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Article

GABA Modulates Frequency-Dependent Plasticity in Humans

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HIGHLIGHTS

In the context of repetitive sensory stimulation, GABA release is frequency dependent

Stimulating above the resonance frequency of the somatosensory cortex reduces GABA

Perceptual learning is associated with a reduction in GABA

Early GABA reduction opens a window for plasticity and learning

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Article

GABA Modulates Frequency-Dependent Plasticity in Humans

Caroline A. Lea-Carnall,[1,4,*] Stephen R. Williams,[2] Faezeh Sanaei-Nezhad,[2] Nelson J. Trujillo-Barreto,[1] Marcelo A. Montemurro,[1] Wael El-Deredy,[3,*] and Laura M. Parkes[1]

SUMMARY

Frequency-dependent reorganization of the primary somatosensory cortex, together with perceptual changes, arises following repetitive sensory stimulation. Here, we investigate the role of GABA in this process. We co-stimulated two finger tips and measured GABA and Glx using magnetic resonance (MR) spectroscopy at the beginning and end of the stimulation. Participants performed a perceptual learning task before and after stimulation. There were 2 sessions with stimulation frequency either at or above the resonance frequency of the primary somatosensory cortex (23 and 39 Hz, respectively). Perceptual learning occurred following above resonance stimulation only, while GABA reduced during this condition. Lower levels of early GABA were associated with greater perceptual learning. One possible mechanism underlying this finding is that cortical disinhibition "unmasks" lateral connections within the cortex to permit adaptation to the sensory environment. These results provide evidence in humans for a frequency-dependent inhibitory mechanism underlying learning and suggest a mechanism-based approach for optimizing neurostimulation frequency.

INTRODUCTION

Plasticity is the process of altering synaptic efficacy via long-term potentiation (LTP) or long-term depression (LTD) which result in strengthening or weakening of the synapses, respectively (Malenka and Bear, 2004). A number of studies have indicated that the frequency of synaptic activation modulates plasticity at both excitatory and inhibitory synapses. At many glutamatergic synapses, high-frequency stimulation has been observed to induce LTP, whereas low-frequency stimulation promotes depression, a phenomenon known as frequency-dependent plasticity (FDP). FDP has been observed in various animal models (Bear and Abraham, 1996; Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973; Kemp and Manahan-Vaughan, 2004; Kirkwood and Bear, 1994) with evidence that it may extend to sensory stimulation in humans (Lea-Carnall et al., 2017).

In humans, it has been shown that high-frequency tactile stimulation (20 Hz) delivered to the tip of the index finger improved 2-point tactile discrimination while low-frequency stimulation (1 Hz) impaired performance which was attributed to LTP-like changes in the primary somatosensory cortex (SI) (Ragert et al., 2008). It was noted in our recent work that 20 Hz is close to the stimulation frequency that evokes the maximum neural response of the somatosensory cortex, the resonance frequency (Snyder, 1992; Tobimatsu et al., 1999), and that the impaired performance at 1 Hz also applied to higher off-resonance frequencies (39 Hz) using tactile stimulation applied to two digits rather than one (Lea-Carnall et al., 2017), although this is likely via different mechanisms. Functional imaging showed that when the two digits were stimulated at the higher frequency (39 Hz), the digit representations in SI fused closer together which was associated with impaired performance on the tactile discrimination task, an observation interpreted by neural models as a result of strengthened connections between the regions. Conversely, when the two digits were stimulated close to the resonance frequency (23 Hz), performance was facilitated (as in (Ragert et al., 2008)) which was not accompanied by any shifting of the digit regions. Resonance is observed at every scale of cortical organization, from the individual synapse to complete brain regions across multiple animal models (Buzsáki et al.,

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2013; Hutcheon and Yarom, 2000; von Stein and Sarnthein, 2000). Cortical resonance is thought to be an emergent property of the network related to its features such as connection density, network size, and the biophysical properties of the constituent neurons (Hutcheon and Yarom, 2000; Lea-Carnall et al., 2016). It could be argued that such a property evolved in order to facilitate entrainment to the rhythmicity of the external environment (Hutcheon and Yarom, 2000).

Indeed, neural entrainment, the coupling of neural oscillators to an external stimulus, has been shown to be involved in a number of cognitive processes, regardless of sensory modality (Helfrich et al., 2019; Kösem et al., 2018; Obleser and Kayser, 2019; Pérez et al., 2019). Neural entrainment to the environment is crucial for structuring incoming information streams for further processing, including, attention selection, auditory sampling, and coupling neural rhythms to motor output (Lakatos et al., 2019). Entrainment is argued to be a basic mechanism that imparts temporal predictability and shapes sensory perception. Therefore, the selective neural and behavioral effects of entrainment at or away from the resonance frequency raise a number of questions about efficiency and adaptation of the brain's response to the environment. Understanding the mechanism of how or why stimulating at or off resonance results in different plasticity effects may help us to optimize stimulation paradigms in experimental and clinical settings.

There is growing evidence that the neurotransmitters gamma-aminobutyric acid (GABA) and glutamate (Glu) are functionally related to standard learning paradigms. For example, GABAergic disinhibition appears to be crucial to learning in the motor cortex (Floyer-Lea et al., 2006; Kolasinski et al., 2019; Stagg et al., 2011), and there appears to be a positive relationship between resting state Glu, glutamine (Gln), or GABA levels and plasticity in the visual and sensory cortices (Edden et al., 2009; Heba et al., 2016; Wijtenburg et al., 2017). In the case of perceptual learning, it has been shown that plasticity changes induced by presentation of repetitive stimulation applied to the finger tips is abolished in the presence of lorazepam, a $GABA_A$ receptor agonist (Dinse et al., 2003a). A similar study found that application of memantine, an N-methyl-D-aspartate (NMDA) receptor blocker, completely eliminated plastic changes (and impaired performance) (Dinse et al., 2003b). A further study tested the effect of scopolamine, a cholinergic antagonist thought to inhibit NMDA-receptor activity (Falsafi et al., 2012), and found that too eliminated the effects of stimulation on plasticity compared to a control group (Bliem et al., 2008). Taken together, these results indicate a mechanistic role for neurotransmitters GABA and glutamate (which act on GABA and NMDA receptors, respectively) in perceptual learning.

Here, we tested the relationship between frequency-dependent plasticity and perceptual learning by co-stimulating two digits of the right hand at one of two frequencies: 23 Hz (at-resonance) and 39 Hz (above-resonance) while quantifying $GABA_+$ (GABA + co-edited macromolecules) and Glx concentrations in contralateral SI using magnetic resonance spectroscopy (MRS). To test the consequence of the stimulation on perceptual learning, participants performed a forced-choice tactile discrimination test before and after stimulation. We predicted that performance on the tactile discrimination test would decline following above-resonance stimulation, in line with previous findings (Lea-Carnall et al., 2017). Furthermore, we predicted that $GABA_+$ concentration would decrease during this condition only, supporting the idea that frequency-dependent perceptual learning is facilitated by frequency-dependent $GABA_+$ reduction unmasking plasticity mechanisms. Additionally, we investigated the relationship between the change in $GABA_+$ and Glx versus change in performance on the psychophysics test and we hypothesized, as in (Stagg et al., 2011), that we would find a correlation between the reduction in $GABA_+$ and learning. Finally, we correlated $GABA_+$ in the early phase of the experiment to overall changes in performance on the test and similarly we hypothesized that early levels of GABA+ might predict overall learning rates, as found in (Kolasinski et al., 2019).

RESULTS

During each visit, participants completed a forced-choice tactile discrimination test immediately before and after the repetitive stimulation. The test required subjects to locate the site of a brief vibrational pulse delivered to one of two fingers. Each participant was scanned twice and received either the at-resonance or above-resonance stimulation, in an order counter-balanced across participants. MRS data were acquired for the first (early) and last (late) 12 min of co-stimulation from a voxel within contralateral SI. The MRS voxel placement was determined in the axial plane as being centered on the "hand knob" area of the post-central gyrus and rotated in both the sagittal and coronal planes so that it was aligned to the cortical surface (Puts et al., 2011; Yousry et al., 1997), please see Methods for further details.

Frequency-Dependent Perceptual Learning

Error rates for the forced-choice tactile discrimination tests were found to be 8.4 ± 5.5% prior to at-resonance stimulation (23 Hz) and 8.9 ± 6.9% prior to above-resonance stimulation (39 Hz). Intraclass correlation coefficient (ICC) verified the consistency of these mislocalization (ML) error rates over the two visits, see Equation (1). ICC(3,1) = 0.78 (p < 0.001, lower bound 0.45, upper bound 0.92). Post-stimulation ML error rates increased to 10.7 ± 8.5% after at-resonance and 15.8 ± 10.7% after the above-resonance co-stimulation.

Intersession reliability was assessed using ICC(3,1) (Shrout and Fleiss, 1979):

$$ICC(3, 1) = MSB - MSE/MSE + (k - 1) * MSE \qquad \text{(Equation 1)}$$

where MSB is the between-subjects mean square, MSE is the error mean square, and k is the number of repeated sessions.

Linear mixed-model analysis revealed a main effect of discrimination test time (pre/post) (F = 12.6, p = 0.004), no main effect of stimulation frequency (at-resonance/above-resonance) (F = 2.8, p = 0.12), and discrimination test time by stimulation frequency interaction (F = 6.1, p = 0.03) on the error rate. Post-hoc linear regression of the difference in ML errors (post-pre) showed a significant increase of 77% (95% CI: 40%, 113%; p < 0.001) after above-resonance stimulation and no statistically significant effect of at-resonance stimulation with a change of 27% (95% CI: −12%, 67%; p = 0.16). That is to say, participants were more likely to confuse the site of a brief pulse delivered to one finger after 46 min of repetitive stimulation, but the increase was only significant after the above-resonance stimulation. We found no effects on reaction times.

Frequency-Dependent Neurotransmitter Modulation

$GABA_+$ and Glx concentrations were estimated from averaged spectra over each of the two MRS blocks acquired during the first (early) and last (late) 12 min of the total 46 min of co-stimulation, see Figure 1D and 1E. Using N-acetylaspartate (NAA) concentration as a reference, we found $GABA_+$:NAA ratio was reduced in response to above-resonance stimulation, coinciding with the impairment in performance on the discrimination test. There was no change in $GABA_+$:NAA in response to at-resonance stimulation.

Linear mixed-model analysis on the $GABA_+$:NAA ratio revealed a main effect of stimulation frequency (at-resonance/above-resonance) (F = 8.3, p = 0.02), a main effect for MRS block (early/late) (F = 8.8, p = 0.008), and an MRS block by stimulation frequency interaction (F = 5.4, p = 0.03). Post-hoc analysis of the differences between the MRS measurements (late-early) for the two stimulation frequencies showed a mean change in $GABA_+$:NAA of −29% (95% CI: −45%, −13%; p = 0.001) for above-resonance stimulation and −3% (95% CI: −18%, 12%; p = 0.60) for at-resonance stimulation.

A similar test on the Glx:NAA concentrations found no effect of frequency (F = 0.03, p = 0.87), a main effect of MRS block (F = 5.9, p = 0.03), and no interaction (F = 0.15, p = 0.70). Post-hoc analysis of the differences (late-early) for each group showed a mean change in GLX:NAA of 17% (95% CI: −1%, 34%; p = 0.07) for above-resonance stimulation and 10% (95% CI: −7%, 29%; p = 0.23) for at-resonance stimulation.

We tested raw NAA in the same way as for the other metabolites and found no effect of stimulation frequency (F = 2.1, p = 0.18), no effect of MRS block (F = 3.3, p = 0.15), and no interaction (F = 2.5, p = 0.22). Finally, we tested the ratio of NAA:tCr and similarly found no effect of stimulation frequency (F = 0.3, p = 0.61), no effect of MRS block (F = 3.1, p = 0.12), and no interaction (F = 4.3, p = 0.09). A typical voxel placement is shown in Figure 1 (a) and an edited spectrum from a single participant is given in (c). We note here that spectral quality was consistent across the sessions, please see Table 1 for details of quality control metrics.

Relationship between GABA, Glx, and Perceptual Learning

Initially, we tested the relationship between $GABA_+$:NAA quantified from the first 12 min of the stimulation with the percentage difference in performance on the ML task (pre to post stimulation) over both conditions combined using Pearson correlation analysis. There was a significant negative correlation between $GABA_+$:NAA at the start of stimulation and change in ML error rate (R = −0.51, p = 0.01) which was maintained for the at-resonance condition (R = −0.75, p = 0.008) but not for the above-resonance condition
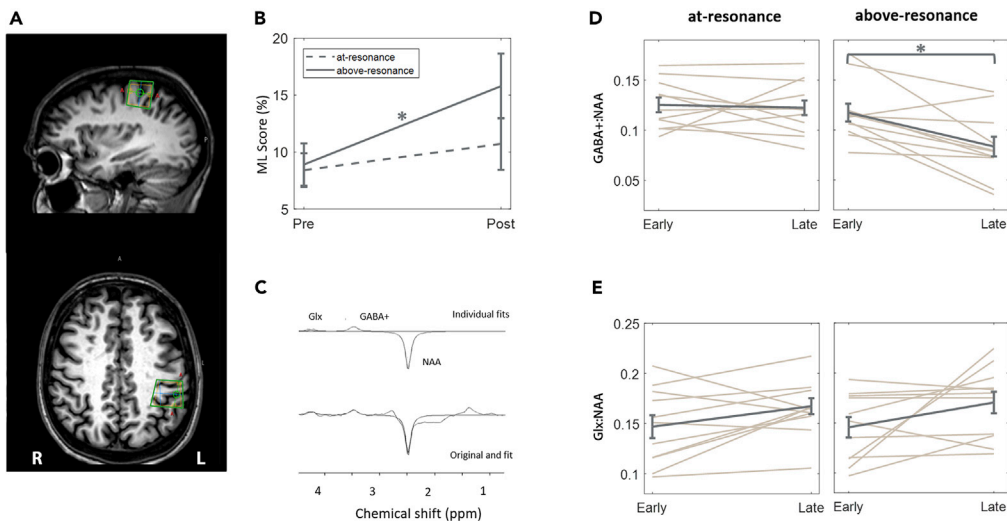
Figure 1. Frequency-Dependent Changes in Perception and Neurotransmitter Concentration

(A) MRS was acquired from left SI using a voxel measuring $20 \times 30 \times 20$ mm^3.

(B) Frequency-dependent perceptual learning: mean (and standard error) mislocalization rates on a forced-choice tactile discrimination task performed before and after 46 min of synchronous co-stimulation of D2 and D4 of the right hand using 23 Hz (at-resonance) and 39 Hz (above-resonance). Only above-resonance stimulation caused impaired performance on the task (F = 13.0, p = 0.003).

(C) Example of an edited spectrum acquired with MEGA-PRESS and its fit by AMARES. The lower trace shows the original data and the fit, while the upper trace shows the components of the fit: NAA, Glx (Glu + Gln), and GABA$_+$ (GABA + co-edited macromolecules).

(D) Mean (and standard error) GABA$_+$:NAA ratios acquired over the first and last 12 min of at-resonance stimulation (left) and above-resonance stimulation (right). A significant drop in GABA$_+$ is observed during above-resonance stimulation only (F = 12.4, p = 0.005).

(E) Mean (and standard error) Glx:NAA ratios acquired over the first and last 12 min of at-resonance stimulation (left) and above-resonance stimulation (right). No significant differences are observed.

(R = −0.42, p = 0.19), see Figure 2. In other words, subjects who had lower levels of GABA$_+$:NAA at the beginning of the stimulation went on to have greater increases in their ML error rates post stimulation. We interpret impairment of task performance as being indicative of plastic changes in SI, as in (Pilz et al., 2004).

Next, we tested whether the percentage difference in GABA$_+$:NAA between the early and late MRS blocks was correlated with the percentage difference in the ML score and found no correlation (R = 0.09, p = 0.70).

These tests were repeated for Glx:NAA and no correlation with learning was found, neither in the early MRS block (R = −0.04, p = 0.88), nor the percentage difference for GLX:NAA between the early and late MRS blocks (R = 0.28, p = 0.21).

DISCUSSION

Evidence from physiological recordings has shown that when endogenous brain oscillations phase align to salient events in a sensory stream, those events are processed and perceived more readily than non-aligned events (Schroeder and Lakatos, 2008). In other words, and regardless of the sensory modality, neural entrainment to the environment is crucial for structuring incoming information streams for further processing (Besle et al., 2011; Helfrich et al., 2019; Lakatos et al., 2019). Neural entrainment imparts temporal predictability that is argued to be a basic mechanism that shapes sensory perception. However, the neural and cognitive processes that arise due to entrainment seem to be frequency dependent (Lea-Carnall et al., 2017; Ragert et al., 2008). Of particular interest is how the frequency of the entraining stimulus selectively enables plasticity mechanisms and the special role of GABA in modulating such frequency-dependent plasticity (Davies et al., 1991).

	At-Resonance		Above-Resonance	
	Early	Late	Early	Late
Subjects recruited	14	14	14	14
Subjects rejected	3	2	3	3
Subjects remaining	11	12	11	11
NAA LW	7.9 ± 1.7 Hz	8.1 ± 1.5 Hz	8.4 ± 3.2 Hz	7.9 ± 1.4 Hz
NAA CRLB (%)	$5.3 \pm 3.9\%$	$5.4 \pm 5.3\%$	$9.4 \pm 8.2\%$	$8.4 \pm 7.5\%$
GABA CRLB	$1.6 \times 10^{-5} \pm 9.7 \times 10^{-6}$	$1.9 \times 10^{-5} \pm 9.2 \times 10^{-6}$	$1.4 \times 10^{-5} \pm 7.4 \times 10^{-6}$	$1.5 \times 10^{-5} \pm 6.2 \times 10^{-6}$
GABA CRLB (%)	22.8 ± 5.8	$24.8 \pm 6.7\%$	$24.7 \pm 7.3\%$	$41.0 \pm 15.9\%$
SNR	13 ± 2.5	13 ± 3.4	15 ± 4.3	14 ± 5.3

Table 1. The Quality Assessment Markers Are Reported Per Group

LW: linewidth; CRLB: Cramer-Rao lower bounds, which is presented as an absolute value for GABA+ and as a percentage of amplitude for both GABA+ and NAA; SNR: time-domain signal-to-noise ratio. Values are presented as mean $\pm$ standard deviation (SD) for the final list of included datasets. Linear mixed-model analysis on the NAA LW and SNR showed no effects of the stimulation.

Here, we combined psychophysics testing with MR spectroscopy to investigate the neuromodulatory basis of frequency-dependent plasticity and its role in perceptual learning. We used a paradigm of digit co-stimulation (Pilz et al., 2004; Vidyasagar et al., 2014) to induce FDP in human SI at two different frequencies. We confirmed frequency specific changes in perception as previously reported (Lea-Carnall et al., 2017). Subjects were more likely to mislocalize a brief pulse delivered to one finger following above-resonance stimulation which is interpreted as an indicator of cortical reorganization (Pilz et al., 2004). We quantified $GABA_+$ and Glx during the first and last 12 min of the co-stimulation to test for frequency-specific changes of these metabolites and whether such changes related to FDP and perceptual learning. Using NAA concentration as a reference, we found that $GABA_+$:NAA ratio was frequency dependent, in that only above-resonance stimulation reduced this ratio during the course of the experiment. No changes in Glx:NAA were found. Further, we found that the magnitude of change in ML errors correlated with the concentration of $GABA_+$:NAA during the first 12 min of co-stimulation which may be an indication that early disinhibition of the GABAergic systems is important to learning as has been found in the motor cortex (Kolasinski et al., 2019).

Variants of the digit co-stimulation paradigm have been used to study plasticity. Synchronous co-stimulation of multiple points on a single digit (or paw) has been shown to improve 2-point tactile discrimination in humans and animals, accompanied by topographical changes in SI evidenced by electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI) (Godde et al., 2003, 1996; 2000; Hodzic et al., 2004; Pleger et al., 2001). Synchronous co-stimulation of multiple digits has been shown to impair 2-digit discrimination and fused or reduced the distance between the cortical representations of digit regions as measured by fMRI, whereas asynchronous stimulation has the opposing effect (Pilz et al., 2004; Vidyasagar et al., 2014). In this study, we tested the effect of stimulation frequency on plasticity outcomes and found that above-resonance stimulation led to worsening of performance on a tactile discrimination test, whereas at-resonance stimulation did not, in agreement with previous findings (Lea-Carnall et al., 2017). We interpret a worsening error rate after stimulation as being the result of increased lateral connectivity between these regions due to the stimulation (Pilz et al., 2004).

It has been suggested that the mechanisms underlying perceptual learning are the same as those underlying LTP in general which rely on NMDA receptor activation (Dinse et al., 2003b). This is supported by pharmacological studies by Dinse et al. who showed that lorazepam, a $GABA_A$ agonist, completely eliminated the effects of a digit stimulation protocol known to induce plasticity (Dinse et al., 2003a). We observed a
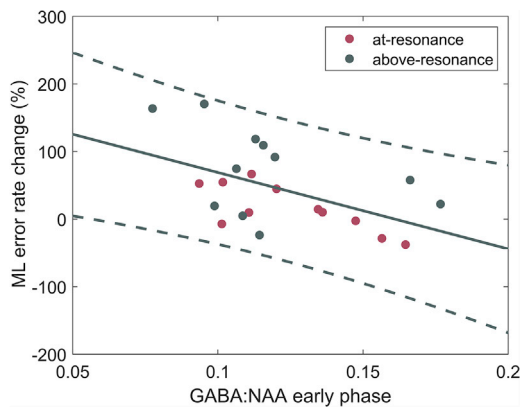
reduction in GABA₊ concentration in the above-resonance condition only, which was associated with impaired performance on the behavioral task. fMRI has shown that above-resonance stimulation causes the digit regions to shift closer together and functional connectivity between them to increase, supported by modeling work indicating that this is commensurate with a strengthening of lateral connections (Lea-Carnall et al., 2017). Lower levels of tonic GABA may facilitate this process. We suggest that by strengthening connections between the digit representations within SI, their local activation maxima broaden and overlap on the cortical surface. An incoming stimulus is then more likely to activate an area within the overlapping region leading to increased ML errors on the task (Pilz et al., 2004; Vidyasagar et al., 2014). More generally, these results are in line with findings from other authors that have observed an association between a reduction in GABA concentration in the relevant brain regions and LTP-like plasticity in animals (Wigstrom and Gustafsson, 1983; Davies et al., 1991), and both healthy (Floyer-Lea et al., 2006; Kolasinski et al., 2019; Stagg et al., 2011) and clinical human populations (Levy et al., 2002; Blicher et al., 2014). With regard to FDP specifically, early animal studies have indicated that inhibitory GABAergic autoreceptors may underlie the phenomenon of FDP (Davies et al., 1991). At low frequencies, excitatory potentiation is inhibited by GABAergic neurons, whereas, high-frequency stimulation causes disinhibition of this system allowing excitatory potentiation to occur. Our results suggest that a similar mechanism may also be present here.

We report a negative correlation between SI GABA₊:NAA in the early MRS block and overall magnitude of perceptual learning across both conditions, with lower early levels of GABA₊:NAA relating to higher levels of impairment on the tactile discrimination test. This correlation persisted for the at-resonance condition but not for the above-resonance condition, which was surprising given the latter condition facilitated the greatest levels of learning as measured by the ML test. In a recent study, Kolasinski et al. similarly showed that GABA concentration acquired in M1 in the early phase of a motor learning task negatively correlated with the overall degree of learning but this effect was not present in a control condition (Kolasinski et al., 2019). We found no evidence of learning for the at-resonance condition at the group level. However, we cannot rule out learning at the level of each individual. As the 23 Hz stimulation was fixed for all participants and was not calibrated individually, it is likely that it was just below or above the actual resonance frequency for each participant. The resonance frequency of SI is thought to be between 20 and 26 Hz (Snyder, 1992; Tobimatsu et al., 1999), meaning there may have been considerable variability between the participants and this may be driving any differences in their response to the stimulation. If early GABA levels are predictive of overall learning, then it may be the case that there is a certain "critical threshold" level for GABA within a neuronal circuit which once breached triggers plasticity cascades which affect further inhibitory processes in a nonlinear way. If this is the case, then the correlation between early GABA₊:NAA and learning in the above-resonance condition might be lost. The general finding, however, that early levels of GABA predict learning and performance fits with our current understanding of the role of GABA in sensory and motor systems. Across different sensory modalities, higher baseline GABA levels are positively associated with enhanced sensory function and acuity (Kolasinski et al., 2017; Edden et al., 2009), as well as induced plasticity changes as assessed by task performance (Heba et al., 2016). Taken together, these results indicate a potential role for early frequency-dependent cortical disinhibition in perceptual learning.

We did not observe a direct correlation between the late minus early change in SI $GABA_+$:NAA and the equivalent change in ML scores. Other studies have shown a relationship between alterations in GABA concentration and plasticity in the motor cortex (Floyer-Lea et al., 2006; Stagg et al., 2011). However, an important difference is that our MRS acquisition started concurrently with the stimulus providing an insight into ongoing GABA processes, whereas in the above studies, GABA concentrations were acquired before and after an intervention. It may also be that the relationship between the change in $GABA_+$:NAA and change in task performance is nonlinear which our correlation analysis would fail to detect. Finally, we note that the physiological basis for a functional change in GABA concentration is unclear (Stagg et al., 2011) and may be related to a change in GABA metabolism or a relative shift of GABA to a state/compartment that is less "visible" to the MR scanner, for example, due to shortened T2* (Mullins, 2018). Altered $GABA_+$:NAA between the early and late blocks may be due to different physiological mechanisms and may therefore relate to plasticity (ML measures) differently.

In a previous study, we found that at-resonance stimulation sped up reaction times (Lea-Carnall et al., 2017); this effect was absent here. While the protocol was the same, the experimental environment differed between the two studies. In (Lea-Carnall et al., 2017), psychophysics data were collected outside the scanner at 20-min intervals over 60 min of co-stimulation and the ML tests were performed immediately after co-stimulation. In the current study, the stimulus was applied while the subjects were in the scanner for 46 min, experiencing concurrent repetitive mechanical and acoustic stimuli, almost certainly off-resonance, which may have interfered with the 23 Hz stimulus. Further, upon completion of the stimulation, there was a 10-min period within which the participants completed an fMRI task that also involved vibrational pulses delivered to the digits and only after that they were removed from the scan room and asked to perform the final ML test.

We do not report any change in Glx:NAA during either stimulation condition. Glx is a composite measure of the combined concentration of Glu and Gln reported due to the difficulty of Glu quantification with AMARES using the GABA-edited MEGA-PRESS sequence which does not generally allow for the separation of these resonance peaks in the spectrum. With regard to the specific role of Glu in plasticity, it is well established that NMDAR activity mediates LTP allowing potentiation of the presynaptic signal via increased receptor trafficking. However, MRS is sensitive to glutamate concentrations rather than measures of receptor density. There is evidence that Glu presynaptic release rates might increase as a result of both Hebbian and homeostatic plasticity, but this is dependent on a number of factors (see (Costa et al., 2017) for a review). It has been shown that by blocking NMDAR activity using memantine (Dinse et al., 2003b) or scopolamine (Bliem et al., 2008), the effects of co-stimulation on perceptual learning are abolished, indicating that NMDARs play a critical role in this type of learning. Glu is abundant in the human brain and is involved in a plethora of neural processes (Ramadan et al., 2013). It may be that changes in glutamate in relation to plasticity are small in magnitude compared to global glutamate levels, and therefore, teasing out the specific effect attributable to plasticity is difficult.

While entrainment to sensory stimulation has been shown to have behavioral and neural consequences (Lakatos et al., 2019), similar results have been achieved using exogenous transcranial alternating current (tACS) stimulation (see (Herrmann et al., 2013; Thut et al., 2011), for reviews). In a recent study, Nowak et al. applied beta (20 Hz, at-resonance) and gamma (75 Hz, above-resonance) tACS stimulation over primary motor cortex (Nowak et al., 2017). They found a duration-dependent reduction in resting state $GABA_A$ inhibition, quantified by short interval intracortical inhibition, following gamma stimulation only which was not present following beta stimulation. These results help corroborate the observation that the triggering of GABAergic mechanisms coincided with above-resonance stimulation, although we acknowledge that the relationship between physiological measurements of inhibition and MRS GABA is unclear (Dyke et al., 2017; Stagg et al., 2011). On the other hand, Vossen et al. attributed the persistence of occipital alpha oscillations following individualized alpha tACS to plasticity, even though the alpha is or is close to the resonance frequency of the occipital cortex (Vossen et al., 2015). Similarly, beta tACS entrainment (at-resonance) has been shown to induce NMDAR-mediated plasticity in the human motor cortex using pharmacological intervention (Wischnewski et al., 2019). However, gamma tACS entrainment of the motor cortex (above-resonance) appears to have the unique effect of boosting plasticity via $GABA_A$ disinhibition as has been replicated in the literature (Guerra et al., 2019, 2018). It has been suggested that tACS targets interneurons and pyramidal cells differently, affecting the overall balance of activity and furthermore that inhibitory subnetworks which exhibit resonance frequencies in the gamma range may be more susceptible to

activation by similar stimulus frequencies (Nowak et al., 2017; Wischnewski et al., 2019). While we are cautious about drawing comparisons between these two stimulus types (tACS and sensory stimulation), we suggest that it is plausible that entrainment via either method using above-resonance frequencies has a similar mechanism of action on inhibitory circuits.

The scan session in this study was particularly long (approx. 70 mins), and therefore, it is expected that participants experienced a decline in attention by the end which may have had an effect on ML test performance. It is generally acknowledged that attention plays a crucial role in functional activation and learning (Ahissar and Hochstein, 1993; Buchner et al., 1999; Recanzone et al., 1992). However, studies have consistently shown that perceptual learning in response to repetitive sensory stimulation (even below detection thresholds) occurs even in the absence of attention in both the visual (Watanabe et al., 2002) and sensory systems (Dinse et al., 2003a; Godde et al., 2003, 2000; Heba et al., 2016; Hodzic et al., 2004; Lea-Carnall et al., 2017; Pleger et al., 2001, 2003). If GABA disinhibition is required for the induction of perceptual learning, then we would expect to observe the changes in $GABA_+$:NAA concentration in this experiment regardless of whether participants attended to the stimulus or not. We also note that participants were reminded before completing each test of the importance of performing to the best of their ability maintaining their integrity as scientific research subjects. If lethargy and lack of attention played a role in the diminished ML scores, then we expect this to be balanced across the conditions.

In a previous study, we showed that at-resonance stimulation consolidates a network, whereas above-resonance recruits a broader network. While the determination of the precise relationship between at/above resonance stimulation and performance of different cognitive tasks remains an open question, the results suggest that in applications where plasticity is of interest, for example, for learning novel environments, entraining at a frequency away from resonance of the target network may be advantageous. Identifying the preferred or resonance frequency of a network, nested within other networks, may not be straightforward but could be determined experimentally and would depend on the context and sensory and cognitive content of the stimuli (Besle et al., 2011; Ding et al., 2006; Lea-Carnall et al., 2016).

In conclusion, our findings suggest that frequency-dependent early changes in inhibitory signaling are correlated with perceptual learning outcomes. Furthermore, we bridge a gap between cellular and systems levels of investigation frequency-dependent plasticity and highlight the importance of distinguishing between at-resonance and off-resonance frequencies of a particular network. From a practical viewpoint, our results suggest that repetitive sensory stimulation could selectively switch plasticity mechanisms by modulating inhibitory processes GABA. This could prove useful in optimizing stimulation strategies to suit different rehabilitation or cognitive enhancement purposes.

Limitations of the Study

In this study, we compared SI MRS $GABA_+$:NAA measured during the first and last 12 min of co-stimulation of the digits. For a complete picture of GABA dynamics, a baseline measurement taken before the onset of stimulation would be optimal. In addition, MRS data were not corrected for GM/WM variations in the tissue, making this a potential confound. However, since the MRS voxel was not re-positioned between the early and late acquisitions, this cannot contribute to the changes seen between the early and late MRS blocks. Our experimental design ensures that any variation in GM/WM ratio or macromolecule concentration due to differing voxel placement between subjects and sessions was unlikely to influence the findings. The "at" and "above" resonance stimulation conditions were performed in a randomization order between participants, and the radiographer placing the voxel was blinded to the condition, meaning there could be no bias in voxel placement between conditions. Finally, the duration of the scan as well as acoustic and mechanical vibrations from the scanner might have interfered with the tactile stimulation, causing fatigue and dampening the frequency-dependent plasticity effect.

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Caroline Lea-Carnall (caroline.lea-carnall@manchester.ac.uk).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The data collected in this study are publicly available at https://doi.org/10.6084/m9.figshare.13026320.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101657.

AUTHOR CONTRIBUTIONS

C.L.C., L.M.P., W.E-.D., N.T.B., and M.M. conceptualized the experiment. C.L.C., L.M.P., and W.E-.D. designed the experiments. C.L.C., S.R.W., L.M.P., and F.S.N. performed the experiments. C.L.C., S.R.W., and F.S.N. analyzed the data. C.L.C., S.R.W., W.E-.D., and L.M.P. wrote the manuscript. All authors edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Ahissar, M., and Hochstein, S. (1993). Attentional control of early perceptual learning. Proc. Natl. Acad. Sci. U S A *90*, 5718–5722.

Bear, M., and Abraham, W. (1996). Long-term depression in hippocampus. Annu. Rev. Neurosci. *19*, 437–462.

Besle, J., Schevon, C., Mehta, A., Lakatos, P., Goodman, R., McKhann, G., Emerson, R., and Schroeder, C. (2011). Tuning of the human neocortex to the temporal dynamics of attended events. J. Neurosci. *31*, 3176–3185.

Blicher, J., Near, J., Næss-Schmidt, E., Stagg, C., Johansen-Berg, H., Nielsen, J., Østergaard, L., and Ho, Y. (2014). GABA levels are decreased after stroke and gaba changes during rehabilitation correlate with motor improvement. Neurorehabil. Neural Repair *29*, 278–286.

Bliem, B., Tegenthoff, M., and Dinse, H. (2008). Cholinergic gating of improvement of tactile acuity induced by peripheral tactile stimulation. Neurosci. Lett. *434*, 129–132.

Bliss, T., and Gardner-Medwin, A. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J. Physiol. *232*, 357–374.

Bliss, T., and Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. *232*, 331–356.

Buchner, H., Reinartz, U., Waberski, T., Gobbelé, R., Noppeney, U., and Scherg, M. (1999). Sustained attention modulates the immediate effect of de-afferentation on the cortical representation of the digits: source localization of somatosensory evoked potentials in humans. Neurosci. Lett. *260*, 57–60.

Buzsáki, G., Logothetis, N., and Singer, W. (2013). Scaling brain size, keeping time: evolutionary preservation of brain rhythms. Neuron *80*, 751–764.

Costa, R., Mizusaki, B., Sjostrom, P., and van Rossum, M. (2017). Functional consequences of pre- and postsynaptic expression of synaptic plasticity. Phil Trans. R. Soc. B *372*, 20160153.

Davies, C., Starkey, S., Pozza, M., and Collinridge, G. (1991). Gaba(b) autoreceptors regulate the induction of LTP. Nature *349*, 609–611.

Ding, J., Sperling, G., and Srinivasan, R. (2006). SSVEP power modulation by attention depends on the network tagged by the flicker frequency. Cereb. Cortex *16*, 1016–1029.

Dinse, H., Ragert, P., Pleger, B., Schwenkreis, P., and Tegenthoff, M. (2003a). Gabaergic mechanisms gate tactile discrimination learning. Neuroreport *14*, 1747–1751.

Dinse, H., Ragert, P., Pleger, B., Schwenkreis, P., and Tegenthoff, M. (2003b). Pharmacological modulation of perceptual learning and associated cortical reorganization. Science *301*, 91–94.

Dyke, K., Pépés, S., Chen, C., Kim, S., Sigurdsson, H., Draper, A., Husain, M., Nachev, P., Gowland, P., Morris, P., and Jackson, S. (2017). Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. NeuroImage *152*, 360–370.

Edden, R., Muthukumaraswamy, S., Freeman, T., and Singh, K. (2009). Orientation discrimination performance is predicted by GABA concentration and gamma oscillation frequency in human primary visual cortex. J. Neurosci. *29*, 15721–15726.

Falsafi, S., Deli, A., Höger, H., Pollak, A., and Lubec, G. (2012). Scopolamine administration modulates muscarinic, nicotinic and nmda receptor systems. PLoS One *7*, e32082.

Godde, B., Spengler, F., and Dinse, H. (1996). Associative pairing of tactile stimulation induces somatosensory cortical reorganization in rats and humans. Neuroreport *8*, 281–285.

Godde, B., Stauffenberg, B., Spengler, F., and Dinse, H. (2000). Tactile coactivation-induced changes in spatial discrimination performance. J. Neurosci. *20*, 1597–1604.

Floyer-Lea, A., Wylezinska, M., Kincses, T., and Matthews, P. (2006). Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J. Neurophysiol. *95*, 1639–1644.

Godde, B., Ehrhardt, J., and Braun, C. (2003). Behavioral significance of input-dependent plasticity of human somatosensory cortex. Neuroreport *14*, 543–546.

Guerra, A., Suppa, A., Bologna, M., D'Onofrio, V., Bianchini, E., Brown, P., Di Lazzaro, V., and Berardelli, A. (2018). Boosting the LTP-like plasticity effect of intermittent theta-burst stimulation using gamma transcranial alternating current stimulation. Brain Stim. *11*, 734–742.

Guerra, A., Suppa, A., Asci, F., De Marco, G., D'Onofrio, V., Bologna, M., Di Lazzaro, V., and Berardelli, A. (2019). LTD-like plasticity of the human primary motor cortex can be reversed by -tACS. Brain Stim *12*, 1490–1499.

Heba, S., Puts, N., Kalisch, T., Glaubitz, B., Haag, L., Lenz, M., Dinse, H., Edden, R., Tegenthoff, M., and Schmidt-Wilcke, T. (2016). Local GABA concentration predicts perceptual improvements after repetitive sensory stimulation in humans. Cereb. Cortex *26*, 1295–1301.

Helfrich, R., Breska, A., and Knight, R. (2019). Neural entrainment and network resonance in support of top-down guided attention. Curr. Opin. Psychol. *29*, 82–89.

Herrmann, C., Rach, S., Neuling, T., and Strüber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front. Hum. Neurosci. *7*, 279.

Hodzic, A., Veit, R., Karim, A., Erb, M., and Godde, B. (2004). Improvement and decline in tactile discrimination behavior after cortical plasticity induced by passive tactile coactivation. J. Neurosci. *24*, 442–446.

Hutcheon, B., and Yarom, Y. (2000). Resonance, oscillation and the intrinsic frequency preferences of neurons. Trends Neurosci. *23*, 216–222.

Kemp, A., and Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. Proc. Natl. Acad. Sci. U S A *101*, 8192–8197.

Kirkwood, A., and Bear, M. (1994). Homosynaptic long-term depression in the visual cortex. J. Neurosci. *74*, 34043412.

Kolasinski, J., Hinson, E., Zand, A., Rizov, A., Emir, U., and Stagg, C. (2019). The dynamics of cortical GABA in human motor learning. J. Physiol. *597*, 271–282.

Kolasinski, J., Logan, J., Hinson, E., Manners, D., Zand, A., Makin, T., Emir, U., and Stagg, C. (2017). A mechanistic link from gaba to cortical architecture and perception. Curr. Biol. *27*, 1685–1691.

Kösem, A., Bosker, H., Takashima, A., Meyer, A., Jensen, O., and Hagoort, P. (2018). Neural entrainment determines the words we hear. Curr. Biol. *28*, 2867–2875.

Lakatos, P., Gross, J., and Thut, G. (2019). A new unifying account of the roles of neuronal entrainment. Curr. Biol. *29*, 890–905.

Lea-Carnall, C., Montemurro, M., Trujillo-Barreto, N., Parkes, L., and El-Deredy, W. (2016). Cortical resonance frequencies emerge from network size and connectivity. Plos Comput. Biol. *12*, e1004740.

Lea-Carnall, C., Trujillo-Barreto, N., Montemurro, M., El-Deredy, W., and Parkes, L. (2017). Evidence for frequency-dependent cortical plasticity in the human brain. Proc. Natl. Acad. Sci. U S A *114*, 8871–8876.

Levy, L., Ziemann, U., Chen, R., and Cohen, L. (2002). Rapid modulation of gaba in sensorimotor cortex induced by acute deafferentation. Ann. Neurol. *52*, 755–761.

Malenka, R., and Bear, M. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Mullins, P. (2018). Towards a theory of functional magnetic resonance spectroscopy (fmrs): a meta-analysis and discussion of using mrs to measure changes in neurotransmitters in real time. Scand. J. Psychol. *59*, 91–103.

Nowak, M., Hinson, E., van Ede, F., Pogosyan, A., Guerra, A., Quinn, A., Brown, P., and Stagg, C. (2017). Driving human motor cortical oscillations leads to behaviorally relevant changes in local GABAA inhibition: a tACS-TMS study. J. Neurosci. *37*, 4481–4492.

Obleser, J., and Kayser, C. (2019). Neural entrainment and attentional selection in the listening brain. Trends Cogn. Sci. *23*, 913–926.

Pérez, A., Dumas, G., Karadag, M., and Duñabeitiach, J. (2019). Differential brain-to-brain entrainment while speaking and listening in native and foreign languages. Cortex *111*, 303–315.

Pilz, K., Veit, R., Braun, B., and Godde, G. (2004). Effects of co-activation on cortical organization and discrimination performance. Neuroreport *15*, 2669–2672.

Pleger, B., Dinse, H., Ragert, P., Schwenkreis, P., Malin, J., and Tegenthoff, M. (2001). Shifts in cortical representations predict human discrimination improvement. Proc. Natl. Acad. Sci. U S A *98*, 12255–12260.

Pleger, B., Foerster, A., Ragert, P., Dinse, H., Schwenkreis, P., Malin, J., Nicolas, V., and Tegenthoff, M. (2003). Functional imaging of perceptual learning in human primary and secondary somatosensory cortex. Neuron *40*, 643–653.

Puts, N., Edden, R., Evans, C., McGlone, F., and McGonigle, D. (2011). Regionally specific human gaba concentration correlates with tactile discrimination thresholds. J. Neurosci. *31*, 16556:16560.

Ragert, R., Kalisch, T., Bliem, B., Franzkowiak, S., and Dinse, H. (2008). Differential effects of tactile high- and low-frequency stimulation on tactile discrimination in human subjects. BMC Neurosci. *9*, 9.

Ramadan, S., Lin, A., and Stanwell, P. (2013). Glutamate and glutamine: a review of in vivo MRS in the human brain. NMR Biomed. *26*, 1630–1646.

Recanzone, G., Merzenich, M., Jenkins, W., Grajski, K., and Dinse, H. (1992). Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. J. Neurophysiol. *67*, 1031–1056.

Schroeder, C., and Lakatos, P. (2008). Low-frequency neural oscillations as instruments of sensory selection. Trends Neurosci. *32*, 9–18.

Shrout, P., and Fleiss, J. (1979). Intraclass correlations: uses in assessing rater reliability. Psychol. Bull. *86*, 420–428.

Snyder, A. (1992). Steady-state vibration evoked potentials: description of technique and characterization of responses. Electroencephalogr. Clin. Neurophysiol. *84*, 257–268.

Stagg, C., Bachtiar, V., and Johansen-Berg, H. (2011a). The role of GABA in human motor learning. Curr. Biol. *21*, 480–484.

Stagg, C., Bachtiar, V., and Johansen-Berg, H. (2011b). What are we measuring with GABA magnetic resonance spectroscopy? Commun. Integr. Biol. *4*, 573–575.

von Stein, A., and Sarnthein, J. (2000). Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. Int. J. Psychophysiol. *38*, 301–313.

Stagg, C., Bestmann, S., Constantinescu, A., Moreno, L., Allman, C., Mekle, R., and Woolrich, M. (2011c). Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. J. Physiol. (Lond) *589*, 5845–5855.

Thut, G., Schyns, P., and Gross, J. (2011). Entrainment of perceptually relevant brain

oscillations by non-invasive rhythmic stimulation of the human brain. Front. Psychol. *2*, 170.

Tobimatsu, S., Zhang, Y., and Kato, M. (1999). Steady-state vibration somatosensory evoked potentials: physiological characteristics and tuning function. Clin. Neurophysiol. *110*, 1953–1958.

Vidyasagar, R., Folger, S., and Parkes, L. (2014). Re-wiring the brain: increased functional connectivity within primary somatosensory cortex following synchronous co-activation. NeuroImage *92*, 19–26.

Vossen, A., Gross, J., and Thut, G. (2015). Alpha power increase after transcranial alternating

current stimulation at alpha frequency (α-tacs) reflects plastic changes rather than entrainment. Brain Stim *8*, 499–508.

Watanabe, T., Náñez, .,J., Sr., Koyama, S., Mukai, I., Liederman, J., and Sasaki, Y. (2002). Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. Nat. Neurosci. *5*, 1003–1009.

Wigstrom, H., and Gustafsson, B. (1983). Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. Nature *301*, 603–604.

Wijtenburg, S., West, J., Korenic, S., Kuhney, F., Gaston, F., Chen, H., Roberts, M.,

Kochunov, P., Hong, L., and Rowland, L. (2017). Glutamatergic metabolites are associated with visual plasticity in humans. Neurosci. Lett. *644*, 30–36.

Wischnewski, M., Engelhardt, M., Salehinejad, M., Schutter, D., Kuo, M., and Nitsche, M. (2019). NMDA receptor-mediated motor cortex plasticity after 20 hz transcranial alternating current stimulation. Cereb. Cortex *29*, 2924–2931.

Yousry, T., Schmid, U., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., and Winkler, P. (1997). Localization of the motor hand area to a knob on the precentral gyrus: a new landmark. Brain *120*, 141–157.

Supplemental Information

GABA Modulates

Frequency-Dependent

Plasticity in Humans

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Supplementary Information

1 Transparent Methods

1.1 Subjects and ethics statement

This study was approved by the University of Manchester Research Ethics Committee (UREC Ref 2018-4395-6120) and fully informed written consent was obtained from all subjects prior to participation. Fourteen healthy right-handed subjects (7 male, age range 20-49 years old) were scanned twice using a 3 T Philips Achieva MRI with an 8-channel head coil.

1.2 Experimental protocol

A schematic design of the study can be seen in Fig. S1 which indicates the temporal order of the scans and tests used in the study. MRS data was collected during tactile co-stimulation at 2 different frequencies in order to measure changes in $GABA_+$ and Glx. A tactile discrimination task was performed before and after scanning to measure the effect of co-stimulation on tactile perception with worsening scores assumed to be an indicator of plastic change (Pilz et al., 2004). Participants first completed the tactile discrimination test while seated in a quiet room near to the scanner in order to measure their baseline ability. They were then immediately positioned in the scanner and vibrotactile stimulators were placed in the middle of the fleshy pads of digits 2 and 4 (the index and ring fingers) of the right hand and secured with tape. A T_1-weighted image with 1 mm isotropic resolution was acquired followed by fMRI (not reported here). There followed a period of 46 minutes of simultaneous in-phase tactile co-stimulation of digits 2 and 4 using either at-resonance (23 Hz) or above-resonance (39 Hz) frequency stimulation in order to bring about plastic change as reported in our earlier study (Lea-Carnall et al., 2017). MRS data was acquired for the first (early) and last (late) 12 minutes of co-stimulation in order to measure any changes in $GABA_+$ or Glx due to the ongoing application of the co-stimulation. Resting state fMRI was collected in the gap between MRS. Upon completion of scanning, participants were removed from the scanner and immediately moved to the nearby testing room to repeat the tactile discrimination test. The tactile stimulators consist of a ceramic box containing a vibrating probe driven by a piezoceramic wafer (Dancer Designs Ltd) which were controlled using Matlab software (http://www.mathworks.com). Each digit had its own stimulator which was driven with the correct vibrational frequency delivered in-phase to each digit with un-loaded peak to peak amplitude of 250 m. Each participant was scanned twice at least 2 weeks apart and received either the at-resonance or above-resonance stimulation, in an order counter-balanced across participants.

1.3 Tactile discrimination test

Participants were seated and the vibrotactile stimulators were placed in the middle of the fleshy pads of digits 2 and 4 of the right hand and secured with tape. Short pulses (500 ms) were delivered randomly to one of the two digits, at one of three intensity levels, and participants were asked to decide which digit they felt the pulse
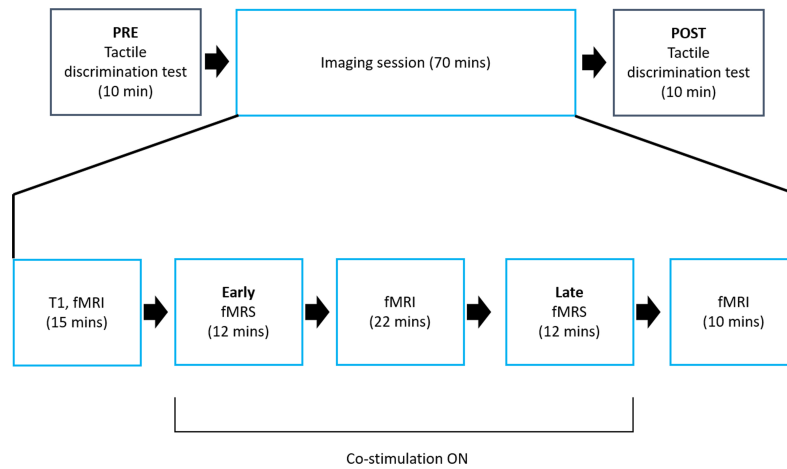
Figure S1: Schematic design of the study. Related to Figure 1.
Schematic of one (of two) scans of the experimental protocol. Initially, participants completed a forced-choice tactile discrimination test outside the scanner. The test required them to detect which finger received a short vibrational pulse (500 ms). Once inside the scanner, a high-resolution T1-weighted image was acquired to guide the placement of the spectroscopy voxel. MRS was collected during the first and last 12 mins of 46 mins total of co-stimulation at one of the two frequencies (at or above resonance). Once participants left the scanner, they immediately repeated the forced-choice tactile discrimination test. fMRI was also collected and that is reported elsewhere Lea-Carnall et al. (2017). The scan session and testing was repeated using the other stimulation frequency a minimum of two weeks later.

in. The pulse intensities determined detection difficulty and were defined in terms of the displacement distance of the probe (Easy 8.3 m; Medium 5.6 m; and Hard 2.8 m) above the average detection threshold calculated from data collected previously (Lea-Carnall et al., 2017) (mean 16.1 m, SD 4 m, calculated for both digits from 15 participants with a similar age and gender distribution to the participants in this study). The test was programmed using Presentation (Neurobehavioural Systems, www.neurobs.com). A total of 180 pulses were delivered in a random order with an inter-trial interval of 2s. Responses were recorded with the left hand using a keyboard. The tactile mislocalisation rate is defined as the percentage of times a participant incorrectly classified the stimulus as belong to a specific digit (non-responses allocated at 50% accuracy). The stimulus was applied at a range of difficulty levels in order to try to capture an intensity just above the participant's true detection threshold given that we used an estimated value for this experimental parameter. For this reason, mislocalisation test results are reported as means averaged across difficulty levels for each participant. Reaction times were also recorded.

1.4 Data acquisition

GABA-edited spectra were acquired using a J-difference-edited MEshcher–GArwood Point RESolved Spectroscopy (MEGA-PRESS) sequence (Mescher et al., 1998) from participants' left SI with a voxel size of $20 \times 30 \times 20$ mm^3 for the first and last 12 minutes of the tactile stimulation. A 1 mm isotropic T1-weighted image was acquired to aid the positioning of the 1H MRS voxel. A typical voxel placement is shown in Fig. 1 (a). The MRS voxel placement within SI was determined in the axial plane as being centred on the "hand knob" area of

the post-central gyrus and rotated in both the sagittal and coronal planes so that it was aligned to the cortical surface (Puts et al., 2011; Yousry et al., 1997). The MRS voxel was placed at the beginning of the scan session and was not moved between the early and late MRS blocks. Placement of the MRS voxel was replicated between experimental conditions (the 2 scan sessions relating to the 2 stimulation frequencies) by eye by referring to images of the initial placement in 3 planes by a radiographer who was blinded to the conditions and was then checked by another researcher. Great care was taken to minimise error and as the conditions were performed in a randomised order between participants, there was no bias for positioning between the conditions or movement of the participant between early and late MRS blocks.

In the initial phase of the study (10 subjects), the spectra were acquired in blocks of 4 acquisitions when the MEGA pulse was set at 1.89 ppm (MEGA-on), referred to as a single dynamic, followed by 4 acquisitions of MEGA pulse set at 7.6 ppm (MEGA-off) There were 90 dynamics in total (45 ON and 45 OFF). For the final 4 subjects, a spectrometer upgrade enabled us to interleave single spectra of MEGA-on and MEGA-off off with 352 spectra in total (176 ON and 176 OFF). The dynamics were then repeated in an interleaved manner for 12 mins. Echo time (TE) was 70 ms, repetition time (TR) was 2000 ms with 1024 samples. Receiver bandwidth was 2000 Hz, water suppression method was excitation with a window of 140 Hz and the shimming was second order pencil beam. MRS acquisitions followed EPI and whilst it is known that EPI can induce frequency instability in MRS acquisitions, we overcame any effects of this by frequency aligning dynamics before calculating the edited spectra. It should also be noted that any effects of EPI in the data would be present in both conditions. Using data from power calculations given in (Sanaei Nezhad et al., 2020) we used a sample size of 14 which powered our data to detect a 20% change in $GABA_+$ in SI (using the motor cortex as a reference).

1.5 Metabolite quantification

The acquired MEGA-PRESS spectra were exported from the scanner as single time-domain free induction decays for processing using jMRUI v6.0 (Naressi et al., 2001; Stefan et al., 2009). The spectra were aligned using the 'automatic alignment' option in the jMRUI pre-processing menu and were then summed (effectively subtracting the MEGA-OFF from the MEGA-ON spectrum due to the 180 degree phase shift in the MEGA-OFF acquisition). The resultant edited spectra was phase-corrected automatically by jMRUI using the co-acquired non-water suppressed acquisition. The N-acetylaspartate (NAA) peak at 2.02 ppm was used for frequency referencing.

$GABA_+$, conventionally taken to mean GABA plus co-edited macromolecules (Mullins et al., 2014) was estimated from the edited spectrum using the AMARES routine (Mierisova et al., 1998), which fits single resonances in the time-domain using frequency and line shape prior knowledge. AMARES analysis also provides a measurement of glutamate + glutamine (conventionally abbreviated as Glx). Data are presented as ratios to NAA in the MEGA-edited spectrum as this provides an internal correction for any variations in editing efficiency.

1.6 Statistical analysis

Tactile mislocalisation rates for each participant were averaged across the three difficulty levels for each testing session. Initially, we evaluated intersession reliability of the mislocalisation baseline (comparing pre condition measured before scanning and repeated at least two weeks apart for each participant) measurement using the ICC (please see Equation 1 for definition).

Due to missing values for some of the subjects, linear mixed model analyses were used to assess the main effect of MRS Block (early/late - relating to the first and last 12 mins of co-stimulation) and Stimulation Frequency (at-resonance/above-resonance), and an interaction between MRS Block and Stimulation Frequency on the $GABA_+$

and Glx levels. We conducted a similar test to assess the main effects of Mislocalisation Test Time (pre/post) and Stimulation Frequency (at-resonance/above-resonance), and an interaction between Mislocalisation Test Time and Stimulation Frequency on the participants' error rates and reaction times. Finally we tested the reference metabolite NAA as well as the ratio NAA:tCr (creatine + phosphocreatine) in the same way.

Normality was assessed using the Wilk-Shapiro test on the residuals, and Mauchly's sphericity test was conducted to examine whether the assumption of sphericity had been violated. No violations were found in the present study.

Finally, we assessed whether levels of SI $GABA_+$:NAA and Glx:NAA in the early MRS block or the magnitude of its change across the early and late acquisitions predicted subsequent learning using Pearson correlation analyses. We calculated correlations for each condition separately and combined. All statistics were computed in R (R Core Team, 2013).

1.7 Quality control and exclusion criteria

Edited MEGA-PRESS spectra were initially inspected by eye to identify any of poor quality to be excluded from analysis. A number of spectra were seen to be noisy so signal-to-noise ratio (SNR) in the time-domain was calculated for all spectra using AMARES. Spectra were excluded if SNR < (mean SNR - minus two standard deviations (SD)), in which the mean was calculated without excluded spectra. Practically this involved rejecting spectra which fell outside 2 SD below the global mean, then recalculating the mean and SD excluding the rejected spectra and iteratively removing new outliers. 11/72 spectra were rejected and a table of quality control metrics per group is given in Table 1.

References

Lea-Carnall, C., Trujillo-Barreto, N., Montemurro, M., El-Deredy, W. and Parkes, L. (2017), 'Evidence for frequency-dependent cortical plasticity in the human brain', *Proc Natl Acad Sci U S A* **114**(33), 8871–8876.

Mescher, M., Merkle, H., Kirsch, J., Garwood, M. and Gruetter, R. (1998), 'Simultaneous in vivo spectral editing and water suppression', *NMR Biomed* **11**(6), 266–272.

Mierisova, S., van den Boogaart, A., Tkac, I., Van Hecke, P., Vanhamme, L. and Liptaj, T. (1998), 'New approach for quantitation of short echo time in vivo 1h mr spectra of brain using amares', *NMR Biomed* **11**, 32–39.

Mullins, P., McGonigle, D., O'Gorman, R., Puts, N., Vidyasagar, R., Evans, C., on MRS of GABA, C. S. and Edden, R. (2014), 'Current practice in the use of mega-press spectroscopy for the detection of gaba', *Neuroimage* **1**(86), 43–52.

Naressi, A., Couturier, C., Castang, I., de Beer, R. and Graveron-Demilly, D. (2001), 'Java-based graphical user interface for mrui, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals', *Comput Biol Med* **31**, 269–286.

Pilz, K., Veit, R., Braun, B. and Godde, G. (2004), 'Effects of co-activation on cortical organization and discrimination performance', *Neuroreport* **15**, 2669–2672.

Puts, N., Edden, R., Evans, C., McGlone, F. and McGonigle, D. (2011), 'Regionally specific human gaba concentration correlates with tactile discrimination thresholds', *J Neurosci* **31**(46), 16556:16560.

R Core Team (2013), *R: A language and environment for statistical computing.*, R Foundation for Statistical Computing, Vienna, Austria.

Sanaei Nezhad, F., Lea-Carnall, C., Anton, A., Jung, J., Michou, E., Williams, S. and Parkes, L. (2020), 'Number of subjects required in common study designs for functional gaba magnetic resonance spectroscopy in the human brain at 3 tesla', *Eur J Neurosci* **51**(8), 1784–1793.

Stefan, D., Di Cesare, F., Andrasescu, A., Popa, E., Lazariev, A., Vescovo, E., Strbak, O., Williams, S., Starcuk, Z., Cabanas, M., van Ormondt, D. and Graveron-Demilly, D. (2009), 'Quantitation of magnetic resonance spectroscopy signals: the jmrui software package', *Measurement Science and Technology* **20**(10), 104035–104044.

Yousry, T., Schmid, U., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A. and Winkler, P. (1997), 'Localization of the motor hand area to a knob on the precentral gyrus: a new landmark', *Brain* **120**(1), 141–157.