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Abnormal relationship between GABA, neurophysiology and impulsive behavior in neurofibromatosis type 1

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Abstract

Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder characterized by a broad spectrum of cognitive deficits. In particular, executive dysfunction is recognized as a core deficit of NF1, including impairments in executive attention and inhibitory control. Yet, the neural mechanisms behind these important deficits are still unknown.

Here, we studied inhibitory control in a visual go/no-go task in children and adolescents with NF1 and age- and gender-matched controls ($n = 16$ per group). We applied a multimodal approach using high-density electroencephalography (EEG), to study the evoked brain responses, and magnetic resonance spectroscopy (MRS) to measure the levels of GABA and glutamate + glutamine in the medial frontal cortex, a brain region that plays a pivotal role in inhibitory control, and also in a control region, the occipital cortex. Finally, we run correlation analyses to identify the relationship between inhibitory control, levels of neurotransmitters, and EEG markers of neural function.

Individuals with NF1 showed impaired impulse control and reduced EEG correlates of early visual processing (parieto-occipital P1) and inhibitory control (frontal P3). MRS data revealed a reduction in medial frontal GABA+/tCr (total Creatine) levels in the NF1 group, in parallel with the already reported reduced occipital GABA levels. In contrast, glutamate + glutamine/tCr levels were normal, suggesting the existence of abnormal inhibition/excitation balance in this disorder. Notably, medial frontal but not occipital GABA levels correlated with general intellectual abilities (IQ) in NF1, and inhibitory control in both groups. Surprisingly, the relationship between inhibitory control and medial frontal GABA was reversed in NF1: higher GABA was associated with a faster response style whereas in controls it was related to a cautious strategy.

Abnormal GABAergic physiology appears, thus, as an important factor underlying impaired cognition in NF1, in a level and region dependent manner.

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Keywords

Electroencephalography; Impulse control disorders; Prefrontal cortex; Magnetic resonance spectroscopy; Gamma-aminobutyric acid; Neurofibromatosis type 1

1. Introduction

Neurofibromatosis type 1 (NF1) is a multisystem neuro-developmental disorder caused by mutations in the *NF1* gene (Tonsgard, 2006). In particular, NF1 affects the structure, function and neurochemistry of the central nervous system, leading to learning impairments (Payne, Moharir, Webster, & North, 2010; Violante, Ribeiro, Edden, et al., 2013). Individuals with NF1 present increased white matter volume, particularly within the frontal lobe and the corpus callosum, as well as increased grey matter volume, particularly in the thalamus and right caudate nucleus (Duarte et al., 2014; Payne et al., 2010; Violante, Ribeiro, Silva, & Castelo-Branco, 2013). Reduced integrity of white matter microstructure is also found in NF1, suggesting a reduction in effective structural connectivity (Karlsgodt et al., 2012). Functional magnetic resonance imaging (fMRI) studies have suggested functional deficits in several brain regions including deficient visually evoked activation of occipital, temporal and parietal brain regions (Clements-Stephens, Rimrodt, Gaur, & Cutting, 2008; Violante et al., 2012) and abnormal engagement of the frontal lobe (Billingsley et al., 2004; Clements-Stephens et al., 2008; Shilyansky et al., 2010; Violante et al., 2012). Furthermore, magnetic resonance spectroscopy (MRS), an *in vivo* tool capable of non-invasively measuring brain metabolites, has revealed neurochemical anomalies in patients with NF1 (Nicita et al., 2014; Violante, Ribeiro, Edden, et al., 2013). In particular, GABA levels have been shown to be significantly reduced in the occipital cortex of individuals with NF1 (Violante, Ribeiro, Edden, et al., 2013). This finding is important because research in NF1 mice models suggests that abnormal GABAergic neurotransmission is the main cause of NF1 cognitive deficits (Costa et al., 2002; Li et al., 2005).

The distributed structural and functional neural anomalies observed in NF1 are consistent with the lack of consensus regarding the NF1 cognitive profile. Indeed, deficits in visual abilities, attention, memory, motor skills, language and executive function indicate a broad cognitive phenotype (Descheemaeker, Ghesquiere, Symons, Fryns, & Legius, 2005; Hyman, Shores, & North, 2005; Levine, Materek, Abel, O'Donnell, & Cutting, 2006; Ozonoff, 1999). Nevertheless, executive dysfunction is increasingly recognized as a core deficit in NF1 with difficulties in response inhibition commonly observed (Ferner, Hughes, & Weinman, 1996; Gilboa et al., 2011; Huijbregts, Swaab, & de Sonnevile, 2010; Rowbotham, Pit-ten Cate, Sonuga-Barke, & Huijbregts, 2009). Deficient inhibitory control has been associated with low quality of life and poor academic and job achievements (Diamond, 2013). In NF1, impaired inhibitory control is related with social and emotional problems (Huijbregts & de Sonnevile, 2011). Yet, the neural mechanisms behind impaired inhibitory control in NF1 are still unknown.

Being one of the most common monogenic disorders affecting brain function, NF1 provides a unique genetic model to identify and dissect mechanistically the neurochemical and brain functional bases underlying cognitive dysfunction. The main aim of this study was to

characterize the neural correlates of impaired response inhibition in NF1 by examining both neurochemistry and the neurophysiologic responses in relation to a behavioral experimental model of impulsivity – a visual go/no-go task. Go/no-go tasks involve the ability to control impulsive behavior and the performance metrics correlate with symptoms of inattention, hyperactivity and impulsivity (Epstein et al., 2003). Given the previously described deficit in response inhibition in NF1, we hypothesized that individuals with NF1 would show difficulties in inhibiting the motor response in no-go trials. fMRI, electroencephalography (EEG) and lesion studies suggest the involvement of the medial frontal cortex including the anterior cingulate and pre-supplementary motor area in go/no-go task performance (Huster, Westerhausen, Pantev, & Konrad, 2010; Picton et al., 2007; Rubia et al., 2001; Simmonds, Pekar, & Mostofsky, 2008). Reduced activity within a medial frontal cluster comprising the supplementary motor area and the anterior cingulate cortex, has been linked with poor inhibitory control in patients with attention-deficit/hyperactivity disorder (ADHD) (Hart, Radua, Nakao, Mataix-Cols, & Rubia, 2013). Moreover, several studies show that disruption of the activity of superior medial frontal cortex affects inhibitory control (Chen, Muggleton, Tzeng, Hung, & Juan, 2009; Obeso, Robles, Marron, & Redolar-Ripoll, 2013). Interestingly, during adolescence, poor go/no-go performance in healthy individuals has been shown to be related with lower GABA levels in the anterior cingulate cortex (Silveri et al., 2013). Thus, the region of the medial frontal cortex comprising the anterior cingulate cortex, the pre-supplementary motor area and the supplementary motor area appears as a fundamental part of the circuit underlying inhibitory control. Given the GABAergic deficits already described in NF1, we sought to determine whether abnormal GABA levels in the medial frontal cortex, comprising the anterior cingulate cortex and the pre-supplementary motor area, might underlie inhibitory deficits in NF1. We hypothesized that the role of GABA in inhibitory control is region specific and therefore we also tested whether reduced levels of GABA in a control region, the occipital cortex, correlated with inhibitory control. The study of GABA levels in these two brain regions, allowed the investigation of the regional specificity of GABAergic alterations and their contribution to behavioral deficits. The EEG recordings aimed at determining if the GABA levels were related with the bioelectrical responses thus linking biochemistry to brain functional measures. In addition, the EEG data enabled the analysis of the spatiotemporal dynamics of the different stages of sensorimotor processing elicited during go/no-go task performance. In particular, we analyzed the neural correlates of sensory processing, stimulus evaluation and response inhibition. To assess sensory processing, we studied the visual evoked potentials (VEPs) P1 and N1 over parieto-occipital scalp sites. These responses are sensitive to low-level stimulus properties, as well as attentional control including selective attention to task-relevant stimuli and state of arousal (Codispoti, Ferrari, Junghofer, & Schupp, 2006; Dockree, Kelly, Robertson, Reilly, & Foxe, 2005; Vogel & Luck, 2000). Evaluation of task-relevant stimuli is reflected in P3b, a late positive deflection observed over the parietal scalp (Kok, 2001; Polich, 2007). Thus, to assess cognitive stimulus evaluation processes, we compared the amplitudes of the P3b response of both groups. Finally, to study the neural correlates of response inhibition, we studied the fronto-central N2 and P3 responses. These two event-related potentials (ERPs) have been implicated in response inhibition processes and are believed to originate from regions within the medial frontal cortex (Huster, Enriquez-Geppert, Lavalée, Falkenstein, &

Herrmann, 2013; Huster et al., 2010; Nieuwenhuis, Yeung, van den Wildenberg, & Ridderinkhof, 2003).

2. Methods and materials

2.1. Participants

Children and adolescents with NF1 were recruited in collaboration with the Genetics Department of the Pediatric Hospital of Coimbra in Portugal. All participants met the National Institutes of Health Consensus Development Conference clinical criteria for NF1 (NIH, 1988). We excluded patients with known brain pathology other than NF1 or ophthalmological problems (e.g., amblyopia) as assessed by ophthalmological examination (see below). Furthermore, in order to ensure that the patients included in the study had no unknown brain pathology (e.g., optic gliomas), structural magnetic resonance imaging (MPRAGE and FLAIR sequences) was performed and reviewed by an experienced neuroradiologist. Only children and adolescents with NF1 without significant structural anomalies, besides T2-hyperintensities, were included in the study. Note that, none of the individuals with NF1 included in this study presented T2-hyperintensities in the regions where the spectroscopy voxel was prescribed.

In addition, all patients with NF1 were submitted to a complete ophthalmic examination, including best-corrected visual acuity, stereopsis evaluation, slit lamp examination of anterior chamber structures and fundus examination. No anomalies that could affect vision were found.

For the control group, we recruited typically developing participants. These had no history of learning, developmental, cognitive, neurological or psychiatric problems and their academic records were in accordance with chronological age, indicating adequate intellectual functioning.

For analysis, we included 16 children and adolescents with NF1 and 16 control children and adolescents, age- and gender-matched with the participants with NF1. Handedness was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). General intellectual functioning (full-scale IQ) of the participants with NF1 had already been assessed for a previous study (Ribeiro et al., 2012). Full-scale IQ of control participants were assessed using the Portuguese adapted versions of the Wechsler Intelligence Scale for Children – 3rd edition (WISC-III) or Wechsler Adult Intelligence Scale – 3rd edition (WAIS-III) (Wechsler, 2003, 2008). IQ scores from one participant with NF1 and two participants from the control group were not available. Characteristics of participants are summarized in Table 1. There were no significant differences between the groups for age (*t*-test), sex ratios or handedness (chi-square tests). IQ scores were, as expected, significantly reduced in the NF1 group (*t*-test, $p = .003$).

Children prescribed with stimulant medication (methylphenidate) were not medicated on the day of testing ($n = 4$). None of the participants were taking any other type of medication.

2.2. Protocol approvals and patient consents

The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committees of the Faculty of Medicine of the University of Coimbra and of the Children's Hospital of Coimbra. Written informed consent was obtained from the legal representatives of the participants, after explanation of the nature and possible consequences of the study. In addition, all participants gave written or oral informed consent.

2.3. Go/no-go task and behavioral analysis

The go/no-go task was designed using the Psychophysics Toolbox for Matlab (Brainard, 1997). Visual stimuli were white single digits between 1 and 9 presented randomly with equal probability of 11%, in the centre of the computer screen, on a black background. Each stimulus was presented for 250 msec with an intertrial interval of 1500 msec. Between the offset of one digit and the onset of the next, a white fixation cross was presented in the centre of the screen. Digits were presented in Helvetica font and their size could be any of 5 different sizes (48, 72, 94, 100, 120 font size). Sizes were allocated randomly with equal probability in each trial. Random allocation of different digit sizes aimed at increasing the demands for processing the numerical value and minimizing the possibility that participants would set a search template for some perceptual feature of the target no-go trial. This design followed the rationale of previous studies (Dockree et al., 2005). Participants were asked to respond to all digits except “3” by pressing a button with the right index finger. Thus, digits “1, 2” and “4–9” were the go stimuli and digit “3” the no-go stimulus, and no-go probability was 11%. The presence or absence of a button-press was assessed for each trial, as well as the reaction time for go trials. No-go trials (trials where the digit “3” was presented) were considered *correct withholds* when there was no button press or *errors of commission* (failures to withhold the motor response) when there was a button-press. Go trials with no button press were considered *errors of omission*.

The experimental procedure consisted of two runs of 5.63 min each. Each run contained 225 trials including 25 no-go trials and 200 go trials. Behavioral and EEG data of both runs were appended together for analysis.

2.4. EEG acquisition and analysis

EEG signal was recorded using a 64-channel Neuroscan system with scalp electrodes placed according to the International 10–20 electrode placement standard and with reference between the electrodes CPZ and CZ and ground between FPZ and FZ. Acquisition rate was 1000 Hz. Vertical and horizontal electrooculograms were recorded in order to correct and/or reject artifacts caused by blinking and eye movements. A trigger pulse was generated at the onset of each stimulus and at every button-press.

EEG data analysis was performed with Analyzer 2.0 from Brain Products GmbH. The data from the two runs were appended together and downsampled to 256 Hz, re-referenced to average reference and bandpass filtered using the Butter-worth Zero Phase Filter with cutoff frequencies of .5 and 100 Hz and attenuation of 12 dB/octave. Ocular artifacts were corrected using an independent component analysis-based correction process. The

continuous data were cut into segments locked with the stimuli onset and segments contaminated with muscle activity were rejected using a semi-automatic routine. A baseline was set from -200 msec to stimulus onset. For quantification and topography assessment, the EEG segments were transformed to current source density (CSD) (Perrin, Pernier, Bertrand, & Echallier, 1989). The CSD transform produces reference-free signals and spatial enhancement of the recorded EEG activity, which is important for a reliable topography analysis (Yordanova, Kolev, Hohnsbein, & Falkenstein, 2004).

We compared the CSD responses between go and no-go stimuli. The number of segments averaged in the go condition was equated to the number of segments averaged in the no-go condition for each participant by randomly selecting segments from artifact-free go trials. The number of segments averaged was not significantly different across the groups [mean number of trials averaged (standard deviation, SD): NF1 = 46 (4), CNT = 47 (2); data normally distributed; *t*-test comparisons between groups, n.s.].

The control grand-average CSD waveforms were visually inspected to delineate the latency windows of the ERPs of interest and determine the scalp-sites of maximal amplitude for each response (Dockree et al., 2005; Wylie, Javitt, & Foxe, 2003). The standard VEPs P1 and N1 were observed over parieto-occipital scalp sites; the classical N2/P3 complex was detected over fronto-central midline scalp sites (Huster et al., 2013) and P3b over centro-parietal scalp (Polich, 2007). For analysis, we chose the three electrodes that best represented the maximal topography of the peak of interest (Wylie et al., 2003). Peak amplitude measures for each electrode were derived by calculating the area under the average CSD waveform (compared to the baseline) within a latency window centered on the mean peak latency (as determined through inspection of grand average waveforms). The width of the latency window was chosen depending on the duration and spatial extent of each peak (Dockree et al., 2005; Wylie et al., 2003). The time windows and electrodes chosen for analysis are shown in Table 2. VEPs P1 and N1 showed bilateral responses and thus 3 homologous electrodes in each hemisphere were chosen for analysis.

2.5. Acquisition of MRI data

The MRI data described here was acquired as part of a larger study, part of which has already been published (Violante, Ribeiro, Edden, et al., 2013). The MRI acquisition session consisted of a T₁-weighted MPRAGE sequence 1 mm³ isotropic voxel, repetition time (TR) 2.3 sec, echo time (TE) 2.98 msec, inversion time (TI) 900 msec, flip angle (FA) 9°, field of view (FOV) 256 × 256, 160 slices, GRAPPA acceleration factor = 2, a T₂-weighted FLAIR sequence, 1 mm³ isotropic voxel, TR 5 sec, TE 2.98 msec, TI 1.8 sec, FOV 250 × 250, 160 slices, GRAPPA acceleration factor = 2, a functional MRI acquisition and two GABA-edited magnetic resonance spectra using the MEGA-PRESS method (Edden & Barker, 2007; Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998), one spectrum positioned within the occipital cortex, as described in (Violante, Ribeiro, Edden, et al., 2013) and the other positioned within the medial frontal cortex. The medial frontal voxel was placed over the midline in a region comprising the bilateral dorsal anterior cingulate cortex, between the cingulate and the pericallosal sulci. Both voxels were (3 cm)³ isotropic, TE 68 msec, TR 1.5 sec, 196 averages, 1024 data points. During odd number acquisitions a frequency-selective

inversion pulse was applied to the GABA-C3 resonance at 1.9 ppm (“On resonance”). During even number acquisitions the pulse was applied at 7.5 ppm (“Off resonance”). In this manuscript, we present the MRS data acquired within the medial frontal cortex. T1-weighted images were used for MRS voxel placement and image segmentation.

2.6. Brain structural MRI data analysis

Segmentation of T1-weighted images was performed using in-house software written in Matlab7 (The MathWorks Inc) and the VBM8 toolbox in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) and applied to determine the relative proportions of grey matter, white matter and cerebrospinal fluid in the voxel.

2.7. MRS data analysis

Spectra from all subjects were inspected for movement artifacts. A difference spectrum was generated for each participant (“On resonance”-“Off resonance”). Using the Gannet software written in Matlab (Edden, Puts, Harris, Barker, & Evans, 2014), we applied 4 Hz exponential line broadening to all spectra. Peak integration was used to quantify GABA+ (3 ppm) and the combined glutamate plus glutamine peaks (Glx; 3.75 ppm) in the difference spectra and total creatine peak (tCr; 3 ppm) in the summed spectra. In this manuscript the signal corresponding to GABA is labeled GABA + rather than GABA to indicate the potential contribution of macromolecules and homocarnosine at 3.02 ppm (Rothman, Behar, Prichard, & Petroff, 1997).

Integrals of GABA+, Glx and tCr peaks were automatically calculated using a linear fit of the baseline and Gaussian (GABA+), Gaussian doublet (Glx) and Lorentzian (tCr) models to fit the peaks. GABA+ and Glx peaks were well edited for most participants. Spectra with poor signal to noise ratio due to participants’ movement during MRS acquisition were excluded from spectroscopic analysis (medial frontal cortex MRS – 3 control participants and 3 individuals with NF1; occipital cortex MRS – 1 control participant). Therefore, for medial frontal MRS analysis, we included 13 control participants and 13 children and adolescents with NF1 and for occipital cortex MRS analysis, we included 15 control participants and 16 children and adolescents with NF1.

Ratios of GABA+/tCr and Glx/tCr were calculated for each subject. GABA+/tCr provides reliable GABA concentration estimates and reduces inter-subject variability attributable to differences in signal-to-noise ratio and cerebrospinal fluid fraction within the voxel (Bogner et al., 2010).

Analyses of GABA+/tCr and Glx/tCr within the occipital cortex were presented in our previous study in the context of early visual processing (Violante, Ribeiro, Edden, et al., 2013). In here, we reanalyzed these data in relation to inhibitory control.

2.8. Statistics

All statistical analyses were performed with IBM SPSS Statistics version 19 software. We verified the normality assumption for the different parameters using the Shapiro–Wilk test. All measures were normally distributed except the percentage of errors of omission. We

used, as appropriate, repeated-measures analysis of variance (ANOVA), parametric *t*-tests, Mann–Whitney test and partial correlation analyses. When the data did not meet assumptions of sphericity, the Greenhouse-Geisser correction was used, with original degrees of freedom and corrected confidence probabilities (*p*) being reported.

To investigate the influence of general intellectual ability (full-scale IQ) on the behavioral measures, we used the general linear model including IQ as a covariate. Correlation analyses between behavioral measures (errors of commission and reaction time) were performed controlling for the effect of age, gender and full-scale IQ, as these factors can affect go/no-go task performance (Conners, Epstein, Angold, & Klaric, 2003).

Given that the grey matter content in the MRS voxel may affect GABA concentration measurements (Jensen, Frederick Bde, & Renshaw, 2005), comparisons of GABA+/tCr and Glx/tCr levels between groups were performed using the general linear model with percentage of grey matter in the spectroscopy voxel as covariate. Correlation analyses between IQ and GABA levels were controlled for grey matter content in the spectroscopy voxel. Correlation analysis between go/no-go task performance and GABA+/tCr levels were controlled for age, gender, full-scale IQ, and grey matter content, as these factors have been shown to affect GABA levels and inhibitory control (Conners et al., 2003; Gao et al., 2013; Jensen et al., 2005).

According to our previous study (Violante, Ribeiro, Edden, et al., 2013), to assess the effect of genotype on GABA levels, we first transformed GABA+/tCr measurements of all participants to Z-scores and performed a multiple regression analysis using a forced entry model, in which all predictors are forced into the model simultaneously. Predictors included age, gender and grey matter content (factors known to affect GABA levels) and predictors constructed from categorical variables relative to the type of NF1 mutation (non-sense, missense, splice-site and mutation not found in the *NF1* gene).

For the analyses of the amplitude of each CSD peak, repeated-measures analyses were performed with trial type (go vs no-go) and *Electrode* (the three electrodes chosen for analysis) as within-subjects factors and *Group* (NF1 vs control) as between-subjects factor. For parietal peaks (P1 and N1) *Hemisphere* (left vs right) was also included as a within-subjects factor.

Correlations analyses between behavioral measures and CSD amplitude measures were performed controlling for the effect of age, gender, and full-scale IQ as these factors might affect both measures (Conners et al., 2003; van Dinteren, Arns, Jongsma, & Kessels, 2014).

Data were inspected for outliers ($|z\text{-score}| > 3$). Outliers were found in P1 amplitude data (1 NF1), N2 amplitude data (1 control and 1 NF1) and P3 amplitude data (1 NF1). These data points were removed from the statistical analyses.

3. Results

3.1. Behavioral performance

In comparison with control participants, children and adolescents with NF1 committed a significantly higher number of commission errors, i.e., failed to inhibit the button press in no-go trials [mean percentage of errors (standard error, SE): control, 33.6 (5.5); NF1, 56.6 (6.0); $p = .008$; Fig. 1A]. Errors of omission (failures to respond to go stimuli) were rare (less than 3% on average). Nevertheless, these were significantly higher in the NF1 group than in the control group [mean percentage of errors (SE): control, .53 (.15); NF1, 2.36 (.50); $p = .001$; Fig. 1B]. In addition, individuals with NF1 responded significantly faster to go stimuli than control subjects [mean reaction time (SE): control, .46 (.10) sec; NF1, .39 (.09) sec; $p = .05$; Fig. 1C]. Importantly, reaction times to go stimuli were strongly negatively correlated with the number of errors of commission, so that individuals that responded slower to go stimuli were better at inhibiting the response to no-go stimuli ($r = -.852$, $p < .001$; Fig. 1D). In order to investigate the relationship between general intellectual ability (full-scale IQ) and inhibitory control, we performed correlation analyses. The control group presented a significant correlation between IQ and number of commission errors ($r = -.698$, $p = .005$) with higher IQ related to better inhibitory control. Yet, this relationship was not observed in the NF1 group ($p = .990$), indicating that poorer intellectual abilities and inhibitory deficits are dissociated in this disorder. In fact, after controlling for full scale-IQ, the two groups still differed significantly in the number of commission errors ($p = .018$), and reaction time ($p = .006$), further supporting the idea that the deficit in inhibitory control observed in NF1 is independent of deficits in general intellectual abilities.

3.2. GABA levels and go/no-go performance

We measured GABA+/tCr and Glx/tCr levels in two brain regions: a posterior medial prefrontal region known to be relevant for the correct performance of go/no-go tasks (Huster et al., 2013) and the occipital cortex, where GABA levels have been shown to correlate with basic visual processing (Edden, Muthukumaraswamy, Freeman, & Singh, 2009; Violante, Ribeiro, Edden, et al., 2013; Yoon et al., 2010) (Fig. 2A and B). We found that, in these brain regions, GABA+/tCr levels were significantly reduced in children and adolescents with NF1 when compared with control levels [estimated marginal means (SE): control frontal GABA+/tCr, .115 (.004); NF1 frontal GABA+/tCr, .100 (.004); $F_{(1, 25)} = 5.601$, $p = .027$; control occipital GABA+/tCr, .148 (.004); NF1 occipital GABA+/tCr, .137 (.003); $F_{(1, 30)} = 3.950$, $p = .057$; Fig. 2C], while Glx/tCr levels were not significantly modulated [estimated marginal means (SE): control frontal Glx/tCr, .135 (.005); NF1 frontal Glx/tCr, .125 (.005); $F_{(1, 25)} = 2.046$, $p = .166$; control occipital Glx/tCr, .127 (.005); NF1 occipital Glx/tCr, .123 (.005); $F_{(1, 30)} = .284$, $p = .599$].

Next, we investigated the relationship between GABA and full-scale IQ. In the NF1 group, frontal GABA+/tCr correlated positively with IQ ($r = .685$, $p = .020$; Fig. 2D) suggesting that decreased GABA levels in that brain region in these patients lead to deficits in a global set of intellectual functions. No correlation was observed in the control group or for the occipital GABA+/tCr levels. As expected, the GABA group differences were abolished after

correcting for IQ levels, further emphasizing the close link between frontal GABA and intellectual abilities.

Furthermore, we sought to determine if, beyond the relationship with general intellectual abilities, GABA levels were specifically related to go/no-go behavioral performance, and whether this relationship was specific to medial frontal GABA or if inhibitory control was also related to occipital GABA. In the control group, frontal GABA+/tCr levels correlated negatively with the number of commission errors ($r = -.783, p = .037$; Fig. 2D) and positively with reaction time ($r = .830, p = .021$; Fig. 2D), indicating that higher levels of frontal GABA were associated with a more cautious response style. However, surprisingly, the opposite relationships were observed in individuals with NF1, with an association with a less cautious response style: medial frontal GABA+/tCr levels correlated positively with the number of commission errors ($r = .761, p = .028$; Fig. 2D) and negatively with reaction time ($r = -.719, p = .044$; Fig. 2D). Occipital GABA+/tCr levels did not correlate with inhibitory control in either group indicating that the influence of GABA on cognitive performance is region specific.

3.3. GABA levels and NF1 mutational profile

In our previous study, occipital GABA+/tCr levels were found to be significantly related to NF1 mutation type (Violante, Ribeiro, Edden, et al., 2013). In here, we performed a similar analysis to determine if NF1 mutation type also explained medial frontal GABA levels. The NF1 mutations found in our cohort of patients have been described before (Violante, Ribeiro, Edden, et al., 2013). In particular, the 16 patients included in this study presented 4 non-sense mutations, 6 splice-site mutations and 2 missense mutations. In 4 patients no mutation was found by whole-gene sequencing. These individuals are likely to have a mutation missed by whole-gene sequencing but affecting gene expression, given that the criteria for diagnosis were fulfilled. We did not observe a significant relationship between mutation type and frontal GABA. This finding is in line with the idea that other factors, like gene expression and epigenetic factors, need to be taken in consideration to fully explain phenotypic heterogeneity.

3.4. Neuroelectric responses

CSD analyses of the EEG data enabled the characterization of the different stages of neural processing associated with go/no-go task performance: early visual processing, cognitive stimulus evaluation, and inhibitory control.

3.4.1. Early visual processing—P1 and N1 parieto-occipital peaks were observed bilaterally in both groups of participants reflecting early visual processing. P1 amplitude was significantly reduced in the NF1 group [$F_{(1, 29)} = 4.548, p = .042$; Fig. 3A and B; Table 2]. Interestingly, P1 was also sensitive to trial type being significantly smaller in no-go trials [$F_{(1, 29)} = 4.275, p = .048$; Table 2]. There was no interaction between the effects of trial type and group indicating that amplitude reduction observed in no-go trials was preserved in the NF1 group (Fig. 3B). Furthermore, P1 presented a significant effect of electrode [$F_{(2, 58)} = 11.44, p < .001$], a significant interaction between electrode and hemisphere [$F_{(1, 29)} = 12.35, p < .001$] and a significant 3-way interaction between hemisphere, electrode and

group [$F_{(2, 58)} = 4.590, p = .014$] suggesting that the topography of the signal was affected in the NF1 group. N1 amplitude did not show a significant effect of group [$F_{(1, 30)} = 1.987, p = .169$] but showed, as for P1, a significant effect of trial type [$F_{(1, 30)} = 7.113, p = .012$; Fig. 3A and B; Table 2]. These modulations with trial type of both P1 and N1 amplitudes appeared as a push of P1 and N1 amplitudes towards negative values and resembled the negative deflection over temporo-occipital regions associated with engagement of feature-based attentional mechanisms (Codispoti et al., 2006). Importantly, in both peaks, there was no interaction between trial type and group suggesting that this modulation is not significantly affected in NF1. N1 amplitude showed a significant interaction between trial type and hemisphere [$F_{(1, 30)} = 5.46, p = .026$] reflecting the stronger modulation observed in the right hemisphere in comparison with the left (Fig. 3A and B). There was no 3-way interaction with group indicating that this lateralization effect was preserved in children with NF1.

3.4.2. Cognitive stimulus evaluation—No-go stimuli elicited a positive late response over centro-parietal scalp sites that we interpreted as the P3b wave elicited by deviant events (Polich, 2007). This positive deflection was virtually absent in go trials (Fig. 4).

Accordingly, P3b amplitude showed a very significant effect of trial type [$F_{(1, 30)} = 65.14, p < .001$; Table 2]. P3b did not show an effect of group or interaction between trial type and group, indicating that this neural response was not significantly affected in NF1.

3.4.3. Inhibitory control—The N2/P3 complex was visible over frontal midline scalp sites (Fig. 5A). These two peaks have been associated with go/no-go and stop signal tasks performance (Huster et al., 2013). As expected, N2 showed highest absolute amplitude values over midline frontal electrodes (Table 2). Statistical analyses of its amplitude effects did not reveal a significant effect of group, any group interactions or significant effect of trial type [see Table 2 for mean (SE) values; Fig. 5A and B]. In contrast, P3 was significantly reduced in the NF1 group [$F_{(1, 29)} = 6.384, p = .017$; Table 2; Fig. 5A and C] and presented a significant effect of trial type [$F_{(1, 29)} = 15.011, p = .001$; Fig. 5C]. The effect of trial type did not show a significant interaction with group [$F_{(1, 29)} = .330, p = .570$], indicating that although with reduced amplitude the NF1 response was equally sensitive to no-go stimuli.

3.4.4. Relation between neuroelectric responses, full-scale IQ, GABA and behavior—Next, we investigated if the amplitude reduction of frontal P3 neuroelectric signal might be related to the reduced early visual response (P1). We found no correlation between these two signals suggesting independent neuronal mechanisms. In addition, IQ did not correlate with any of the electrophysiological deficits observed. Nevertheless, reduced GABA levels in the medial frontal cortex observed in individuals with NF1 might be related to the electrophysiological deficits. Previous studies have suggested that the generators of the frontal N2 and P3 EEG signals are localized within medial frontal regions (Huster et al., 2010). The reduction of P3 amplitude in NF1 might, thus, be related to the reduction in GABA levels observed in this region. Correlation analyses revealed a significant negative correlation between GABA+/tCr levels and no-go P3 amplitude, but only in the NF1 group ($r = -.754, p = .019$), suggesting that impaired activation of these brain regions might be

related to the abnormal GABA system. This correlation was, however, no longer significant when IQ was included as a covariate. No correlation was found between GABA+/tCr levels and N2 amplitude or between the behavioral measures and the amplitude of the ERPs studied (P1, N1, P3b, N2 and P3).

4. Discussion

In this study, we investigated the neural correlates of impaired inhibitory control in children and adolescents with NF1. Behaviorally, individuals with NF1 showed increased number of errors of commission (false alarms) and faster reaction times in go trials indicating an impulsive response style. We studied the neural correlates of this impairment by examining both medial frontal and occipital neurochemistry and neurophysiologic responses. We found that GABA, but not Glx, was significantly reduced in the medial frontal and occipital cortices of children and adolescents with NF1. Notably, in individuals with NF1, medial frontal (but not occipital) GABA+/tCr levels correlated with general intellectual abilities (IQ) indicating an important relationship between regional GABA dysfunction and the cognitive profile of these patients. Furthermore, the relationship between GABA and inhibitory control was found to be changed in NF1. Indeed, whilst in the control group higher levels of medial frontal GABA+/tCr levels were related with a more cautious response style, in NF1 this relationship was reversed: higher levels of frontal GABA+/tCr were related with faster and less accurate responses. These findings suggest an abnormal link between GABA and behavior in NF1. Furthermore, occipital GABA+/tCr levels did not correlate with IQ or task performance indicating regional specificity for the relation between GABA and cognitive functions. Our EEG/CSD analyses focused on the study of the neural correlates of basic visual processing, conflict monitoring/inhibitory control and cognitive stimulus evaluation. Two correlates were found impaired: parieto-occipital P1, reflecting abnormal early visual processing, and frontal P3, a correlate of response inhibition. In contrast, frontal N2, a signal that has been implicated with inhibitory control, and P3b, a correlate of stimulus categorization, were not affected.

4.1. Behavioral deficit in go/no-go task performance

The faster reaction times observed in the NF1 group are unlikely to reflect differences in motor function, as previous studies found either slowing of visuomotor responses or no impairments in simple finger tapping tasks (Huijbregts et al., 2010; Rowbotham et al., 2009). Instead, faster responses indicate differences in response styles with patients adopting a less cautious, more impulsive strategy, thus suggesting deficits in impulse control.

4.2. The spatiotemporal pattern of sensorimotor processing

Our CSD findings revealed a distributed pattern of brain functional deficits in NF1 associated with go/no-go task performance.

The earliest deficit was a reduction in the amplitude of visual P1 observed over occipito-parietal scalp sites. This deficit in visual cortex activation is compatible with fMRI findings from earlier reports (Clements-Stephens et al., 2008; Violante et al., 2012) and might be related to deficits in basic visual processing or, alternatively, deficient allocation of spatial

attention (Hillyard & Anllo-Vento, 1998) or reduced arousal levels (Vogel & Luck, 2000). Interestingly, the visual responses P1 and N1 showed significant modulations with trial type (go vs no-go) resembling the selection negativity, a correlate of feature-based attention (Codispoti et al., 2006; Hillyard & Anllo-Vento, 1998; Zhang & Luck, 2009). Given the right lateralization of the N1 modulation with trial type, it is likely that this response reflects engagement of the right ventral frontoparietal network associated with target (no-go) detection (Corbetta & Shulman, 2002). Notably, P1 and N1 modulations with trial type were preserved in the NF1 group suggesting that the cortical mechanisms of feature-based attentional control and target detection are not significantly affected in these children.

N2 amplitude did not show a significant modulation with trial type and was not affected in NF1. N2 is a frontal midline negative deflection that has been extensively studied in the context of go/no-go tasks (Huster et al., 2013). This electro-physiological response is thought to be an important signal regarding the success of response inhibition (Falkenstein, Hoormann, & Hohnsbein, 1999). However, the cognitive function subserved by the N2 response is still a subject of debate. N2 might underlie motor response inhibition, response selection/conflict monitoring or context updating/revision (Huster et al., 2013; Nieuwenhuis et al., 2003; Ouyang, Schacht, Zhou, & Sommer, 2013; Smith, Smith, Provost, & Heathcote, 2010). Alternatively, N2 might reflect the detection of a mismatch between the stimulus perceptual features and a mental template set to match the most frequently presented stimulus (Naatanen, Kujala, & Winkler, 2011). This alternative model would explain why no-go N2 depends on the probability of no-go trials (Bruin & Wijers, 2002; Nieuwenhuis et al., 2003) and on the perceptual overlap between go and no-go stimuli (Nieuwenhuis, Yeung, & Cohen, 2004). Notably, the perceptual mismatch model might explain the lack of N2 modulation in our study. In contrast to the more common go/no-go paradigms that use one stimulus for go trials and another for no-go trials, we used a high number of equiprobable visual stimuli (single digits between 1 and 9 presented in 5 different sizes allocated randomly in each trial). The difference in go/no-go probability was achieved by assigning only one number to the no-go condition and eight numbers to the go condition. The perceptual differences between the different go stimuli were the same as the perceptual differences between any of the go and the no-go stimulus. Thus, if N2 reflects the detection of perceptual differences then, under these conditions, go and no-go trials should elicit N2 signals with similar amplitude. This was what we found. Notably, a similar lack of N2 go/no-go modulation was observed by Smith, Jamadar, Provost, and Michie (2013) using a similar design to ours, with five different equiprobable visual stimuli (Smith et al., 2013). Importantly, the fact that N2 amplitude was not affected in individuals with NF1, but inhibitory control was, further suggests that this signal is not directly related with response inhibition. This has also been suggested by several other studies (Smith et al., 2013; Smith et al., 2010; Wiersma & Roeyers, 2009).

Late positive deflections were clearly observed in our data in response to no-go stimuli over parietal and frontal sites. We interpreted these signals as part of the P300 complex (Falkenstein et al., 1999; Huster et al., 2013; Kok, 2001; Polich, 2007). This complex consists of a parietal component that appears in response to rare task relevant stimuli (P3b) and a fronto-central component (P3a or no-go P3) that is elicited by rare and/or novel stimuli

when no response is required, including no-go stimuli in go/no-go tasks (Goldstein, Spencer, & Donchin, 2002; Huster et al., 2013; Polich, 2007). In our analysis, frontal no-go P3 and parietal P3b displayed different time courses and different group effects. Parietal P3b presented a very significant effect of trial type but no significant effect of group or interaction between group and trial type, suggesting that the cognitive function subserved by this neuroelectric signal is not affected in NF1. P3b is considered to be a correlate of allocation of attention during event-categorization, i.e., a correlate of the awareness that the stimulus perceived belongs to a task-relevant category (Kok, 2001). Therefore, the lack of group effect indicates that the difficulties in inhibitory control observed in children and adolescents with NF1 were not related with impaired recognition and/or categorization of no-go stimuli, i.e., individuals with NF1 were able to recognize the no-go trials yet failed to inhibit the response. The frontal peak (P3) also showed a significant modulation with trial type, yet, in contrast to P3b, it was significantly reduced in the NF1 group in response to both go and no-go stimuli. Importantly, reduction in early visual responses (P1 amplitude) did not explain the reduction in frontal P3 amplitude suggesting that these two deficits are independent. Frontal P3 has been associated with inhibition of the motor response. It is more prominent when a motor response has been inhibited (Smith et al., 2013; Smith, Johnstone, & Barry, 2008) and it correlates negatively with the number of commission errors (Wiersema & Roeyers, 2009). Furthermore, in stop-signal tasks successful motor inhibition, in comparison with failed motor inhibition trials, elicits a frontal P3 with higher amplitude (Dimoska, Johnstone, & Barry, 2006). In contrast, the parietal P3b response does not modulate with the success of the response inhibition (Dimoska et al., 2006). Importantly, in children, the amplitude of no-go P3 correlates with the outcomes of questionnaires on effortful control, persistence, impulsive behavior and attention focusing (Wiersema & Roeyers, 2009). Accordingly, go/no-go P3 amplitude increases with increased motivational incentives (Groom et al., 2010). Thus, P3 appears to be a correlate of motivational state and self-regulation of arousal. Also, children scoring high on ADHD symptoms show smaller no-go P3 amplitudes (Fallgatter et al., 2004; Groom et al., 2010; Wiersema & Roeyers, 2009). Thus, reduced frontal P3 amplitude in NF1 might reflect impairments in self-regulation.

4.3. Abnormal GABA in the medial frontal cortex

We found that levels of GABA+/tCr were decreased in the medial prefrontal cortex of children and adolescents with NF1, while levels of Glx/tCr were not significantly affected; suggesting that the medial frontal inhibition/excitation balance is abnormal in NF1. This finding is in line with our previous study that showed significantly reduced GABA levels in the visual cortex of the same group of children (Violante, Ribeiro, Edden, et al., 2013). Thus, inhibitory neurotransmission in individuals with NF1 appears abnormal in independent brain regions, as has been observed in Nf1 mutant mice (Costa et al., 2002; Shilyansky et al., 2010). Given that GABA levels increase with age during childhood and adolescence (Gaetz et al., 2014; Silveri et al., 2013), the lower levels of GABA observed in NF1 might reflect a degree of developmental delay. It would be interesting to determine if the reduction in GABA levels persists into adulthood in this disorder. In alternative, these differences in GABA levels might reflect compensatory mechanisms. Indeed, the NF1 mice models suggest the existence of enhanced GABAergic transmission in the NF1 brain related

to increased GABA release (Costa et al., 2002; Cui et al., 2008). GABAergic inhibition is modulated by alterations in GABA metabolism, which determines the cytosolic concentration of the neurotransmitter (Golan, Talpalar, Schleifstein-Attias, & Grossman, 1996). GABA concentration measured by MRS provides information about the overall concentration of GABA (mainly the cytosolic, extracellular and vesicular pools). It is, thus, plausible that the point of equilibrium is changed in NF1 with an increased synaptic GABA release for the same levels of overall GABA. The lower levels of overall GABA observed might be a consequence of a compensatory mechanism working towards reducing and thus normalizing the levels of synaptic GABA release. Indeed, decreased cortical GABA levels have been observed in healthy individuals following facilitation of GABA_A receptor function by acute administration of benzodiazepine or ethanol (Goddard et al., 2004; Gomez et al., 2012).

Lower frontal GABA levels were related with lower IQ levels in individuals with NF1, indicating that GABAergic deficits in this brain region partially explain the impaired intellectual abilities characteristic of this disorder. Accordingly, several previous studies suggest that in healthy individuals lower GABA levels are associated with poorer cognitive function (Boy et al., 2011; Edden et al., 2009; Puts, Edden, Evans, McGlone, & McGonigle, 2011; Quetscher et al., 2014; Silveri et al., 2013; Sumner, Edden, Bompas, Evans, & Singh, 2010). In addition, other disorders affecting brain function are also associated with reduced levels of GABA (Edden, Crocetti, Zhu, Gilbert, & Mostofsky, 2012; Long et al., 2013; Smigielska-Kuzia, Bockowski, Sobaniec, Kulak, & Sendrowski, 2010; Yoon et al., 2010). Yet, in NF1 our results suggest a dissociation between global intellectual abilities (full-scale IQ) and response style/inhibitory control. Indeed, in this disorder, lower GABA was associated with both poorer general intellectual abilities and a more cautious response style characterized by higher go/no-go accuracy levels. This observation supports the idea that the relationships between GABA and specific cognitive functions are specific rather than global, and suggests a complex relationship between GABA and cognitive function in NF1.

Medial frontal but not occipital GABA presented a significant relationship with measures of cognitive function, indicating frontal dysfunction as an important neural correlate of the NF1 cognitive profile. In addition, it suggests the existence of regional specificity in the relationship between GABA and specific cognitive functions, as opposed to a globally correlated system, and according with previous studies (Boy et al., 2010). Further in line with this dissociation between occipital and medial frontal GABA levels, the relationship between NF1 mutation type and occipital GABA observed in our earlier study (Violante, Ribeiro, Edden, et al., 2013) was not observed for the medial frontal GABA levels. This observation further emphasize the idea that significant genotype – phenotype correlations are more the exception than the rule, in particular in heterogeneous genetic disorders with variable genetic penetrance.

Regions within the medial frontal cortex underlie the EEG signal generation of the N2 and P3 responses (Huster et al., 2013). Accordingly, medial frontal GABA+/tCr levels were negatively correlated with the no-go P3 amplitude but again only in the NF1 group. This correlation further supported the idea that high levels of GABA were impairing to children

with NF1 as these were associated with lower P3 amplitudes that in turn are related to poor self control, as mentioned above.

The finding that the relationship between medial frontal GABA and inhibitory control is distinct in NF1 and controls suggests that the function of these medial frontal areas is abnormal in NF1. In the healthy brain, voluntary action control engages a frontal cortical-basal ganglia-thalamo-cortical circuit involving frontal cortex projections to the globus pallidus pars interna/externa, via the striatum or the subthalamic nucleus, and then back to the cortex (via the thalamus) (Aron, 2011; Jahfari et al., 2011). Children and adolescents with NF1 present abnormally large striatal and thalamic structures (Violante, Ribeiro, Silva, et al., 2013) as well as white matter microstructural alterations involving particularly the anterior thalamic radiations which connect the thalamus with the frontal lobes, and the cingulate bundle that contains all connections to the cingulate gyrus (Karlsgodt et al., 2012). Abnormal subcortical structure and impaired subcortical–prefrontal connectivity might lead to an abnormal reliance on prefrontal areas for appropriate inhibitory control. This hypothesis is a possible explanation for the distinct relationship between medial prefrontal GABA levels and inhibitory control in the NF1 group.

4.4. Conclusions

In summary, children and adolescents with NF1 showed impaired impulse control, reduced GABA levels in the medial frontal cortex, and reduced EEG correlates of early visual processing (parieto-occipital P1) and inhibitory control (frontal P3), suggesting impaired self-regulation of arousal levels. In individuals with NF1, lower levels of medial frontal GABA were associated with lower IQ and a more cautious response style. This was in contrast to the control group where lower GABA was associated with a more impulsive response style. This abnormal effect of GABA on behavior suggests that the hypothesized GABAergic dysfunction goes beyond the observed reduction in GABA levels.

This study contributes towards the understanding of how in disorders of brain function changes in the balance between excitatory and inhibitory neurotransmission relate to cognitive performance. Importantly, although reduction in the levels of GABA appears to be a feature of several disorders (Rojas, Singel, Steinmetz, Hepburn, & Brown, 2014; Smigielska-Kuzia et al., 2010; Yoon et al., 2010), our findings indicate that in NF1 the relationship between GABA levels and performance are dependent on the type of cognitive function. This suggests that therapies increasing GABAergic function might not necessarily be appropriate to improve all cognitive functions.

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References

- Aron AR. From reactive to proactive and selective control: developing a richer model for stopping inappropriate responses. *Biological Psychiatry*. 2011; 69(12):e55–68. [PubMed: 20932513]
- Billingsley RL, Jackson EF, Slopis JM, Swank PR, Mahankali S, Moore BD. Functional MRI of visual-spatial processing in neurofibromatosis, type I. *Neuropsychologia*. 2004; 42(3):395–404. [PubMed: 14670578]
- Bogner W, Gruber S, Doelken M, Stadlbauer A, Ganslandt O, Boettcher U, et al. In vivo quantification of intracerebral GABA by single-voxel (1)H-MRS-How reproducible are the results? *European Journal of Radiology*. 2010; 73(3):526–531. [PubMed: 19201120]
- Boy F, Evans CJ, Edden RA, Lawrence AD, Singh KD, Husain M, et al. Dorsolateral prefrontal gamma-aminobutyric acid in men predicts individual differences in rash impulsivity. *Biological Psychiatry*. 2011; 70(9):866–872. [PubMed: 21757187]
- Boy F, Evans CJ, Edden RA, Singh KD, Husain M, Sumner P. Individual differences in subconscious motor control predicted by GABA concentration in SMA. *Current Biology*. 2010; 20(19):1779–1785. [PubMed: 20888227]
- Brainard DH. The psychophysics toolbox. *Spatial Vision*. 1997; 10(4):433–436. [PubMed: 9176952]
- Bruin KJ, Wijers AA. Inhibition, response mode, and stimulus probability: a comparative event-related potential study. *Clinical Neurophysiology*. 2002; 113(7):1172–1182. [PubMed: 12088714]
- Chen CY, Muggleton NG, Tzeng OJ, Hung DL, Juan CH. Control of prepotent responses by the superior medial frontal cortex. *NeuroImage*. 2009; 44(2):537–545. [PubMed: 18852054]
- Clements-Stephens AM, Rimrodt SL, Gaur P, Cutting LE. Visuospatial processing in children with neurofibromatosis type 1. *Neuropsychologia*. 2008; 46(2):690–697. [PubMed: 17988695]
- Codispoti M, Ferrari V, Junghofer M, Schupp HT. The categorization of natural scenes: brain attention networks revealed by dense sensor ERPs. *NeuroImage*. 2006; 32(2):583–591. [PubMed: 16750397]
- Conners CK, Epstein JN, Angold A, Klaric J. Continuous performance test performance in a normative epidemiological sample. *Journal of Abnormal Child Psychology*. 2003; 31(5):555–562. [PubMed: 14561062]
- Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain. *Nature Reviews Neuroscience*. 2002; 3(3):201–215. [PubMed: 11994752]
- Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, et al. Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. *Nature*. 2002; 415(6871):526–530. [PubMed: 11793011]
- Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, et al. Neurofibromin regulation of ERK signaling modulates GABA release and learning. *Cell*. 2008; 135(3):549–560. [PubMed: 18984165]
- Descheemaeker MJ, Ghesquiere P, Symons H, Fryns JP, Legius E. Behavioural, academic and neuropsychological profile of normally gifted Neurofibromatosis type 1 children. *Journal of Intellectual Disability Research*. 2005; 49(Pt 1):33–46. [PubMed: 15634310]
- Diamond A. Executive functions. *Annual Review of Psychology*. 2013; 64:135–168.
- Dimoska A, Johnstone SJ, Barry RJ. The auditory-evoked N2 and P3 components in the stop-signal task: indices of inhibition, response-conflict or error-detection? *Brain and Cognition*. 2006; 62(2):98–112. [PubMed: 16814442]
- van Dinteren R, Arns M, Jongsma ML, Kessels RP. P300 development across the lifespan: a systematic review and meta-analysis. *PLoS One*. 2014; 9(2):e87347. [PubMed: 24551055]
- Dockree PM, Kelly SP, Robertson IH, Reilly RB, Foxe JJ. Neurophysiological markers of alert responding during goal-directed behavior: a high-density electrical mapping study. *NeuroImage*. 2005; 27(3):587–601. [PubMed: 16024257]
- Duarte JV, Ribeiro MJ, Violante IR, Cunha G, Silva E, Castelo-Branco M. Multivariate pattern analysis reveals subtle brain anomalies relevant to the cognitive phenotype in neurofibromatosis type 1. *Human Brain Mapping*. 2014; 35(1):89–106. [PubMed: 22965669]

- Edden RA, Barker PB. Spatial effects in the detection of gamma-aminobutyric acid: improved sensitivity at high fields using inner volume saturation. *Magnetic Resonance in Medicine*. 2007; 58(6):1276–1282. [PubMed: 17969062]
- Edden RA, Crocetti D, Zhu H, Gilbert DL, Mostofsky SH. Reduced GABA concentration in attention-deficit/hyperactivity disorder. *Archives of General Psychiatry*. 2012; 69(7):750–753. [PubMed: 22752239]
- Edden RA, Muthukumaraswamy SD, Freeman TC, Singh KD. Orientation discrimination performance is predicted by GABA concentration and gamma oscillation frequency in human primary visual cortex. *Journal of Neuroscience*. 2009; 29(50):15721–15726. [PubMed: 20016087]
- Edden RA, Puts NAJ, Harris AD, Barker PB, Evans CJ. Gannet: a batch-processing tool for the quantitative analysis of gamma-aminobutyric acid – edited MR spectroscopy spectra. *Journal of Magnetic Resonance Imaging*. 2014; 40:1445–1452. [PubMed: 25548816]
- Epstein JN, Erkanli A, Conners CK, Klaric J, Costello JE, Angold A. Relations between Continuous Performance Test performance measures and ADHD behaviors. *Journal of Abnormal Child Psychology*. 2003; 31(5):543–554. [PubMed: 14561061]
- Falkenstein M, Hoormann J, Hohnsbein J. ERP components in Go/Nogo tasks and their relation to inhibition. *Acta Psychologica (Amsterdam)*. 1999; 101(2–3):267–291.
- Fallgatter AJ, Ehlis AC, Seifert J, Strik WK, Scheuerpflug P, Zillesen KE, et al. Altered response control and anterior cingulate function in attention-deficit/hyperactivity disorder boys. *Clinical Neurophysiology*. 2004; 115(4):973–981. [PubMed: 15003781]
- Ferner RE, Hughes RA, Weinman J. Intellectual impairment in neurofibromatosis 1. *Journal of the Neurological Sciences*. 1996; 138(1–2):125–133. [PubMed: 8791250]
- Gaetz W, Bloy L, Wang DJ, Port RG, Blaskey L, Levy SE, et al. GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. *NeuroImage*. 2014; 86:1–9. [PubMed: 23707581]
- Gao F, Edden RA, Li M, Puts NA, Wang G, Liu C, et al. Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *NeuroImage*. 2013; 78:75–82. [PubMed: 23587685]
- Gilboa Y, Rosenblum S, Fattal-Valevski A, Toledano-Alhadeif H, Rizzo AS, Josman N. Using a virtual classroom environment to describe the attention deficits profile of children with neurofibromatosis type 1. *Research in Developmental Disabilities*. 2011; 32(6):2608–2613. [PubMed: 21757320]
- Goddard AW, Mason GF, Appel M, Rothman DL, Gueorguieva R, Behar KL, et al. Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. *American Journal of Psychiatry*. 2004; 161(12):2186–2193. [PubMed: 15569888]
- Golan H, Talpalar AE, Schleifstein-Attias D, Grossman Y. GABA metabolism controls inhibition efficacy in the mammalian CNS. *Neuroscience Letters*. 1996; 217(1):25–28. [PubMed: 8905731]
- Goldstein A, Spencer KM, Donchin E. The influence of stimulus deviance and novelty on the P300 and novelty P3. *Psychophysiology*. 2002; 39(6):781–790. [PubMed: 12462506]
- Gomez R, Behar KL, Watzl J, Weinzier SA, Gulanski B, Sanacora G, et al. Intravenous ethanol infusion decreases human cortical gamma-aminobutyric acid and N-acetylaspartate as measured with proton magnetic resonance spectroscopy at 4 tesla. *Biological Psychiatry*. 2012; 71(3):239–246. [PubMed: 21855054]
- Groom MJ, Scerif G, Liddle PF, Batty MJ, Liddle EB, Roberts KL, et al. Effects of motivation and medication on electrophysiological markers of response inhibition in children with attention-deficit/hyperactivity disorder. *Biological Psychiatry*. 2010; 67(7):624–631. [PubMed: 19914599]
- Hart H, Radua J, Nakao T, Mataix-Cols D, Rubia K. Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: exploring task-specific, stimulant medication, and age effects. *JAMA Psychiatry*. 2013; 70(2):185–198. [PubMed: 23247506]
- Hillyard SA, Anllo-Vento L. Event-related brain potentials in the study of visual selective attention. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95(3):781–787. [PubMed: 9448241]

- Huijbregts S, Swaab H, de Sonnevile L. Cognitive and motor control in neurofibromatosis type I: influence of maturation and hyperactivity-inattention. *Developmental Neuropsychology*. 2010; 35(6):737–751. [PubMed: 21038163]
- Huijbregts SC, de Sonnevile LM. Does cognitive impairment explain behavioral and social problems of children with neurofibromatosis type 1? *Behavior Genetics*. 2011; 41(3):430–436. [PubMed: 21184163]
- Huster RJ, Enriquez-Geppert S, Lavallee CF, Falkenstein M, Herrmann CS. Electroencephalography of response inhibition tasks: functional networks and cognitive contributions. *International Journal of Psychophysiology*. 2013; 87(3):217–233. [PubMed: 22906815]
- Huster RJ, Westerhausen R, Pantev C, Konrad C. The role of the cingulate cortex as neural generator of the N200 and P300 in a tactile response inhibition task. *Human Brain Mapping*. 2010; 31(8):1260–1271. [PubMed: 20063362]
- Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology*. 2005; 65(7):1037–1044. [PubMed: 16217056]
- Jahfari S, Waldorp L, van den Wildenberg WP, Scholte HS, Ridderinkhof KR, Forstmann BU. Effective connectivity reveals important roles for both the hyperdirect (fronto-subthalamic) and the indirect (fronto-striatal-pallidal) fronto-basal ganglia pathways during response inhibition. *Journal of Neuroscience*. 2011; 31(18):6891–6899. [PubMed: 21543619]
- Jensen JE, Frederick Bde B, Renshaw PF. Grey and white matter GABA level differences in the human brain using two-dimensional, J-resolved spectroscopic imaging. *NMR in Biomedicine*. 2005; 18(8):570–576. [PubMed: 16273508]
- Karlsgodt KH, Rosser T, Lutkenhoff ES, Cannon TD, Silva A, Bearden CE. Alterations in white matter microstructure in neurofibromatosis-1. *PLoS One*. 2012; 7(10):e47854. [PubMed: 23094098]
- Kok A. On the utility of P3 amplitude as a measure of processing capacity. *Psychophysiology*. 2001; 38(3):557–577. [PubMed: 11352145]
- Levine TM, Materek A, Abel J, O'Donnell M, Cutting LE. Cognitive profile of neurofibromatosis type 1. *Seminars in Pediatric Neurology*. 2006; 13(1):8–20. [PubMed: 16818171]
- Li W, Cui Y, Kushner SA, Brown RA, Jentsch JD, Frankland PW, et al. The HMG-CoA reductase inhibitor lovastatin reverses the learning and attention deficits in a mouse model of neurofibromatosis type 1. *Current Biology*. 2005; 15(21):1961–1967. [PubMed: 16271875]
- Long Z, Medlock C, Dziedzic M, Shin YW, Goddard AW, Dydak U. Decreased GABA levels in anterior cingulate cortex/medial prefrontal cortex in panic disorder. *Progress In Neuro-Psychopharmacology & Biological Psychiatry*. 2013; 44C:131–135. [PubMed: 23391588]
- Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine*. 1998; 11(6):266–272. [PubMed: 9802468]
- Naatanen R, Kujala T, Winkler I. Auditory processing that leads to conscious perception: a unique window to central auditory processing opened by the mismatch negativity and related responses. *Psychophysiology*. 2011; 48(1):4–22. [PubMed: 20880261]
- Nicita F, Di Biasi C, Sollaku S, Cecchini S, Salpietro V, Pittalis A, et al. Evaluation of the basal ganglia in neurofibromatosis type 1. *Child's Nervous System*. 2014; 30(2):319–325.
- Nieuwenhuis S, Yeung N, Cohen JD. Stimulus modality, perceptual overlap, and the go/no-go N2. *Psychophysiology*. 2004; 41(1):157–160. [PubMed: 14693011]
- Nieuwenhuis S, Yeung N, van den Wildenberg W, Ridderinkhof KR. Electrophysiological correlates of anterior cingulate function in a go/no-go task: effects of response conflict and trial type frequency. *Cognitive, Affective, & Behavioral Neuroscience*. 2003; 3(1):17–26.
- NIH. Neurofibromatosis conference statement, national institutes of health consensus development conference. 1988. From http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3128965
- Obeso I, Robles N, Marron EM, Redolar-Ripoll D. Dissociating the role of the pre-SMA in response inhibition and switching: a combined online and offline TMS approach. *Frontiers in Human Neuroscience*. 2013; 7:150. [PubMed: 23616761]
- Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*. 1971; 9(1):97–113. [PubMed: 5146491]

- Ouyang G, Schacht A, Zhou C, Sommer W. Overcoming limitations of the ERP method with Residue Iteration Decomposition (RIDE): a demonstration in go/no-go experiments. *Psychophysiology*. 2013; 50(3):253–265. [PubMed: 23316862]
- Ozonoff S. Cognitive impairment in neurofibromatosis type 1. *American Journal of Medical Genetics*. 1999; 89(1):45–52. [PubMed: 10469436]
- Payne JM, Moharir MD, Webster R, North KN. Brain structure and function in neurofibromatosis type 1: current concepts and future directions. *Journal of Neurology Neurosurgery and Psychiatry*. 2010; 81(3):304–309.
- Perrin F, Pernier J, Bertrand O, Echallier JF. Spherical splines for scalp potential and current density mapping. *Electroencephalography and Clinical Neurophysiology*. 1989; 72(2):184–187. [PubMed: 2464490]
- Picton TW, Stuss DT, Alexander MP, Shallice T, Binns MA, Gillingham S. Effects of focal frontal lesions on response inhibition. *Cerebral Cortex*. 2007; 17(4):826–838. [PubMed: 16699079]
- Polich J. Updating P300: an integrative theory of P3a and P3b. *Clinical Neurophysiology*. 2007; 118(10):2128–2148. [PubMed: 17573239]
- Puts NA, Edden RA, Evans CJ, McGlone F, McGonigle DJ. Regionally specific human GABA concentration correlates with tactile discrimination thresholds. *Journal of Neuroscience*. 2011; 31(46):16556–16560. [PubMed: 22090482]
- Quetscher C, Yildiz A, Dharmadhikari S, Glaubitz B, Schmidt-Wilcke T, Dydak U, et al. Striatal GABA-MRS predicts response inhibition performance and its cortical electrophysiological correlates. *Brain Structure & Function*. 2014 Epub ahead of print.
- Ribeiro MJ, Violante IR, Bernardino I, Ramos F, Saraiva J, Reviriego P, et al. Abnormal achromatic and chromatic contrast sensitivity in neurofibromatosis type 1. *Investigative Ophthalmology & Visual Science*. 2012; 53(1):287–293. [PubMed: 22190595]
- Rojas DC, Singel D, Steinmetz S, Hepburn S, Brown MS. Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. *NeuroImage*. 2014; 86:28–34. [PubMed: 23370056]
- Rothman DL, Behar KL, Prichard JW, Petroff OA. Homocarnosine and the measurement of neuronal pH in patients with epilepsy. *Magnetic Resonance in Medicine*. 1997; 38(6):924–929. [PubMed: 9402193]
- Rowbotham I, Pit-ten Cate IM, Sonuga-Barke EJ, Huijbregts SC. Cognitive control in adolescents with neurofibromatosis type 1. *Neuropsychology*. 2009; 23(1):50–60. [PubMed: 19210032]
- Rubia K, Russell T, Overmeyer S, Brammer MJ, Bullmore ET, Sharma T, et al. Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. *NeuroImage*. 2001; 13(2):250–261. [PubMed: 11162266]
- Shilyansky C, Karlsgodt KH, Cummings DM, Sidiropoulou K, Hardt M, James AS, et al. Neurofibromin regulates corticostriatal inhibitory networks during working memory performance. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(29):13141–13146. [PubMed: 20624961]
- Silveri MM, Sneider JT, Crowley DJ, Covell MJ, Acharya D, Rosso IM, et al. Frontal lobe gamma-aminobutyric acid levels during adolescence: associations with impulsivity and response inhibition. *Biological Psychiatry*. 2013; 74(4):296–304. [PubMed: 23498139]
- Simmonds DJ, Pekar JJ, Mostofsky SH. Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent. *Neuropsychologia*. 2008; 46(1):224–232. [PubMed: 17850833]
- Smigielska-Kuzia J, Bockowski L, Sobaniec W, Kulak W, Sendrowski K. Amino acid metabolic processes in the temporal lobes assessed by proton magnetic resonance spectroscopy (1H MRS) in children with Down syndrome. *Pharmacological Reports*. 2010; 62(6):1070–1077. [PubMed: 21273664]
- Smith JL, Jamadar S, Provost AL, Michie PT. Motor and non-motor inhibition in the Go/NoGo task: an ERP and fMRI study. *International Journal of Psychophysiology*. 2013; 87(3):244–253. [PubMed: 22885679]

- Smith JL, Johnstone SJ, Barry RJ. Movement-related potentials in the Go/NoGo task: the P3 reflects both cognitive and motor inhibition. *Clinical Neurophysiology*. 2008; 119(3):704–714. [PubMed: 18164657]
- Smith JL, Smith EA, Provost AL, Heathcote A. Sequence effects support the conflict theory of N2 and P3 in the Go/NoGo task. *International Journal of Psychophysiology*. 2010; 75(3):217–226. [PubMed: 19951723]
- Sumner P, Edden RA, Bompas A, Evans CJ, Singh KD. More GABA, less distraction: a neurochemical predictor of motor decision speed. *Nature Neuroscience*. 2010; 13(7):825–827. [PubMed: 20512136]
- Tongsgard JH. Clinical manifestations and management of neurofibromatosis type 1. *Seminars in Pediatric Neurology*. 2006; 13(1):2–7. [PubMed: 16818170]
- Violante IR, Ribeiro MJ, Cunha G, Bernardino I, Duarte JV, Ramos F, et al. Abnormal brain activation in neurofibromatosis type 1: a link between visual processing and the default mode network. *PLoS One*. 2012; 7(6):e38785. [PubMed: 22723888]
- Violante IR, Ribeiro MJ, Edden RA, Guimaraes P, Bernardino I, Rebola J, et al. GABA deficit in the visual cortex of patients with neurofibromatosis type 1: genotype-phenotype correlations and functional impact. *Brain*. 2013; 136(Pt 3):918–925. [PubMed: 23404336]
- Violante IR, Ribeiro MJ, Silva ED, Castelo-Branco M. Gyrification, cortical and subcortical morphometry in neurofibromatosis type 1: an uneven profile of developmental abnormalities. *Journal of Neurodevelopmental Disorders*. 2013; 5(1):3. [PubMed: 23406822]
- Vogel EK, Luck SJ. The visual N1 component as an index of a discrimination process. *Psychophysiology*. 2000; 37(2):190–203. [PubMed: 10731769]
- Wechsler, D. Manual for intelligence scale for children. Lisbon: Cegoc-Tea; 2003.
- Wechsler, D. Manual for intelligence scale for adults. Lisbon: Cegoc-Tea; 2008.
- Wiersema JR, Roeyers H. ERP correlates of effortful control in children with varying levels of ADHD symptoms. *Journal of Abnormal Child Psychology*. 2009; 37(3):327–336. [PubMed: 19037722]
- Wylie GR, Javitt DC, Foxe JJ. Task switching: a high-density electrical mapping study. *NeuroImage*. 2003; 20(4):2322–2342. [PubMed: 14683733]
- Yoon JH, Maddock RJ, Rokem A, Silver MA, Minzenberg MJ, Ragland JD, et al. GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. *Journal of Neuroscience*. 2010; 30(10):3777–3781. [PubMed: 20220012]
- Yordanova J, Kolev V, Hohnsbein J, Falkenstein M. Sensorimotor slowing with ageing is mediated by a functional dysregulation of motor-generation processes: evidence from high-resolution event-related potentials. *Brain*. 2004; 127(Pt 2):351–362. [PubMed: 14607784]
- Zhang W, Luck SJ. Feature-based attention modulates feedforward visual processing. *Nature Neuroscience*. 2009; 12(1):24–25. [PubMed: 19029890]

Fig. 1.

Individuals with NF1 committed more errors and responded faster than control participants. (A) Percentage of errors of commission (failures to inhibit the response to no-go stimuli). (B) Percentage of errors of omission (failures to respond to go stimuli). (C) Reaction time of the correct responses to go stimuli. (A, B and C) Data are represented as mean $\pm$ 1 SE. Control group – white bars; NF1 group – black bars. * $p < .05$, ** $p < .01$. (D) Partial correlation controlled for the effect of age, gender, and IQ between reaction time and number of errors of commission.

Fig. 2.

The GABA+/total creatine (tCr) levels of the medial frontal and occipital cortices of individuals with NF1 were significantly reduced. (A) Localization of the two MRS voxels (white squares) in the medial frontal and occipital cortices of a representative participant. (B) Edited MRS spectrum from a representative participant showing clearly resolved peaks for GABA+ and glutamine + glutamate (Glx). (C) GABA+/tCr and Glx/tCr levels adjusted for the percentage of grey matter in the spectroscopy voxels, for the control (white bars) and NF1 (black bars) groups. Data are represented as mean $\pm$ 1 SE. * $p < .05$ # $p = .057$. (D) Upper graphs, partial correlations between levels of medial frontal GABA+/tCr and IQ,

controlled for the effect of grey matter within the spectroscopy voxel. Middle and lower graphs, partial correlations between levels of medial frontal GABA+/tCr and number of errors of commission (middle) and between levels of medial frontal GABA+/tCr and reaction time (bottom), controlled for the effect of age, gender, IQ, and grey matter within the spectroscopy voxel. Graphs presenting NF1 data are shown on the left, control data are shown on the right.

Fig. 3.

(A) Reference-free CSD ($\mu\text{V}/\text{m}^2$) control and NF1 grand average traces showing the P1–N1 complex for go and no-go trials. Waveforms shown were pooled across electrodes used in the N1 analysis (left: PO5, P5, P3; right: PO6, P6, P4). (B) Graphs represent mean and SEs of the P1 (left) and N1 (right) CSD amplitudes calculated at each electrode site.

Fig. 4.

(A) Reference-free CSD ($\mu\text{V}/\text{m}^2$) grand average traces detected over centro-parietal electrodes, showing the P3b positive deflection highly enhanced in no-go trials. Waveforms shown are pooled across the electrodes used for statistical analysis (Cz, CPz, and Pz). (B) Graph represents means and SEs of the P3b CSD amplitudes calculated at each electrode site for each condition.

Fig. 5.

(A) Reference-free CSD ($\mu\text{V}/\text{m}^2$) grand average traces detected over midline frontal electrodes, showing the N2/P3 frontal complex. Waveforms shown were pooled across the electrodes used for P3 statistical analysis (FC1, FCz, FC2). (B and C) Graphs representing mean and SEs of the CSD amplitudes calculated at each electrode site for each CSD peak. (B) Frontal N2. (C) Frontal P3.

Table 1

Characteristics of NF1 and control groups.

Group	Control	NF1
<i>n</i>	16	16
Age (years)		
Mean (SD)	14.1 (2.7)	13.8 (2.7)
Range (min–max)	10.2–19.7	10.4–19.5
Sex ratio (F/M)	12/4	12/4
Handedness (R/L)	16/0	16/0
Full-scale IQ		
<i>n</i>	14	15
Mean (SD)	112 (11)	96 (16)
Range (min–max)	90–124	80–124

SD: standard deviation.

Table 2

Summary of neuroelectric responses.

Peak	Latency window	Electrodes	Control amplitude values		NF1 amplitude values	
			Mean (SE) $\mu\text{V}/\text{m}^2$		Mean (SE) $\mu\text{V}/\text{m}^2$	
			Go	No-go	Go	No-go
Early sensory responses						
P1	109–149 msec	Left: O1, PO3, PO5 Right: O2, PO4, PO6	28.8 (6.5)	23.4 (6.9)	13.2 (3.6)	10.3 (3.9)
N1	164–204 msec	Left: PO5, P5, P3 Right: PO6, P6, P4	−16.2 (4.7)	−19.6 (4.1)	−7.8 (6.5)	−11.0 (6.3)
Sensory evaluation						
P3b	520–620 msec	Pz, CPz, Cz	8.3 (3.5)	37.2 (8.0)	4.9 (3.7)	34.3 (7.7)
Inhibitory control						
N2	380–420 msec	F1, Fz, F2	−9.2 (2.9)	−10.8 (3.2)	−14.2 (4.2)	−13.2 (4.7)
P3	450–550 msec	FC1, FCz, FC2	3.5 (2.7)	16.3 (6.2)	−7.5 (3.5)	1.9 (5.7)

SE: standard error.