the study, analysis, interpretation of the data and drafting of the manuscript. SW contributed to study design, guidance for MRS data collection and analyses, contributed to the drafting of the manuscript. JJ contributed to data analyses and interpretation and input into drafting of the manuscript. GP designed the behavioral tasks, provided input for the use of the intervention and contributed to the drafting of the manuscript. TN contributed to the data analyses and interpretation. BL contributed substantially to acquisition of the data and the initial analyses. GV reviewed all the neuroimaging data for clinically relevant pathology and contributed to the interpretation of the findings. JG & DGE provided input into study design and interpretation of results. LP and CS provided input into study design, designing the neuroimaging protocol, advise regarding the interpretation of findings and drafting of the results. SS contributed to study design, reviewed all the neuroimaging data for clinical interpretation and the drafting of the manuscript. All authors read and approved the submitted manuscript.

Additional information

All the datasets included in the project have been deposited on the Sage Bionetworks data repository <https://www.synapse.org/>. Approved researchers can request to obtain the data which are subject to data sharing agreements.

Ethics approval for the study was obtained from the North West- Greater Manchester South Research Ethics Committee (reference:18/NW/0762). Written informed consent was obtained from the parents and older adolescent participants and assent was obtained from the younger participants.

Conflict of interest

The authors declare that they have no financial or non-financial competing interests.

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Figures

Figure 1

Correlation between working memory accuracy response time (ms) with baseline dorsolateral prefrontal cortex GABA and occipital GABA adjusted for voxel tissue fraction.

Figure 2

Change in neurotransmitters in the active and sham tDCS groups in (A) DLPFC GABA , (B)DLPFC GLx (C) Occ GABA and (D) Occ Glx Box plots showing mean and 95% confidence intervals.

Figure 3

Mean and 95% confidence intervals for the task accuracy and response time on the 2-back task (2-back RT) performed inside the scanner on the Working Memory blocks (R1-4: 4 blocks of n-back tasks)

Figure 4

The relationship between % GABA change and parent reported adaptive functioning in (A) Active and (B) Sham groups

Figure 5

(A) Study design demonstrating the imaging sequences acquired in the scanner. WM1-4: Working memory blocks 1,2,3,4. Stimulation started at end of block 1 and continued during blocks 2 and 3. Each imaging sequence was preceded by checking if participant was ok and providing instructions. (B) Figure showing the placement of the tDCS electrodes, the current and stimulation time (C) Axial, sagittal and coronal images showing the placement of the voxels of interest and corresponding example MEGA-PRESS edited spectra acquired from Dorsolateral Prefrontal Cortex and Occipital Cortex. The original data are shown in red with the fit generated by AMARES for NAA, GABA and Glx overlaid in purple.

Figure 6

Simulated electric field magnitude (normE) induced by atDCS.