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GABA levels relate to BOLD signal in Neurofibromatosis Type 1

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GABA levels relate to BOLD signal in Neurofibromatosis Type 1

Os níveis GABA relacionam-se com o sinal BOLD na Neurofibromatose Tipo I

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“If something exists but the brain can’t learn it, we don’t know it exists”

Pedro Domingos, *in* The Master Algorithm: How the Quest for the Ultimate Learning Machine Will Remake Our World (Basic Books, 2015)

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Abbreviations

ANOVA – Analysis of variance

BOLD – Blood-oxygen-level-dependent

FDR – False discovery rate

fMRI – Functional magnetic resonance imaging

GABA – Gamma-aminobutyric acid

GLM – General Linear Model

Glx – Glutamate + glutamine

IHI – Interhemispheric inhibition

IQ – Intelligence quotient

LCD – Liquid-crystal display

LTP – Long term potentiation

M1 – Primary motor cortex

MEGA-PRESS – Mescher-Garwood point-resolved spectroscopy

MR – Magnetic resonance

MRI – Magnetic resonance imaging

MRS – Magnetic resonance spectroscopy

NF1 – Neurofibromatosis Type 1

PAS – Paired associative stimulation

SD – Standard deviation

TMS – Transcranial magnetic stimulation

VOI – Volume of interest

WAIS – Wechsler Adult Intelligence Scale

Abstract

Neurofibromatosis Type 1 (NF1) is a common autosomal dominant disorder with reduced gamma-aminobutyric acid (GABA) levels in several brain regions and whose clinical manifestations include motor deficits. This study investigates for the first time the relation between GABA levels of the dominant primary motor cortex (M1) and the functional activity of both M1s and the cerebellum during a motor task in NF1.

Twenty-one NF1 subjects and twenty controls executed a finger-tapping task with synchronous and asynchronous movements at increasing rhythms (1 Hz, 3 Hz, and 5 Hz). GABA levels were measured in the dominant M1 using magnetic resonance spectroscopy (MRS) and the functional activity of both M1s and cerebellum was evaluated using functional magnetic resonance imaging (fMRI). We then investigated the existence of a correlation between GABA levels and fMRI activity in each group.

This study showed blood-oxygen-level-dependent (BOLD) signal to be significantly higher in the NF1 group compared to the control group in both M1s and the cerebellum. At asynchronous tapping, GABA levels of the dominant M1 positively correlated with the fMRI activity in both M1s of NF1 patients. That was mainly verified at the highest rhythms of tapping and it was not observed in the control group. In addition, the non-dominant M1 BOLD levels mirrored the dominant M1 in the NF1 group.

In conclusion, neurochemical and activity changes in the M1 and the cerebellum may underlie the motor deficits observed in NF1 patients, which should be further addressed in future studies.

Keywords: Neurofibromatosis Type 1, Motor Skills, Primary Motor Cortex, Cerebellum, fMRI, Magnetic Resonance Spectroscopy, GABA.

Resumo

A neurofibromatose Tipo 1 (NF1) é uma doença autossómica dominante na qual os níveis de ácido gama-aminobutírico (GABA) estão reduzidos em várias regiões cerebrais e cujas manifestações clínicas incluem alterações da motricidade. Este estudo investiga, pela primeira vez, a relação entre os níveis de GABA do córtex motor primário (M1) dominante e a atividade funcional de ambos os M1s e do cerebelo durante uma tarefa motora na NF1.

Vinte e um participantes com NF1 e vinte controlos executaram movimentos síncronos e assíncronos com os dedos indicadores a ritmos crescentes (1 Hz, 3 Hz e 5 Hz). Os níveis de GABA foram medidos no M1 dominante por espetroscopia de ressonância magnética (MRS) e a atividade funcional de ambos os M1s e do cerebelo foi avaliada por ressonância magnética funcional (fMRI). Depois, investigámos a existência de uma correlação entre os níveis de GABA e a atividade fMRI em cada grupo.

Este estudo mostrou que o sinal dependente do nível de oxigenação sanguínea (BOLD) é significativamente mais elevado no grupo NF1 do que no grupo controlo em ambos os M1s e no cerebelo. No movimento assíncrono, os níveis de GABA correlacionaram-se positivamente com a atividade fMRI em ambos os M1s dos doentes com NF1. Essa relação ocorreu sobretudo nos ritmos de tapping mais elevados e não foi observada no grupo controlo. Para além disso, os níveis BOLD do M1 não-dominante espelharam os do M1 dominante no grupo NF1.

Em conclusão, alterações neuroquímicas e/ou funcionais no M1 e no cerebelo poderão ser a causa da diminuição das capacidades motoras observadas na NF1, devendo, por isso, ser objeto de estudos adicionais no futuro.

Palavras-chave: Neurofibromatose 1, Destreza Motora, Córtex Motor, Cerebelo, Imagem por Ressonância Magnética, Espectroscopia de Ressonância Magnética, Ácido gama-Aminobutírico.

Introduction

Neurofibromatosis type 1 (NF1) is a common autosomal dominant neurocutaneous syndrome.[1,2] Beyond the likely clinical manifestations such as café-au-lait macules or neurofibromas,[1,3] NF1 children reveal impairments of several motor domains, including fine motor precision, motor integration, and upper limb coordination.[4] These deficits affect their quality of life[5] and seem to extend into adulthood.[6,7]

The primary motor cortex (M1) plays an essential role in the individuated finger movements.[8] In the M1 of healthy subjects, gamma-aminobutyric acid (GABA) has been shown to be reduced during a motor learning task but not during simple movement.[9] GABA concentration and inhibitory neuronal activity have been suggested to be negatively related with the blood-oxygen-level-dependent (BOLD) signal measured with functional magnetic resonance imaging (fMRI).[10–12] Similar results were observed in the occipital cortex of NF1 patients.[13] However, the relation between GABA levels and BOLD signal in M1 has never been assessed in NF1 patients.

Both M1s connect with each other through the corpus callosum,[14,15] which is enlarged in NF1 children.[16,17] The corpus callosum seems to consist of excitatory fibers which can either exert an excitatory or an inhibitory interhemispheric action, the latter through activation of GABAergic interneurons.[18] An enlargement of the corpus callosum may translate abnormalities in the interhemispheric inhibition (IHI) between M1s, possibly contributing to the motor deficits in NF1 patients.

It has also been postulated that in NF1 there is tonically increased GABAergic inhibition in the brain[19–21] with a trend for increased inhibition in M1 after transcranial magnetic stimulation (TMS) and consequent deficits in the induction of long term potentiation (LTP).[22] This seems not to be in accordance with the Bienenstock-Cooper-Munro rule, in which a strong inhibitory tone should prevent further inhibitory responses and facilitate the excitatory ones,[23] as it has been observed in M1 of healthy subjects.[24]

Using paired associative stimulation (PAS) in healthy volunteers, it has been shown that cerebellar excitation prevents plastic changes in M1, whereas cerebellar inhibition allows M1 to adapt and acquire new elements of a motor program, uncovering the important influence of the cerebellum in M1 plasticity.[25]

Since NF1 patients present GABAergic abnormalities in several brain regions,[13,26,27] GABAergic mechanisms in both M1s and cerebellum may be disrupted. That may be evident in a finger-tapping task, in which both M1s and the cerebellum are recruited.[28]

Thus, this study aims to assess for the first time the relation between GABA levels of the dominant M1 and BOLD signal of both M1s and cerebellum in NF1 patients performing a finger-tapping task.

Materials and methods

Participants

A cross-sectional study was designed, in which a cohort of adult NF1 patients (males and females) was obtained from a database created with the contribution of the Portuguese Association of Neurofibromatosis.[7] All the NF1 patients listed in the database had a clinical or genetic diagnosis of NF1 according to the criteria defined by the National Institutes of Health Consensus Development Conference of 1998,[7] and could potentially enter this study.

Participants who suffered, in that moment or in the past, from another neurologic, psychiatric or neurodevelopment disorder or had been taking medication for mental illness in the previous year or had an intelligence quotient (IQ) lower than 75 were not included. IQ was assessed through the Portuguese-adapted version of the Wechsler Adult Intelligence Scale (WAIS-III).[7]

After meeting the inclusion and exclusion criteria, 21 NF1 patients were assigned to participate in the study. Twenty-one controls who matched NF1 patients in age and gender were recruited through advertisement on the Internet, but one was then excluded due to neurologic disorder. Thus, the final sample that entered the study consisted of 21 NF1 patients (9 males and 12 females, with an average age of 36.68±6.66 years) and 20 controls (8 males and 12 females, with an average age of 36.84±7.07 years).

The Ethics Committee of the Faculty of Medicine of the University of Coimbra approved the study and all the participants signed informed consent.

Brain dominance was inferred through the Edinburgh Handedness Inventory,[7] which revealed that only one participant (NF1) was left-handed.

All the selected participants had normal or corrected visual acuity, as well as normal performance in the Stroop Color and Word Test.

Task / fMRI acquisition

A stimulus was programmed using the Psychophysics Toolbox 3 on Matlab R2013b (MathWorks, Natick, Massachusetts, USA) and fMRI data of both NF1 and controls were acquired during a task of finger-tapping under auditory stimulus received by MR-compatible headphones.[7] Tapping was made at three different rhythms (1, 3 and 5 Hz), each of them having a synchronous condition and an asynchronous condition, a total of 6 conditions. Both index fingers were used at the same time in the synchronous conditions (when the letter “S”

was shown in the MR-compatible LCD) and alternately in the asynchronous conditions (when the letter “A” was shown). The monitor was a 698.40 x 392.85 mm LCD (NordicNeuroLab, Bergen, Norway) placed at 156 cm from the subjects’ eyes.[7]

The subjects performed four repetitions of a sequence composed of baseline (9 seconds), synchronous tapping (9 seconds), baseline (9 seconds) and asynchronous tapping (9 seconds) for 1, 3 and 5 Hz, a total of 7 minutes and 12 seconds (Figures 1 and 2). It was given them time to practice and get familiarized with the task.

The fMRI acquisition was made in a 3 Tesla Magnetom Tim Trio Scanner (Siemens, Erlangen, Germany) after a T1-weighted structural acquisition.[7] Echo-planar sequences with a voxel size of 3 mm^2 were obtained.[7] Slices were parallel to the anterior commissure-posterior commissure line and had 3 mm of thickness.[7]

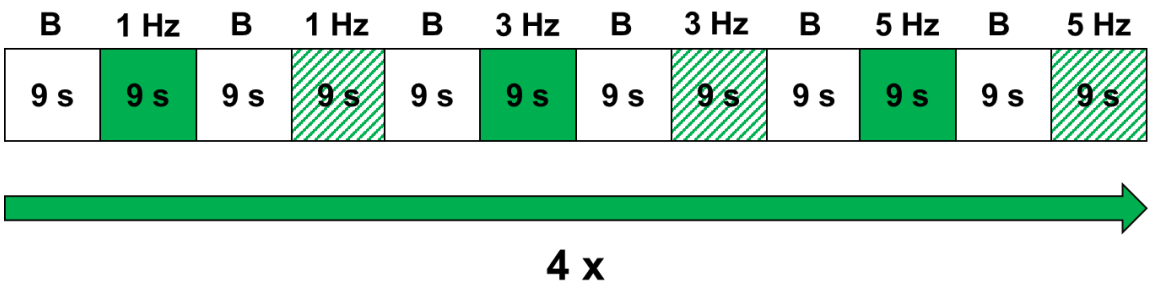

Figure 1. Design of the task. B = baseline; ▭ = synchronous condition; ▨ = asynchronous condition.

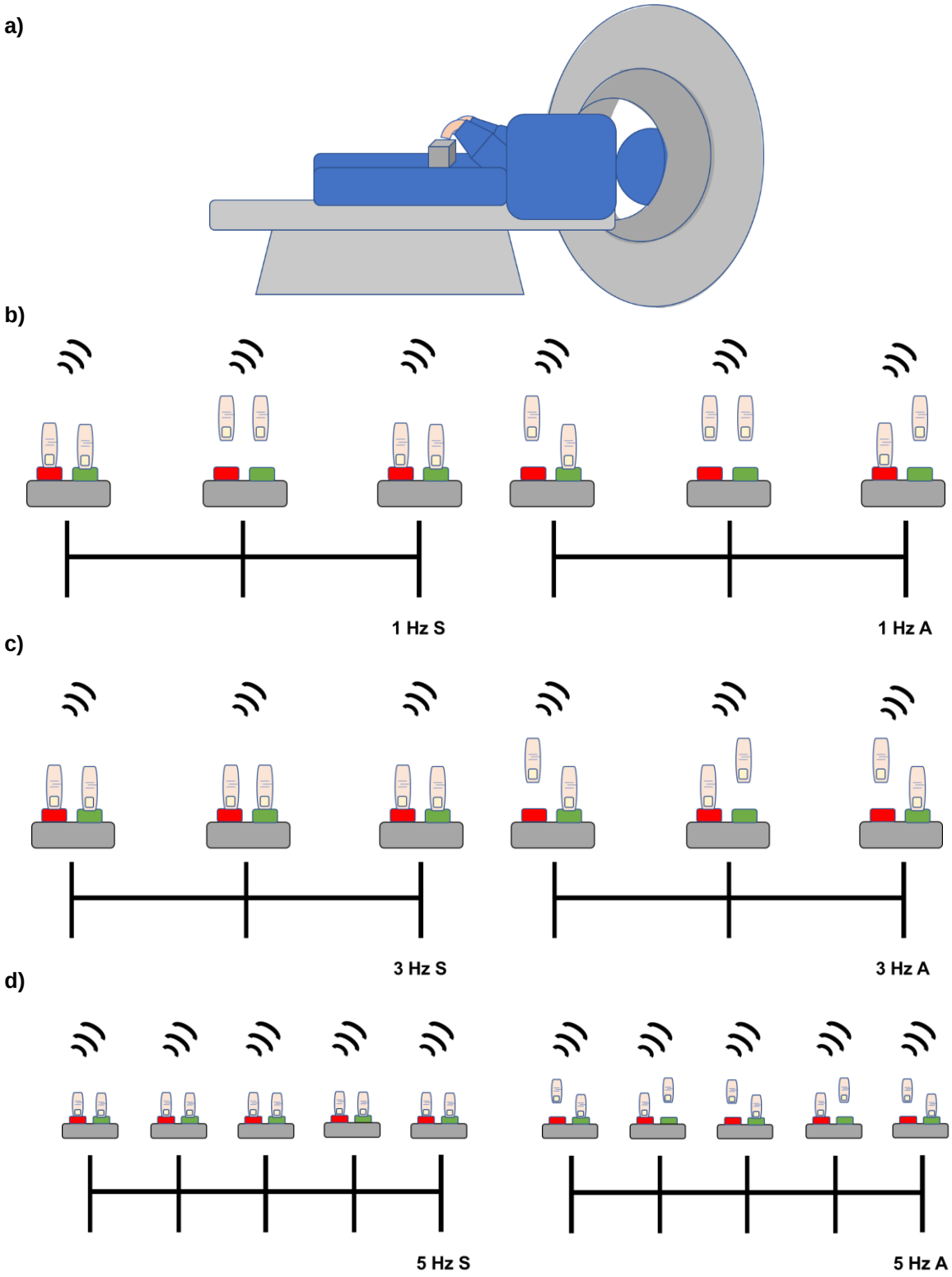

Figure 2. Representation of the task while the fMRI acquisition was made (a). The schemes illustrate the audio-paced finger-tapping at 1 Hz (b), 3 Hz (c) and 5 Hz (d). The synchronous conditions (S) are on the left and the asynchronous conditions (A) are on the right.

Magnetic resonance spectroscopy (MRS)

At the end of the task, a single voxel GABA-edited MRS was individually performed to all the participants, using the MEGA-PRESS spectral editing sequence,[29] with an echo time of 68 milliseconds, repetition time of 1,5 seconds, 196 averages and 1024 data points.[26] To do that, a 3 cm^3 isotropic voxel was defined and placed in the left M1 of 40 participants and in the right M1 of the one left-handed NF1 subject (dominant hemisphere). M1 was identified through fMRI cerebral activation patterns in the expected anatomical location during the functional acquisition.

GABA quantification was made through Gannet 2.0 toolkit, a Matlab-based tool.[29–31] The edited signal detected at 3 ppm corresponds to the sum of the GABA levels with those of homocarnosine and macromolecules (GABA+). GABA+ was then fitted with a Gaussian curve (average fitting error of 10.41±3.57%). Correction for grey and white matters and cerebrospinal fluid was made using T1-weighted sequences.

The sum of glutamate and glutamine (Glx) was measured using the same tools and the ratio between GABA+ and Glx was calculated.

fMRI analysis

The fMRI data were analyzed with BrainVoyager 20.6.2. Functional MRI sequences were corrected for slice scanning time difference, motion artifacts and were then filtered in the time domain. Data were normalized to the Talairach space. Sequences were submitted to spatial smoothing using a Gaussian kernel with a full width at half maximum of 3 mm.[7]

Volumes of interest (VOIs) of each subject were individually defined to non-dominant M1, dominant M1 and cerebellum through a General Linear Model (GLM) Single Study considering the clusters of activation at 1, 3 and 5 Hz (synchronous and asynchronous), in which the convolution of the boxcar function with a standard 2-gamma hemodynamic response function allowed to obtain the predictor's model.[7]

We performed a group GLM with the data of the 39 participants who had less than 3 mm of movement during the fMRI and considering the clusters of activation at 1 and 3 Hz (both synchronous and asynchronous), which allowed to define group VOIs for the non-dominant M1, dominant M1 and cerebellum.

In some cases, the group VOIs or rectangular boxes were intersected with the individual VOIs to include only the voxels that were part of the anatomic brain regions seen in the T1-weighted structural MRI.

In the cerebellum, the activation occurred mainly in its superior part. Therefore, only clusters of activation in the superior part of the cerebellum entered the analysis.

Random effects analysis was made, and β-weights were obtained from the defined individual VOIs as an estimate of the BOLD response. The results were adjusted for false discovery rate (FDR) using a cut-off of 5%.[7] Only clusters with a minimum extension of 4 voxels were considered.

Statistical analysis

Data analysis was separately performed for the non-dominant M1, dominant M1 and cerebellum using the IBM SPSS Statistics version 23.

To assess whether GABA levels and the ratio between GABA+ and Glx differed between groups, Student's t-tests were made. A three-factor ANOVA was performed to evaluate the main effects of group, synchronization, and rhythm, as well as the interaction between them, on BOLD related β-weights. To test in which conditions the difference of β-weights between groups and between synchronizations was greater, post hoc Student's t-tests were made. The normality of the distributions was always assessed to choose the most suitable test and Levene's test allowed to evaluate the homogeneity of variances. There was no reason to exclude outliers.

Linear regression analysis was made to quantify the association between GABA levels and the average β-weight of all the rhythms, in each group.

Linear regression analysis was then performed to quantify the association between GABA levels and the individual β-weights at 1, 3 and 5 Hz for the synchronous and asynchronous conditions separately.

FDR adjustment through the Benjamini-Hochberg procedure was applied to the results of the 116 tests performed in this study, with a cut-off of 8%.

Results

All the participants have shown activation in the dominant M1. Two subjects have not presented activation in the non-dominant M1 (one NF1 and one control) and five subjects (two NF1 and three controls) have not exhibited activation in the cerebellum at the selected threshold. Thus, 21 NF1 and 20 controls entered the statistical analysis for the dominant M1, whereas 20 NF1 and 19 controls entered the analysis for the non-dominant M1. Regarding the cerebellum, 19 NF1 and 17 controls were analyzed.

Group VOIs are shown in Figure 3. Examples of individual VOIs are shown for one participant (control) in Figure 4.

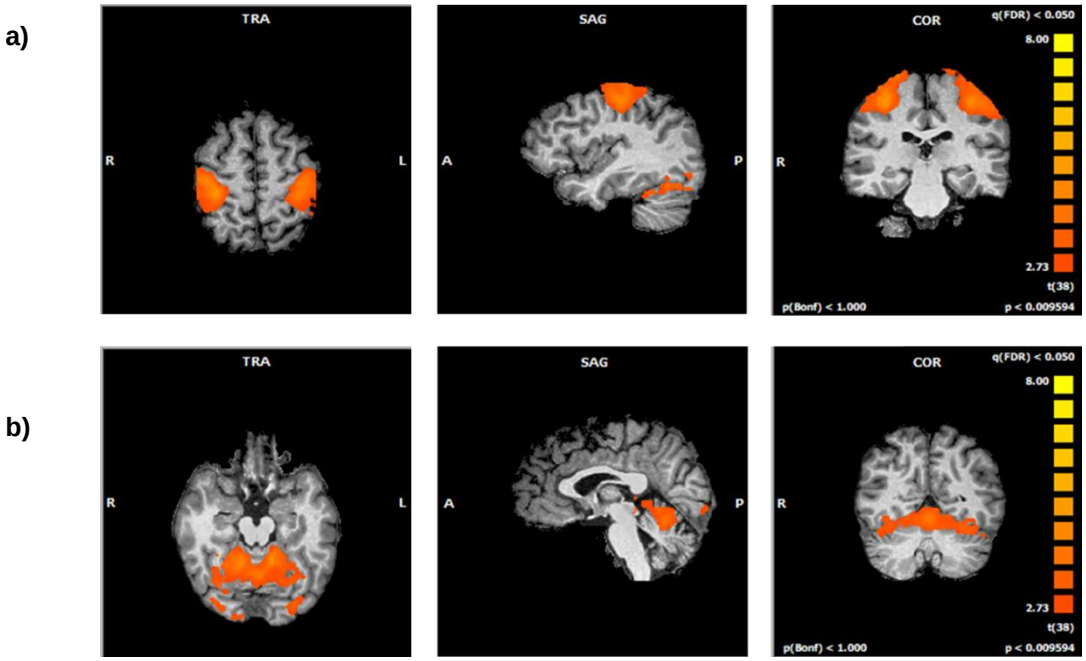

Figure 3. Group VOIs located in the right and left M1 (a) and in the cerebellum (b).

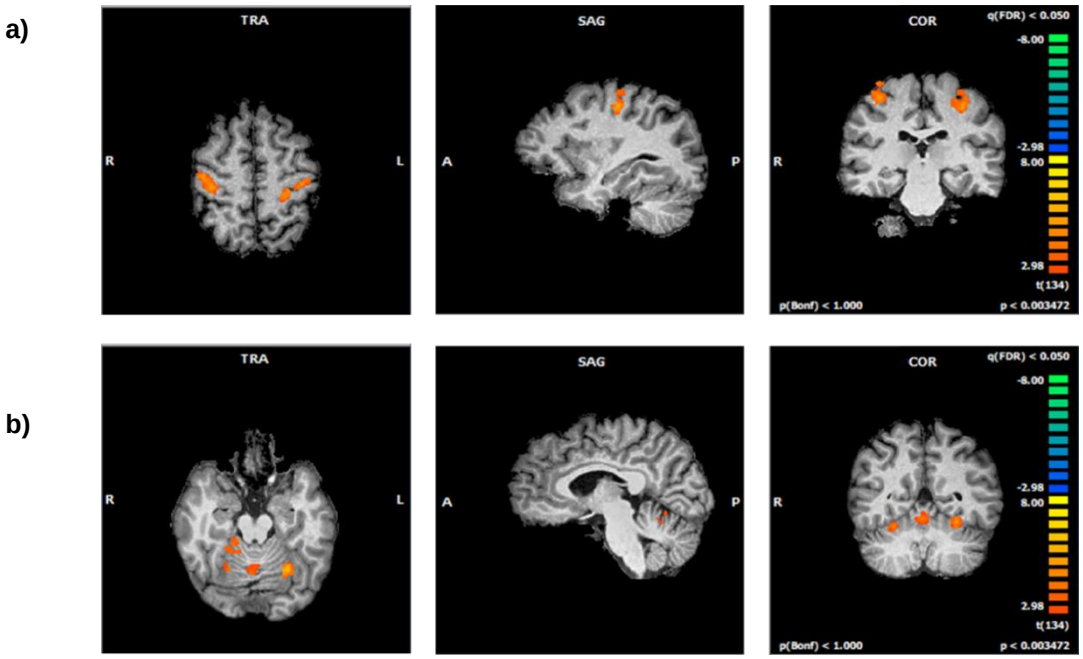

Figure 4. VOIs of one control participant located in the non-dominant and dominant M1 (a) and in the cerebellum (b).

Differences of β-weights of activation between groups, synchronizations, and rhythms

β-weights of the NF1 group significantly differed from those of the control group in the non-dominant M1 [mean(NF1)=1.07, SD=0.03; mean(controls)=0.89, SD=0.03; $F(1,222)=18.28$, $p<0.001$, FDR adjusted $p<0.01$], in the dominant M1 [mean(NF1)=0.96, SD=0.03; mean(controls)=0.81, SD=0.03; $F(1,234)=13.27$, $p<0.001$, FDR adjusted $p<0.01$] and in the cerebellum [mean(NF1)=0.78, SD=0.03; mean(controls)=0.65, SD=0.03; $F(1,204)=7.53$, $p=0.007$, FDR adjusted $p<0.017$] (Table I). Planned analysis showed that such difference was significantly greater at asynchronous 3 Hz of the non-dominant M1 [$t(37)=3.68$, $p=0.001$, FDR adjusted $p<0.013$] and at asynchronous 3 Hz of the dominant M1 [$t(39)=3.39$, $p=0.002$, FDR adjusted $p<0.014$] (Table II). In the cerebellum, there was not any specific condition in which that difference was significantly greater, suggesting that all contributed to the main effect (Table II).

β-weights of the synchronous conditions significantly differed from those of the asynchronous ones in the non-dominant M1 [mean(synchronous)=1.12, SD=0.03; mean(asynchronous)=0.85, SD=0.03; $F(1,222)=40.62$, $p<0.001$, FDR adjusted $p<0.01$], in the dominant M1 [mean(synchronous)=0.96, SD=0.03; mean(asynchronous)=0.81, SD=0.03; $F(1,234)=11.70$, $p=0.001$, FDR adjusted $p<0.013$] but not in the cerebellum (Table I). In the control group, that difference was significantly greater at 1 Hz [$t(36)=3.65$, $p=0.001$, FDR adjusted $p<0.013$], at 3 Hz [$t(36)=3.19$, $p=0.003$, FDR adjusted $p<0.014$] and at 5 Hz

[t(36)=2.97, p=0.005, FDR adjusted p<0.015] in the non-dominant M1, and only at 1 Hz in the dominant M1 [t(38)=2.69, p=0.011, FDR adjusted p<0.018]. In the NF1 group, that difference was significantly greater only at 1 Hz in the non-dominant M1 [t(38)=3.50, p=0.001, FDR adjusted p<0.013] and only at 1 Hz in the dominant M1 [t(40)=2.65, p=0.012, FDR adjusted p<0.019] (Table III).

β-weights significantly differed between rhythms in the non-dominant M1 [F(2,222)=135.71, p<0.001, FDR adjusted p<0.01], in the dominant M1 [F(2,234)=90.07, p<0.001, FDR adjusted p<0.01] and in the cerebellum [F(2,204)=27.32, p<0.001, FDR adjusted p<0.01] (Table I). Post hoc tests have shown β-weights at 5 Hz to be significantly different from β-weights at 3 Hz, both being significantly different from β-weights at 1 Hz in the non-dominant M1 [mean(5 Hz)=1.36, SD=0.04; mean(3 Hz)=1.07, SD=0.04; mean(1 Hz)=0.52, SD=0.04, p<0.001, FDR adjusted p<0.01], in the dominant M1 [mean(5 Hz)=1.20, SD=0.04; mean(3 Hz)=0.95, SD=0.04; mean(1 Hz)=0.50, SD=0.04, p<0.001, FDR adjusted p<0.01] and in the cerebellum [mean(5 Hz)=0.93, SD=0.04; mean(3 Hz)=0.69, SD=0.04; mean(1 Hz)=0.53, SD=0.04, p<0.013, FDR adjusted p<0.019] (Table I).

No interaction effect between group, synchronization or rhythm was significant in any region.

Table I. ANOVA results. Main effect of group, synchronization, and rhythm on β-weights.

When are the β-weights higher?	Non-dominant M1	Dominant M1	Cerebellum
• NF1 > CTR	✓	✓	✓
• SYNC > ASYNC	✓	✓	✗
• 5 Hz > 3 Hz > 1 Hz	✓	✓	✓

CTR = control group; SYNC = synchronous condition; ASYNC = asynchronous condition;

☑ = significant result (FDR<8%); ☒ = non-significant result (FDR<8%).

Table II. T-tests results. When are the differences of β-weights between groups greater?

NF1 β-weights > CTR β-weights But under what conditions are these diferences greater?			
Non-dominant M1	SYNC 1 Hz ✗	SYNC 3 Hz ✗	SYNC 5 Hz ✗
	ASYNC 1 Hz ✗	ASYNC 3 Hz ✓	ASYNC 5 Hz ✗
Dominant M1	SYNC 1 Hz ✗	SYNC 3 Hz ✗	SYNC 5 Hz ✗
	ASYNC 1 Hz ✗	ASYNC 3 Hz ✓	ASYNC 5 Hz ✗
Cerebellum	SYNC 1 Hz ✗	SYNC 3 Hz ✗	SYNC 5 Hz ✗
	ASYNC 1 Hz ✗	ASYNC 3 Hz ✗	ASYNC 5 Hz ✗

CTR = control group; SYNC = synchronous condition; ASYNC = asynchronous condition;

☑ = significant result (FDR<8%); ☒ = non-significant result (FDR<8%).

Table III. T-tests results. When are the differences of β-weights between synchronizations greater?

SYNC β-weights > ASYNC β-weights in both M1s But when are these diferences greater?			
Non-dominant M1	CTR 1 Hz ✓	CTR 3 Hz ✓	CTR 5 Hz ✓
	NF1 1 Hz ✓	NF1 3 Hz ✗	NF1 5 Hz ✗
Dominant M1	CTR 1 Hz ✓	CTR 3 Hz ✗	CTR 5 Hz ✗
	NF1 1 Hz ✓	NF1 3 Hz ✗	NF1 5 Hz ✗

CTR = control group; SYNC = synchronous condition; ASYNC = asynchronous condition;

☑ = significant result (FDR<8%); ☒ = non-significant result (FDR<8%).

GABA levels and the ratio GABA/Glx between groups

GABA levels of all the participants were successfully measured. Glx of one subject (NF1) was not successfully extracted.

GABA levels of the NF1 group [mean=0.61 IU, SD=0.12 IU] were slightly lower than those of the control group [mean=0.66 IU, SD=0.10 IU], although not significantly (Figure 5a). The ratio GABA/Glx of the NF1 group [mean=1.36 IU, SD=0.18 IU] was also slightly lower compared to the control group [mean=1.49 IU, SD=0.23 IU], but not significantly (Figure 5b).

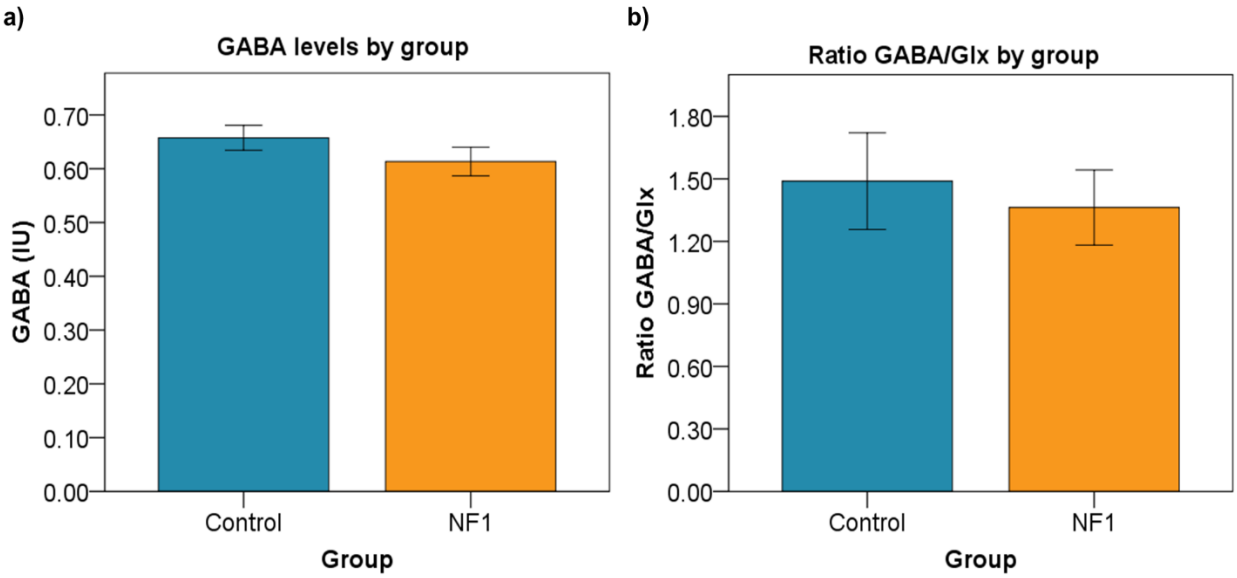

Figure 5. Mean ± standard deviation of the GABA levels (a) and the ratio GABA/Glx (b) in the dominant M1 of all the participants separated by group.

Linear regressions between GABA levels and the average β-weight of activation

Non-dominant M1

In the non-dominant M1 of the NF1 group, GABA levels positively correlated with the average β-weight at asynchronous conditions (r^2=0.355, p=0.006, FDR adjusted p<0.016). At synchronous conditions, no correlation was found. In the control group, GABA levels did not correlate with the average β-weight at synchronous or asynchronous conditions (Figure 6a).

Dominant M1

In the dominant M1 of the NF1 group, GABA levels positively correlated with the average β-weight at asynchronous conditions (r^2=0.280, p=0.014, FDR adjusted p<0.02). At synchronous conditions, no correlation was found. In the control group, GABA levels did not correlate with the average β-weight at synchronous or asynchronous conditions (Figure 6b).

Cerebellum

In the cerebellum of both the NF1 and control groups, GABA levels did not correlate with the average β-weight at synchronous or asynchronous conditions.

Only the graphics with significant results are shown (Figure 6).

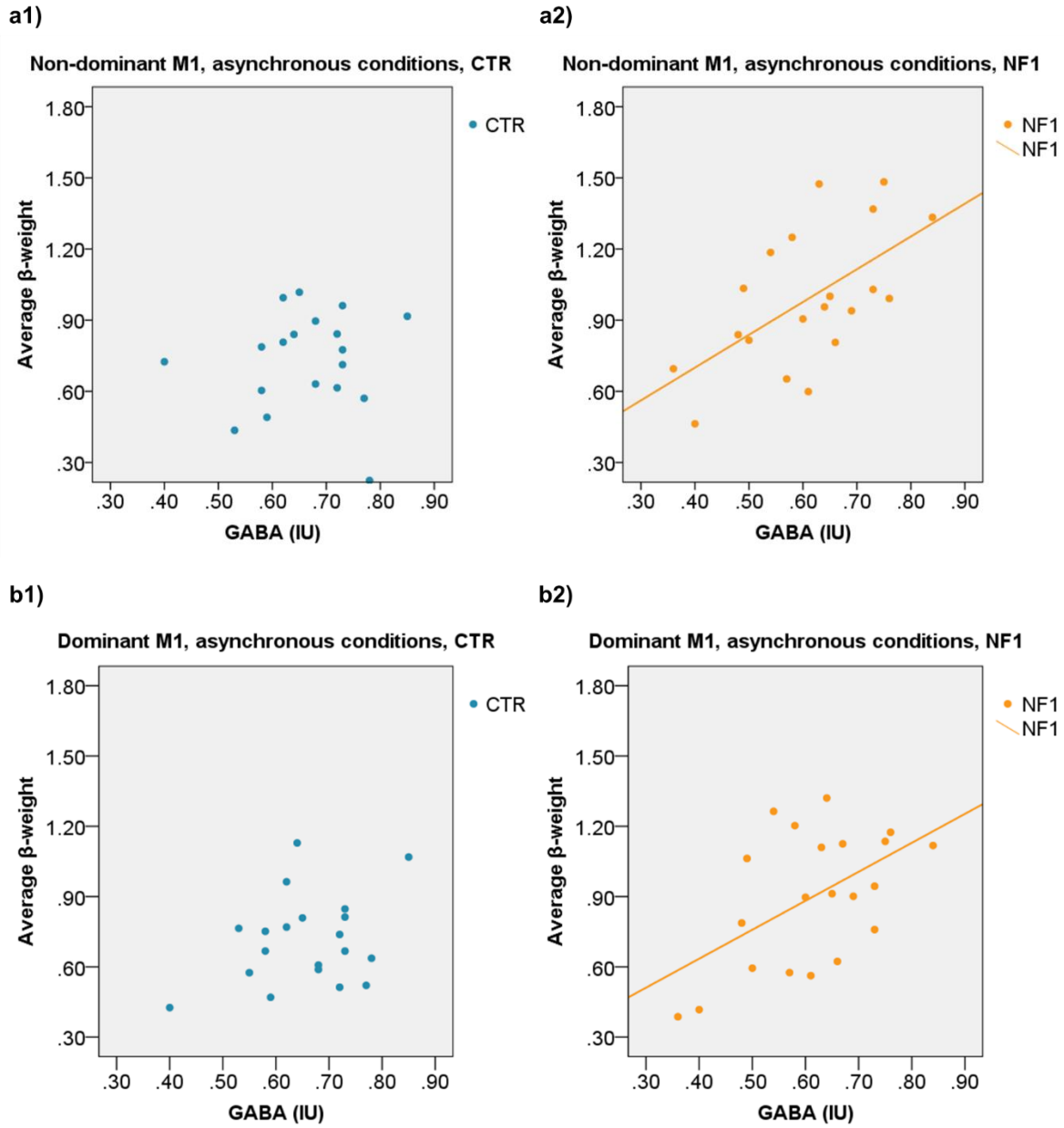

Figure 6. The correlation between GABA levels and the average β-weight at asynchronous conditions in the non-dominant M1 (a) and in the dominant M1 (b). The control group (CTR) is represented on the left (1) and the NF1 group on the right (2). The solid line represents the existence of a significant linear relationship (FDR<8%).

Linear regressions between GABA levels and β-weights of activation at each condition

Non-dominant M1

In the non-dominant M1 of the NF1 group, at asynchronous conditions, GABA levels positively correlated with β-weights at 3 Hz (r^2=0.274, p=0.018, FDR adjusted p=0.02) and 5 Hz (r^2=0.351, p=0.006, FDR adjusted p<0.016). No correlation was found at synchronous conditions nor at asynchronous 1 Hz. In the control group, GABA levels did not correlate with β-weights at any condition (Figures 7a and 8a).

Dominant M1

In the dominant M1 of the NF1 group, at asynchronous conditions, GABA levels appeared to positively correlate with β-weights at 3 Hz (r^2=0.217, p=0.033) and 5 Hz (r^2=0.221, p=0.032), but the FDR adjusted p-values were not significant. No correlation was found at synchronous conditions nor at asynchronous 1 Hz. In the control group, GABA levels did not correlate with β-weights at any condition (Figures 7b and 8b).

Cerebellum

In the cerebellum of both the NF1 and control groups, GABA levels did not correlate with β-weights in any of the synchronous or asynchronous conditions.

Only the graphics with significant results are shown (Figures 7 and 8).

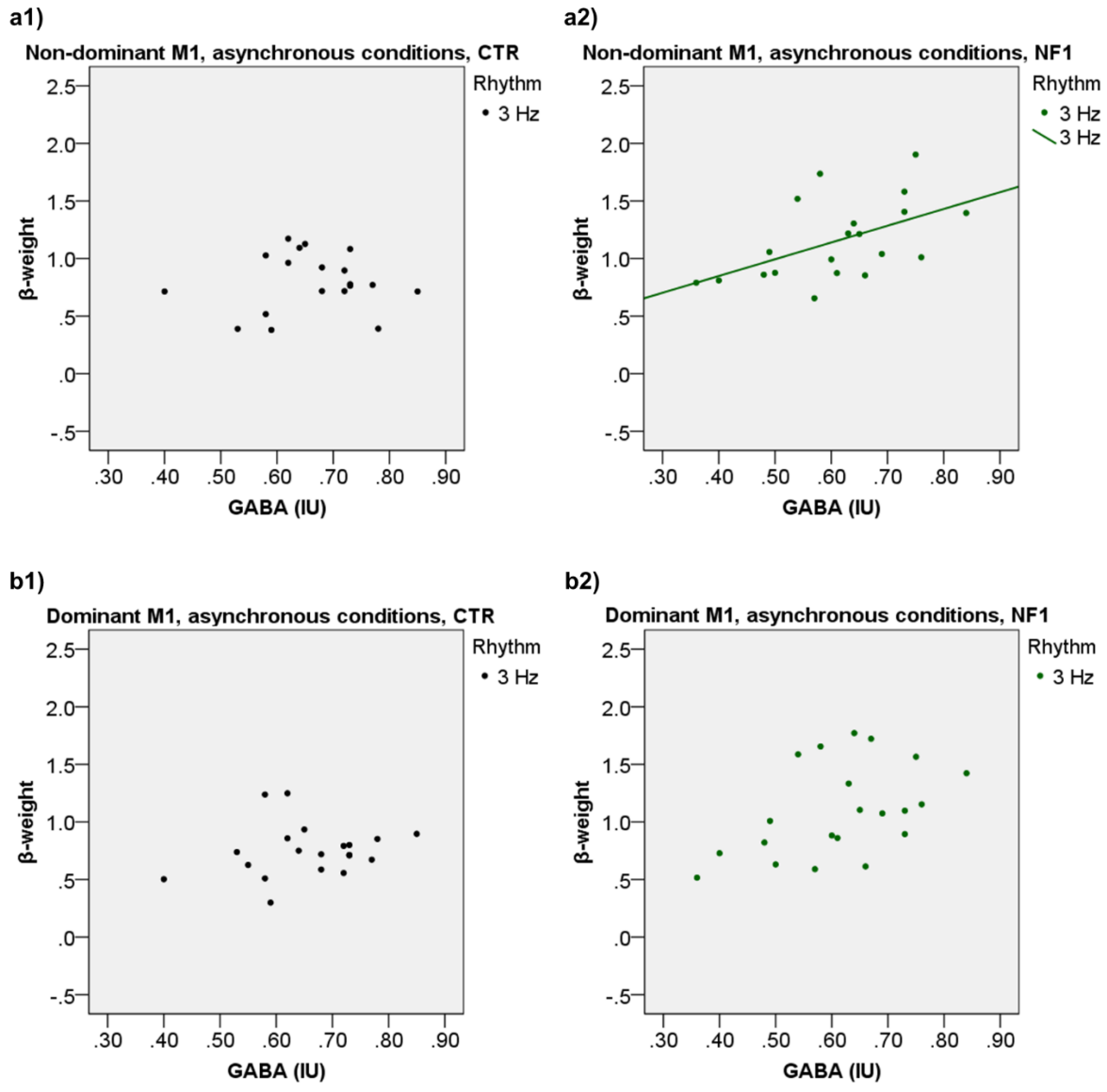

Figure 7. The correlation between GABA levels and β-weights at asynchronous 3 Hz in the non-dominant M1 (a) and in the dominant M1 (b). The control group (CTR) is represented on the left (1) and the NF1 group on the right (2). The solid line represents the existence of a significant linear relationship (FDR<8%).

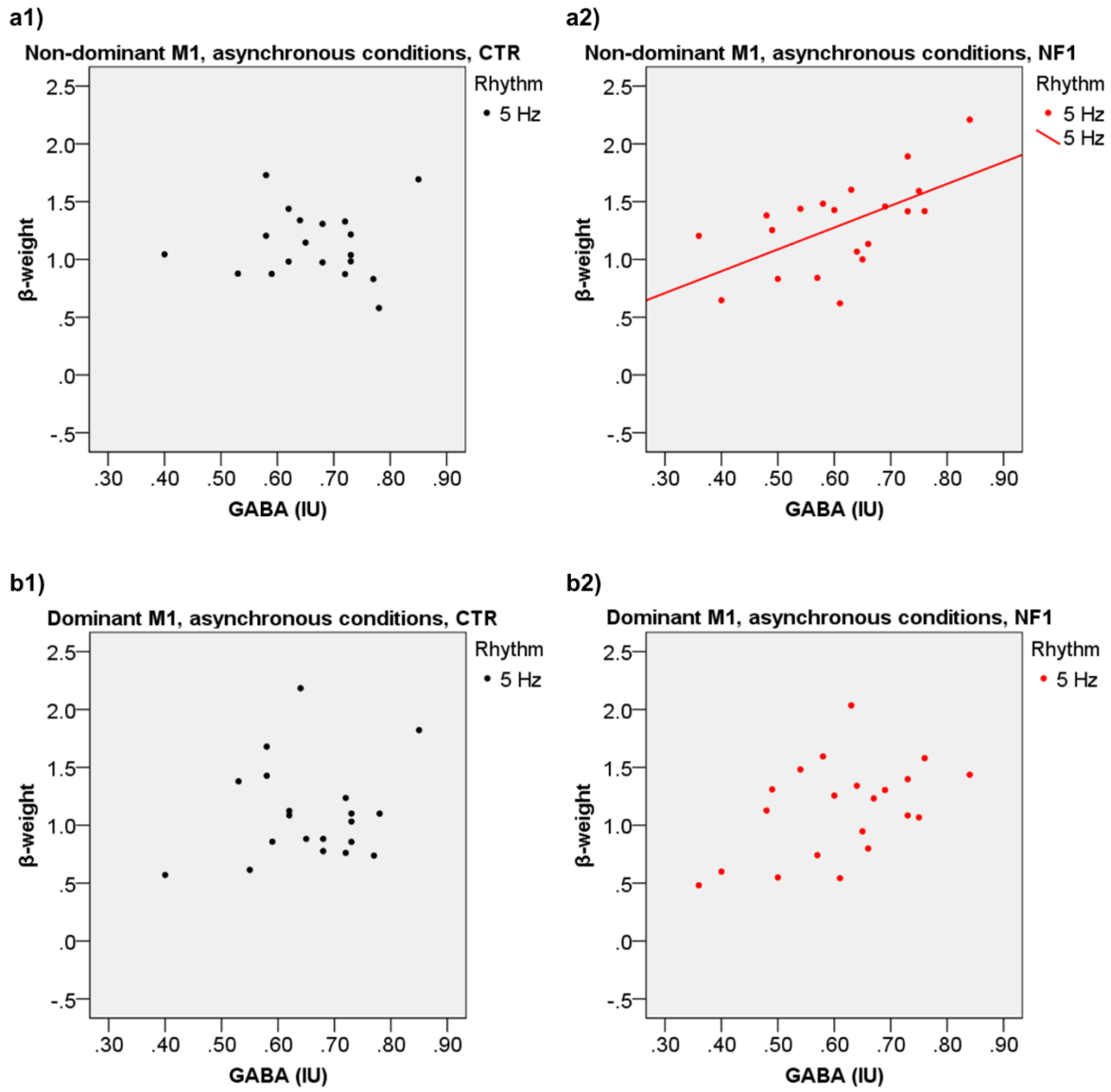

Figure 8. The correlation between GABA levels and β-weights at asynchronous 5 Hz in the non-dominant M1 (a) and in the dominant M1 (b). The control group (CTR) is represented on the left (1) and the NF1 group on the right (2). The solid line represents the existence of a significant linear relationship (FDR<8%).

Discussion

NF1 patients have higher β-weights of activation than controls

NF1 patients significantly have higher β-weights than controls in both M1s and the cerebellum (Table I), with that difference being higher at asynchronous 3 Hz in both M1s (Table II). The BOLD signal reflects hemodynamics in nature,[32] and it is thought to reflect neuronal activation.[32–34] Thus, NF1 patients may need to generate compensatory activity patterns to perform a finger-tapping task with increasing rhythms.

β-weights of activation are higher at the synchronous conditions

β-weights are higher at the synchronous conditions compared to the asynchronous ones in both M1s but not in the cerebellum (Table I). This was expected since asynchronous tapping implies a relative deactivation of the ipsilateral M1.[35,36] In the dominant M1 of both groups that effect is lost at highest rhythms (Table III). The identification of significant effects mainly at the lower frequencies might relate to the fact that these are closer to the temporal resolution of fMRI (in the order of seconds[37]).

Interestingly, at the highest rhythms, that effect is significant in the non-dominant M1 of controls, but not in the non-dominant M1 of NF1 subjects (Table III). In studies of ipsilateral movements,[38,39] healthy subjects had already been shown to exhibit decreased activation in the non-dominant M1 compared to the dominant M1. IHI, in which GABAergic interneurons play a role,[18] has been shown to be involved in that process.[38] Since the BOLD signal is thought to result from the balance between the release of glutamate and GABA,[40] NF1 patients may have a different interhemispheric interaction regarding the excitatory-inhibitory balance.

β-weights of activation increase with increasing rhythms

β-weights at 5 Hz are higher than β-weights at 3 Hz, which in turn are higher than β-weights at 1 Hz in both M1s and the cerebellum (Table I). This rate effect was already known,[41] meaning that higher rhythms require larger neural recruitment and higher neural firing density.

NF1 patients only show a trend for GABA levels and GABA/Glx levels, unlike previously reported in other brain regions

In average, NF1 subjects reveal a slight decrease of GABA in the dominant M1 (Figure 5), being in line with previous studies,[13,26,27] which exhibited similar results in other brain regions of NF1 patients. A prior study in the frontal eye fields showed that NF1 subjects with the highest density of $GABA_A$ receptors have the lowest GABA levels, which are reduced to a significant level,[26] contrary to what was observed in this study. Since inhibitory tone seems to predominate in several brain regions of NF1 patients[19–22] and MRS mainly detects the non-bound GABA,[13] that negative correlation suggests a regulatory mechanism between receptor and neurotransmitter levels. It remains, however, to be demonstrated whether such functional spectroscopy is feasible.[42,43]

In a future study, GABA levels should also be measured before the beginning and during the task and compared with the levels at the end of it.

GABA levels positively correlate with the β-weight of activation at asynchronous conditions in NF1 patients

In both M1s of NF1 subjects, the β-weight of activation significantly increases with the increase of GABA levels at asynchronous conditions. That mainly occurs at the highest rhythms of tapping and it is not verified in controls (Figures 6, 7 and 8).

So, β-weights of NF1 patients seem to depend on GABA levels mainly at faster tappings, which require a quick and intermittent inhibition of the ipsilateral M1. This is not observed in controls, who may also depend on additional mechanisms. These findings are only significant at asynchronous conditions probably because that is when the inhibition is most required. At synchronous conditions, such inhibition in the pauses between tappings is probably less relevant.

In a previous study in which participants performed a visual task, GABA levels negatively correlated with the BOLD signal in the occipital cortex of NF1 patients.[13] In contrast, a positive correlation between GABA levels and BOLD signal was observed in the present study, in which participants performed a motor task with increasing rhythms. Since fMRI activity has been shown to increase with the rate of tapping,[41] and the inhibitory neural activity is thought to contribute to the BOLD signal,[32–34] NF1 patients may have a higher GABAergic demand in this task.

The non-dominant M1 of NF1 patients mirrors the changes of its dominant M1

It is verified that both M1s have their activation increased (Tables I and II). Furthermore, in NF1 patients, unlike controls, the fMRI activity in the non-dominant M1 correlates with GABA levels of the dominant M1 (Figures 6, 7 and 8). So, an interhemispheric correlation is verified in the NF1 group.

IHI presents as an important mechanism to avoid mirroring in one-handed movements[38] and it may be impaired in NF1 patients. Congenital mirror movements have been associated with agenesis of the corpus callosum[44] and the enlargement of the corpus callosum observed in NF1 subjects[16,17] probably translates its dysfunction. In the future, studies addressing the corpus callosum should be performed in NF1 patients to better characterize its changes.

In the cerebellum, NF1 patients have higher β-weights of activation than controls, but no significant correlation with GABA is verified

Although β-weights in the cerebellum of NF1 patients are higher than controls (Table I), the β-weight of activation does not significantly correlate with GABA levels at synchronous or asynchronous conditions. Also, no correlation is verified in the control group. Thus, despite the compensatory activity patterns generated in the cerebellum of NF1 patients, the activity of the cerebellum seems to be independent of GABA levels.

GABAergic changes may occur in other brain structures that mediate the dialogue between M1 and the cerebellum. These brain regions connect with each other through the cerebello-thalamo-cortical pathway.[45] The thalamus is the relay station of several nerve fibers in the brain, including those between the cerebellum and M1.[46] A previous study in NF1 patients showed a reduction of $GABA_A$ receptor density in the thalamus.[26] So, GABAergic mechanisms connecting the M1 and cerebellum may be impaired in NF1 subjects due to abnormalities in the thalamus. In the future, studies addressing the thalamus of NF1 patients should be made.

GABA levels of the non-dominant M1 and cerebellum should also be measured to understand if they suffer the same variations than those of the dominant M1.

Conclusion

This study evaluated for the first time the relation between GABA levels of the dominant M1 and BOLD signal of both M1s and cerebellum in NF1 patients performing a finger-tapping task.

The results showed BOLD signal to be significantly higher in both M1s and the cerebellum of the NF1 group compared to the control group, suggesting a possible compensatory mechanism. In addition, at asynchronous tapping, GABA levels of the dominant M1 positively correlated with the fMRI activity in both M1s of NF1 patients. That was mainly verified at the highest rhythms of tapping and it was not observed in the control group. In addition, the non-dominant M1 of NF1 subjects mirrored the activity of the dominant M1. Therefore, neurochemical and activity changes in the M1 and the cerebellum may underlie the motor deficits observed in NF1 patients, which should be further addressed in future studies.

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