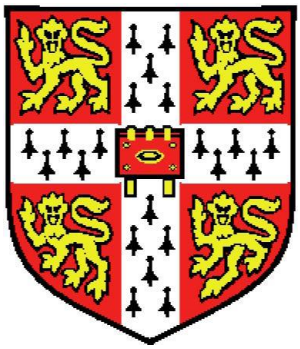
Inhibitory mechanisms for visual learning in the human brain



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To my (not so) little brothers,

Alexandros and Giorgos

Acknowledgments

Achieving one's childhood dreams is a lifelong adventure. With this PhD I come closer to achieving one of mine: learning how the brain works. I am thankful to have had the opportunity to study brain plasticity next to bright minds and close friends.

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Preface

This dissertation is the result of my own work and includes work done in collaboration as declared in this section. Data collection for the experiments of Chapter III was done in collaboration with Dr. Marta Correia (MRC Cognition and Brain Sciences Unit, University of Cambridge). Data collection for the experiments of Chapter IV was done in collaboration with Dr. Charlotte Stagg (Wellcome Centre for Integrative Neuroimaging, University of Oxford). Data analysis on the resting state fMRI data presented in Chapter IV was done in collaboration with Dr. Vasilis Karlaftis and Dr. Ke Jia (Adaptive Brain Lab, University of Cambridge).

This dissertation is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution.

This dissertation does not exceed the prescribed word limit by the Degree Committee for the Faculty of Biology.

Abstract

Identifying targets in cluttered scenes is critical for our interactions in complex environments. Our visual system is challenged to both detect elusive targets that we may want to avoid or chase and discriminate between targets that are highly similar. These tasks require our visual system to become an expert at detecting distinctive features that help us differentiate between indistinguishable targets.

As the human brain is trained on this type of visual tasks, we observe changes in its function that correspond to improved performance. We use functional brain imaging, to measure learning-dependent modulations of brain activation and investigate the processes that mediate functional brain plasticity. I propose that dissociable brain mechanisms are engaged when detecting targets in clutter vs. discriminating between highly similar targets: for the former, background clutter needs to be suppressed for the target to be recognised, whereas for the latter, neurons are tuned to respond to fine differences. Although GABAergic inhibition is known to suppress redundant neuronal populations and tune neuronal representations, its role in visual learning remains largely unexplored. Here, I propose that GABAergic inhibition plays an important role in visual plasticity through training on these tasks.

The purpose of my PhD is to investigate the inhibitory mechanisms that mediate visual perceptual learning; in particular, learning to detect patterns in visual clutter and discriminate between highly similar patterns. I show that BOLD signals as measured by functional Magnetic Resonance Imaging (fMRI) do not differentiate between the two proposed mechanisms. In contrast, Magnetic Resonance Spectroscopy (MRS) provides strong evidence for the distinct involvement of GABAergic inhibition in visual plasticity. Further, my findings show GABA changes during the time-course of learning providing evidence for a distinct role of GABA in learning-dependent plasticity across different brain regions involved in visual learning. Finally, I test the causal link between inhibitory contributions and visual plasticity using a brain stimulation intervention that perturbs the excitation-inhibition balance in the visual cortex and facilitates learning.

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Chapter I

Theoretical background

In this chapter, I introduce the theoretical framework on which the thesis and its respective experimental studies were conceived. In my thesis I investigate the inhibitory mechanisms involved in visual perceptual learning. Here I discuss experience-dependent plasticity in the visual cortex, how it is linked to inhibitory processing, as well as the role of GABA, the main inhibitory neurotransmitter in the human brain.

1. The challenge

Understanding the structure of the world around us entails extracting and discriminating meaningful patterns from cluttered environments. For instance, putting together a jigsaw puzzle requires two separate skills: first, one needs to look in the pile of pieces for the clean-cut corner and frame pieces, detecting target-pieces by suppressing clutter. Then, one needs to discriminate between sometimes hundreds of pieces that look highly similar, processing fine features and creating tuned representations of piece shape categories by suppressing irrelevant features.

To succeed in interpreting cluttered scenes and discriminating the fine features that guide our actions, the brain is challenged to suppress noisy and ambiguous sensory signals. Experience and learning are known to facilitate this ability and improve perceptual judgements. Yet, the inhibitory mechanisms that the human brain employs to suppress such task-irrelevant signals and optimise its perceptual decisions through training remain largely unknown.

2. Visual perceptual learning

Perceptual learning refers to experience-based plasticity that enhances the brain's ability to process the environment and make perceptual judgements about ambiguous sensory inputs. Its effects are long-lasting (Fahle and Poggio, 2002; Gilbert and Li, 2012; Karni and Sagi, 1991) and differ from adaptation or habituation, while it results in improved sensory processing without involving rules, associations or strategies, as for example in higher-order learning tasks (Gold and Watanabe, 2010).

The visual system is an excellent candidate system for studying sensory plasticity. Its high degree of functional specialisation makes it possible to design experiments and visual tasks that target specific functional areas. Visual stimuli are processed at different stages of the visual stream based on their complexity. For example, basic features such as orientation are processed in the primary visual cortex (V1), shapes or objects are processed further down the ventral visual stream, in the lateral occipital cortex (LOC) and moving targets are processed in the dorsal visual stream, in visual area MT.

Visual perceptual learning (VPL) has been shown to improve performance in tasks involving perceptual judgments in a range of tasks that involve extracting features from cluttered backgrounds, discriminating fine feature differences and identifying objects (Fine and Jacobs, 2002; Gilbert et al., 2001; Goldstone, 1998; Goldstone et al., 2001). The effects of VPL are known to be specific to stimulus orientation, spatial-frequency, location (Sagi, 2011), suggesting changes in low-level sensory information in the primary visual cortex (Gilbert and Sigman, 2007; Ito et al., 1998; R. W. Li et al., 2004; Schoups et al., 2001; Sigman et al., 2006). However, the neural locus of perceptual learning and its specificity (Mollon and Danilova, 1996) have been long debated: does VPL cause plastic changes in the functionally specific areas of the visual cortex or does it involve changes in brain-wide networks that support feedback and feedforward processes from and to the visual cortex?

Learning-dependent changes in neural tuning have been reported as more evident in higher than early visual areas (Ghose et al., 2002; Mehta et al., 2000). Electrophysiology recordings on monkeys who were trained on an orientation discrimination task showed larger changes in area V4 rather than V1 for increased orientation selectivity (Raiguel et al., 2006; Yang and Maunsell, 2004a). Further, altered neural sensitivity following VPL has been reported in decision related areas (i.e. lateral intraparietal cortex) rather than visual cortex (Law and Gold, 2010). These findings suggest that VPL involves top-down mechanisms consistent with Ahissar and Hochstein's proposal of reverse hierarchy, implicating high-level areas in easy perceptual judgements, while early visual areas with higher spatial resolution in more difficult perceptual decisions (Ahissar and Hochstein, 2004a). Recent evidence suggests that VPL may alter both information encoding in early visual areas, as well as readout from higher-level areas (Yan et al., 2014), while top-down influences may mediate VPL (Bressler et al., 2008; Gilbert and Li, 2013; W. Li et al., 2004; Piëch et al., 2013). Thus, VPL has been suggested to involve a network of brain regions – including visual areas and decision-related regions in the parietal and frontal cortex– that are thought to re-weight the visual input based on past experience (Dosher and Lu, 1998; Sagi, 2011).

In the past 20 years, functional MRI studies have allowed us to study visual perceptual learning in the human brain. Mechanisms that were once postulated based on psychophysics and physiology are now possible to investigate in humans by measuring changes in brain activation and connections between different cortical areas. Several brain imaging studies have shown learning-dependent activation changes in the visual cortex following VPL (Kourtzi et al., 2005; Mukai et al., 2007; Sigman et al., 2006; Yotsumoto et al., 2008). Specifically, fMRI changes have been shown for detecting targets in clutter: learning to detect low-salience target resulted in increased BOLD after training in early (V1, V2, VP, V4) and late (LOC, pFs) visual areas, while learning to detect high-salience targets resulted in enhanced BOLD in higher areas (LOC,

pFs) (Kourtzi et al, 2005). Learning to detect basic shapes (letters) in the presence of distractors resulted in increased BOLD in the early visual cortex and decreased BOLD in the LOC and parietal cortex (Sigman et al, 2005). fMRI studies also showed BOLD changes for fine feature discrimination: higher BOLD activation and enhanced stimulus selectivity has been shown in LOC following training on highly similar visual patterns (Zhang et al, 2010). Further, Kuai et al (Kuai et al., 2013) showed that shape category templates in the LOC are tuned during training, as revealed by classification image approach on behavioural and fMRI data. Increased BOLD activation was found in area MT after training on motion discrimination (Vaina et al., 1998), while decreased BOLD activation was found in the primary visual cortex following training on contrast discrimination (Mukai et al., 2007). Finally, a study showed increased (initial phase of training) and later decreased (performance was maintained) BOLD activations in the primary visual cortex during training on a texture discrimination task (Yotsumoto et al., 2008).

3. Brain mechanisms for learning-dependent plasticity

Different mechanisms have been suggested to support VPL plasticity. Cortical recruitment, that is the increased number of available neurons that can process a stimulus, has been shown to enhance signal-to-noise ratio and facilitate behavioural improvement (Recanzone et al., 1993, 1992; Reed et al., 2011). In contrast, increasing neuronal sensitivity to stimulus changes and sharper tuning have been shown to enhance stimulus selectivity and facilitate performance in discrimination tasks (Mukai et al., 2007; Schoups et al., 2001). These synaptic changes may be mediated by excitatory and inhibitory mechanisms shaping cortical activity.

Plasticity in the visual cortex has been linked to inhibitory processing in the animal brain. Studies have shown that a decreased inhibition is required for the

induction of LTP-like plasticity in the rat M1 and have causally linked GABAergic inhibition with LTP induction using pharmacological interventions. Castro-Alamancos et al (Castro-Alamancos et al., 1995) used bicuculline methiodide, a GABA antagonist, to reduce GABAergic inhibition locally and induce LTP, while Trepel and Racine (Trepel and Racine, 2000) used diazepam, a GABA agonist, to block LTP induction in the rat motor cortex. The evidence suggests GABA, the main inhibitory neurotransmitter in the brain, plays a critical role in plasticity induction.

Yet, investigating inhibitory processing non-invasively in the human brain has only recently been possible thanks to the development of MR Spectroscopy (MRS) (see also Chapter II). Despite the success of fMRI as a non-invasive tool for studying learning-dependent changes in the human brain, it does not allow us to discriminate excitatory from inhibitory contributions to visual plasticity; that is, BOLD is an aggregate signal reflecting both excitatory and inhibitory processing (Logothetis, 2008). Here, I take advantage of recent developments in MRS to measure GABAergic inhibition non-invasively in the human brain and investigate the inhibitory mechanisms that mediate visual perceptual learning.

4. Thesis outline

In my thesis, I investigate the role of GABAergic inhibition in visual learning. In Chapter II, I describe the visual learning behavioural paradigm employed in my studies and the methods I employed to measure and perturb inhibition. In Chapter III, I combine fMRI and MRS to investigate dissociable GABAergic contributions to visual learning. In Chapter IV, I employ high-field brain imaging to investigate the time course of GABA changes during visual learning. In Chapter V, I employ transcranial direct current stimulation to perturb cortical excitability and causally link GABAergic inhibition to visual learning. Finally, in Chapter VI, I discuss the contribution of my findings to our understanding of visual learning mechanisms and potential future research directions and translational applications.

Chapter II

Methodological background

1. Behavioural paradigm

In this thesis, I present three visual perceptual learning studies where I employ the same perceptual learning paradigm that focuses on two tasks: learning to detect shapes (i.e. Glass patterns) embedded in background noise vs. learning to discriminate highly similar shapes. Here I present in detail these behavioural tasks.

a. Stimuli

Glass patterns were introduced by Leon Glass in 1969 (Glass, 1969). In his seminal paper, he describes how superimposing a rotated random dot pattern on itself creates a concentric pattern, proposing a mechanism by which the human visual system uses local autocorrelations to extract global structure. Glass patterns are the perceived shapes resulting from superimposing onto itself a random dot pattern that has been linearly or non-linearly transformed. Glass and Perez in 1973 showed that if a random dot pattern is uniformly expanded and then superimposed on the original, a radial pattern is generated (Glass and Pérez, 1973). This way, we can think of every dot in the initial pattern as having a "pair" dot in the transformed pattern, forming dot-dipoles. For each dot dipole, the spiral angle can be defined as the angle between the dot dipole orientation and the radius from the centre of the dipole to the centre of the stimulus aperture. In the case of concentric patterns, the dipoles that result from the pattern's rotation are placed perpendicular to radii from the centre of the stimulus to the centre of the dipoles (spiral angle = 90^{o}). In the case of radial

patterns, the dipoles that result from the pattern's uniform expansion are parallel to radii from the centre of the stimulus to the centre of the dipoles (spiral angle = 0^o) (Glass and Smith, 2011).

Glass patterns allow us to maintain local correlations (processed by the early visual cortex), while parametrically manipulating the global structure (processed later in the visual stream). We expect that training to discriminate these patterns will engage shape-processing areas, and in particular the lateral occipital cortex (LOC), rather than local orientation or position detectors in V1. Indeed, fMRI studies have shown that areas in the ventral occipital cortex are selectively activated by Glass patterns vs random patterns (Ostwald et al., 2008).

In my studies, I presented participants with Glass patterns generated using previously described methods (Li et al., 2012; Mayhew et al., 2010) ensuring that coherent form patterns are reliably perceived for the stimulus generation parameters I used (Wilson and Wilkinson, 1998). In particular, stimuli were defined by white dot pairs (dipoles) displayed within a square aperture on a black background. The dot density was 3%, and the Glass shift (i.e., the distance between two dots in a dipole) was 16.2^o arc min. The size of each dot was 2.3° x 2.3° arc min^2 (Figure 2.1).

b. Tasks

Glass patterns allow us to study detection of targets from clutter (Signal-in-noise task) vs discrimination of highly similar targets (Feature differences task) by using the same stimulus and manipulating the global structure while maintaining the local statistics. For the Signal-in-Noise task, I "embed" Glass patterns in noise: while a proportion of the dot dipoles is placed at a determined spiral angle, the remaining dipoles are randomly oriented, serving the purpose of background clutter. For the Feature differences task, I generate pattern categories that are difficult to discriminate: by placing the dipoles at spiral

angles that are close to the boundary between radial and concentric patterns (45 ^{o} spiral angle), classifying the patterns becomes challenging.

I generated radial (0^{o} spiral angle) and concentric (90^{o} spiral angle) Glass patterns by placing dipoles orthogonally (radial stimuli) or tangentially (concentric stimuli) to the circumference of a circle centred on the fixation dot. Further, I generated intermediate patterns between these two Glass pattern types by parametrically varying the spiral angle of the pattern from 0° (radial pattern) to 90° (concentric pattern). I randomized the presentation of clockwise (0° to 90° spiral angle) and counterclockwise patterns (0° to -90° spiral angle) across participants. A new pattern was generated for each stimulus presented in a trial, resulting in stimuli that were locally jittered in their position. Each stimulus comprised dot dipoles that were aligned according to the specified spiral angle (signal dipoles) for a given stimulus and noise dipoles for which the spiral angle was randomly selected. The proportion of signal dipoles defined the stimulus signal level.

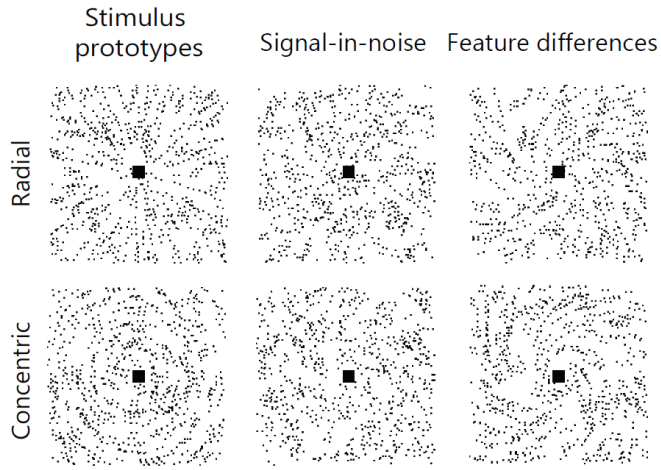


Figure 2.1: Task stimuli examples
Radial and concentric Glass patterns in their prototype version (100% signal, spiral angle 0° for radial and 90° for concentric), in the Signal in noise task version (25% signal, spiral angle 0° for radial and 90° for concentric) and in the Feature-differences task version (100% signal, spiral angle 38° for radial and 52° for concentric). Stimuli are shown inverted for presentation purposes.

For the Signal-in-Noise task, radial and concentric stimuli (spiral angle : 0° and +- 90°) were presented at 24% ± 1% signal level; that is, 76% of the dipoles were presented at a random position and orientation (Figure 2.1). For the Feature-differences task, stimuli were presented at 100% signal and spiral angle of $\pm 38^{o}$ (radial) or $\pm 52^{o}$ (concentric) (Figure 2.1). To control for potential local adaptation due to stimulus repetition, I jittered (± 1-3°) the spiral angle across stimuli.

c. Learning to see in noise vs discriminate fine features: a role for inhibition?

In my thesis, I investigate visual perceptual learning by employing two tasks: detecting targets from visual clutter (Signal-in-Noise task) vs discriminating highly similar targets (Feature differences task). I propose that dissociable brain mechanisms mediate learning in these two tasks and investigate the role of inhibition in shaping learning-dependent plasticity in the visual cortex.

Signal-in-Noise task

Training on the Signal-in-Noise task has been shown to improve sensitivity to visual noise, while increased fMRI activity in LOC has been shown for trained vs untrained stimuli with low salience (Kourtzi et al., 2005). It is possible that increased sensitivity to visual noise involves changes in neuronal gain that are reflected in BOLD changes. At the level of neural populations, information processing can be considered as a function, converting input activity to spike output. A change in neuronal gain describes a change in the function's slope that alters the sensitivity of neurons to the excitatory input.

Modelling of the effects of shunting inhibition on the input-output relationship of granule cells showed that tonic shunting inhibition reduces gain during rate-

coded physiological excitation, while synaptic inhibition is more effective at reducing neuronal gain (Mitchell and Silver, 2003). Further, pharmacological blockade of $GABA_A$ receptors resulted in increased gain in granule cells of rat cerebellar slices (Hamann et al., 2002). Finally, injecting GABA vs GABA antagonists on the inferior colliculus (IC) of anesthetised guinea pigs that is sensitive to interaural time differences had opposite multiplicative effects on IC spike rates, showing that GABAergic inhibition in the IC regulates neural gain (Ingham and McAlpine, 2005). Here, I ask whether GABAergic inhibition is involved in a gain control mechanism that supports learning to detect Glass patterns in visual clutter.

Feature differences task

Training on the Feature differences task has been shown to improve pattern discriminability, while tuning pattern representations in the area of LOC (Zhang et al., 2010). It is possible that increased pattern selectivity involves changes in neuronal tuning that are reflected in BOLD changes. A change in neuronal tuning describes a change in its function that alters the selectivity of the neuron to excitatory inputs.

Single-cell recordings in rats have shown that balanced inhibition, precisely following excitatory input, underlies auditory cortex tuning (Wehr and Zador, 2003), while pharmacological blockade of inhibition in the auditory cortex of bats (Chen and Jen, 2000) and rodents (Wang et al., 2002) by means of a $GABA_A$ antagonist resulted in broader tuning curves. In the occipital cortex, higher GABA baseline levels have been related to increased sensitivity for orientation discrimination in humans (Edden et al., 2009), while a pharmacological challenge in cats showed improved orientation selectivity for a GABA injection vs diminished selectivity for a GABA agonist injection (Li et

al., 2008). Here, I ask whether GABAergic inhibition relates to neuronal tuning that supports learning to discriminate highly similar Glass patterns.

2. Measuring inhibition in the human brain

a. GABA: the main inhibitory neurotransmitter in the brain

γ-aminobutyric acid or GABA is an amino acid and the main inhibitory neurotransmitter in the human brain. GABA is found in the cortex in three distinct pools, where it undertakes a three-fold role: in the cytoplasm it is involved in neuronal metabolism; in the pre-synaptic terminals it is involved in neurotransmission; in the extra-cellular space it can have a neuromodulatory effect on distant neurons. In this section, I discuss the physiology and function of GABA, while in the next sections I present methods to measure and perturb its concentration in the brain.

GABA synthesis takes place in GABAergic interneurons. As part of the glutamate/GABA-glutamine metabolic cycle, glutamine is transformed to glutamate by phosphate-activated glutaminase in both glutamatergic and GABAergic interneurons (Bak et al., 2006). Further, glutamate is a product of the metabolic tricarboxylic acid (TCA) cycle in the cell. In glutamatergic interneurons glutamate is then released into the synaptic cleft, while in GABAergic interneurons glutamate is converted by glutamic acid decarboxylase (GAD) to GABA. There are two GAD isoforms with distinct roles in the brain: tonically active GAD67 produces cytoplasmic GABA that accounts for at least half of the GABA concentration in the cortex (Asada et al., 1997; Martin and Rimvall, 1993), while phasically active GAD65 produces vesicular GABA, found in the presynaptic boutons. GABA synthesis is believed to be the rate-limiting factor in GABA metabolism (Soghomonian and Martin, 1998). Expression of GAD67 has been shown to be decreased when network activity is low, resulting in reduced GABA levels (Lau and Murthy, 2012).

GABA receptors can either be ionotropic or metabotropic. $GABA_A$ receptors are ionotropic and can be found intra- and extrasynaptically. They are thought to mediate fast synaptic neurotransmission and slow tonic extrasynaptic inhibition. Extra-synaptic $GABA_A$ receptors are activated by GABA spillover from the synaptic cleft. When activated, they hyperpolarise the membrane by selectively conducting chloride anions. $GABA_B$ receptors are metabotropic and can be found pre- and post-synaptically. $GABA_B$ inhibition is considered slow and acts by hyperpolarising the cell membrane via activation of potassium channels.

GABA is catabolised by GABA-transaminase (GABA-T) to succinic semialdehyde, which in turn is converted to glutamate via the neuron's TCA cycle and finally aminated to glutamine by glutamine synthetase (Bak et al., 2006). The concentration of GABA inside the cell is believed to be linked to extracellular GABA (Petroff and Rothman, 1998) which is taken up by either neurons or astrocytes to be catabolised.

b. How can we measure human cortical inhibition?

i.Using Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) can elicit motor responses or motor evoked potentials (MEPs), by stimulation of the motor cortex at an intensity above the resting motor threshold. Paired pulse protocols have been used extensively to investigate GABAergic inhibition in the motor cortex (for a review see (Stagg, 2014)), but also in the early visual cortex (Lou et al., 2011) where their applications elicits phosphine perception. In paired-pulse TMS protocols (ppTMS) two consecutive TMS pulses are applied in the same region. The first conditioning pulse is subthreshold and meant to stimulate cortical interneurons, but not pyramidal neurons. The second pulse is suprathreshold and elicits an MEP. Its activation of pyramidal neurons is moderated by the inhibitory interneurons previously stimulated by the conditioning pulse. If the

interstimulus interval (ISI) between the two pulses is 2-4ms, the measured inhibition is believed to reflect fast $GABA_A$ activity (Kujirai et al., 1993; Reis et al., 2008), while an ISI of 50-200ms is thought to reflect slow $GABA_B$ inhibition (Valls-Solé et al., 1992). Finally, an ISI of 1ms has an interestingly distinct mechanism and is thought to reflect extra-synaptic GABAergic tone (Stagg et al., 2011b).

ii. Using MR spectroscopy

Magnetic resonance spectroscopy allows us to quantify the concentration of chemical compounds in the brain by detecting their different resonance frequencies. When a tissue that is found in a magnetic field B_0 is excited, the resulting acquired signal corresponds to a frequency spectrum that consists of the spectral peaks of the different metabolites found in the tissue. ^1H nuclei in the different metabolites do not resonate at the same frequency due to the chemical shift and J-coupling. When a nucleus from the excited tissue belongs to a chemical compound, the surrounding electron structure generates a secondary magnetic field B_{eff} that *shifts* its characteristic frequency (*chemical shift*). Thus, nuclei of different chemical compounds will resonate at shifted resonance frequencies. Further, the B_{eff} experienced by the nucleus is affected by adjacent spins within the compound producing multiple spectral sub-peaks (*J-coupling*). Peak splitting results in lower peak intensity and broader peaks. The strength of the magnetic field affects the chemical shift and the degree of separation between the spectral peaks: the stronger the magnetic field, the better the peaks are resolved (Stagg and Rothman, 2013) .

In order to quantify the concentrations of the different metabolites in an MRS spectrum, spectrum fitting is used. By modelling a priori each metabolite's spectrum as a basis spectrum using numerical methods, we have distinct spectral signatures for the different chemical compounds that can be linearly combined

to produce the final spectra. Then the relative concentrations can be found by the amplitude weightings of the respective subspectra in the combined spectrum (Stagg and Rothman, 2013).

The low concentration of GABA in the brain, as well as the spectral overlap of its peaks, makes its detection and quantification challenging. GABA was first measured in the human brain with MRS in 1993 (Rothman et al., 1993) and it has now been established as a tool in measuring the main inhibitory neurotransmitter in the brain. The most common method of measuring GABA is by defining a 3D volume in the area of interest, the MRS voxel, from where signal will be acquired. While multi-voxel MR spectroscopic imaging (MRSI) has recently emerged (Alger, 2011), the technique is still being developed for detecting and measuring GABA. GABA concentration is reported relative to the concentration of another metabolite, such as NAA or creatine. Using another metabolite acquired in the same spectrum as reference is advantageous to absolute quantification or water referencing, as it accounts for neuronal density and spectral data quality (Stagg and Rothman, 2013), especially in healthy populations where NAA and creatine are believed to be stable (Bogner et al., 2010).

In my thesis I have used two different MRS acquisition sequences. In the study described in chapter III, I employed 2D-JPRESS (Schulte et al., 2006) to measure GABA. This sequence involves spectral editing to achieve spectral peak separation that can reliably detect GABA peaks. J-resolved spectroscopy utilises J-coupling by separating the chemical shift and J-coupling effects: the J-coupled nuclei do not get refocused by the 180^{o} pulse of the spin-echo sequence. 2D MRS combines 1D spectra that have been acquired with different timing parameters from the same sample, into one 2D dataset. More specifically, the last 180^{o} refocusing pulse is shifted and multiple 1D spectra are acquired. 2D basis spectrum fitting is similar to fitting of 1D spectra and can resolve overlapping peaks that can't be separated with 1D spectra (Stagg and Rothman,

2013). 2D-JPRESS has been shown to reliably measure GABA at 3T (Prescot and Renshaw, 2013; Schmitz et al., 2017).

In the study described in chapter IV, I have measured GABA using high-field imaging, at 7 Tesla. The increased spectral resolution and SNR mean spectral editing is not necessary in order to detect GABA. Here I have used a semi-localization by adiabatic selective refocusing (semi-LASER) sequence (Scheenen et al., 2008). semi-LASER has been shown to have minimal chemical shift displacement error, which means artefacts resulting from the differences in spatial encoding for different metabolites are minimised (Scheenen et al., 2008). Further, it has been shown to reliably measure GABA at 7T (Barron et al., 2016; Kolasinski et al., 2017; C Lemke et al., 2015; Lunghi et al., 2015; van de Bank et al., 2015).

c. Linking MRS GABA to behaviour

An increasing number of studies links GABA levels at rest with behavioural performance at different tasks. MRS has been shown to have good intra- and intersubject reproducibility (Bogner et al., 2010), suggesting it can be used to detect interindividual variability in baseline concentration. So far, elevated GABA levels have been related to both better and worse performance. In the motor cortex, higher baseline GABA levels have been linked to slower reaction times (Stagg et al., 2011a) and better tactile acuity (Kolasinski et al., 2017). In the visual cortex, higher GABA levels relate to better orientation discrimination (Edden et al., 2009) and larger orientation illusion magnitude (Song et al., 2017), while they've been shown to underlie the dynamics of bistable perception (van Loon et al., 2013). The above results suggest that GABA does not have a straightforward relationship with behaviour; while some sensory processes are benefited by higher inhibition levels, there are those that are facilitated by lower

concentrations of GABA, suggesting a complex role for GABAergic inhibition in the cortex.

As mentioned in chapter I, changes in GABA have been linked to plasticity and with MRS we are given the opportunity to measure learning-related modulations in GABA concentration. Studies have shown a decrease in GABA in the motor cortex during training (Floyer-Lea et al., 2006; Sampaio-Baptista et al., 2015), while in the visual cortex decreased excitation/inhibition balance is found when overtraining and stabilisation of a visual skill has occurred (Shibata et al., 2017). These results suggest we are able to measure GABAergic contributions to plastic changes in the brain and investigate the role of GABA in learning.

d. What are we measuring with MR spectroscopy?

While MRS allows us to quantify the concentration of neurotransmitters non-invasively, it is still unclear what exactly the measurement reflects. Earlier, I described the complex three-fold role of GABA in the brain, with the different GABAergic pools having distinct mechanisms of action. Using MRS, we measure the total concentration of GABA in a predefined volume of about 8-27 ml, without being able to distinguish between these three different GABAergic pools. The aggregate signal measured includes contributions from all three pools, each being responsible for an unknown percentage of the quantified concentration. It is believed that MRS is sensitive to mobile GABA and less sensitive to immobilised GABA, as for example in the presynaptic vesicles (Floyer-Lea et al., 2006). In the animal literature it has been shown that MRS GABA reflects extra-synaptic GABA tone rather than synaptic GABA activity (Mason et al., 2001), while in the human a paired pulse transcranial magnetic stimulation (TMS) protocol thought to reflect extra-syaptic GABAergic tone was selectively related to MRS GABA measurements (Stagg et al., 2011b).

Converging evidence suggests that MRS measures the concentration of extra-synaptic GABA; however it's important to keep in mind that the different GABAergic pools are directly linked. MRS measured GABA changes do not reflect plastic changes independently in extra-synaptic GABA, but are more likely to reflect changes in $GABA_A$ activity (Stagg, 2014). A change in GABA concentration can be explained in three ways: a change in GABA metabolism, a change in GABA catabolism or a shift of GABA from or into an MRS-invisible pool (Stagg, 2014). While it is not possible to distinguish between these three possible mechanisms, there is evidence from the animal (Lau and Murthy, 2012) and human literature (Stagg et al., 2010) suggesting reduced GAD activity may account for reduced presynaptic GABA in LTP-like plasticity and its modulation may explain the changes measured with MRS (Stagg, 2014). Further research is required in order to understand the exact mechanisms behind MRS-measured GABA changes, using pharmacological interventions that target specific GABAergic pools and stages of the GABA cycle.

e. Linking MRS GABA to other brain signals

While fMRI does not allow us to differentiate between excitatory and inhibitory changes in activation, when combined with MRS it provides new evidence that may explain the variations observed in brain activation due to task execution or plasticity. MRS has been widely used in conjunction with fMRI, with recent work combining the two acquisitions in a new technique, namely fMRI-MRS (Ip et al., 2017). In the majority of studies, however, baseline GABA levels are related to subsequent changes in fMRI BOLD. A negative relationship between GABA concentration and BOLD responses has been observed in human (Barron et al., 2016; Muthukumaraswamy et al., 2009; Northoff et al., 2007; Walter et al., 2016) and animal studies (Chen et al., 2005). In particular, attenuated BOLD responses have been suggested to result from GABAergic inhibition. Further, a positive relationship between baseline GABA measurements and BOLD

contrast has been reported (Harris et al., 2015; Lipp et al., 2015). Finally, lack of a relationship between baseline GABA and task related BOLD has been shown in both healthy and patient populations (Bhattacharyya et al., 2017). In a study measuring BOLD, cerebral blood flow (CBF)-weighted arterial spin labelling (ASL), cerebral blood volume (CBV)-weighted vascular-space-occupancy (VASO), and arterial CBV (aCBV)-weighted inflow VASO (iVASO) in the visual cortex, baseline GABA concentration was found to correlate negatively with BOLD reactivity and magnitude CBV-weighted VASO reactivity, while positively with baseline CBF-weighted ASL signal and ASL time-to-peak (Donahue et al., 2010). The contrasting results suggest that the link between GABA concentration and fMRI BOLD signal may be more complex than initially thought and may not reflect simply inhibitory effects of GABA, but may be also influenced by vascular factors, or task choice.

Further, levels of GABAergic inhibition have also been linked to resting state functional connectivity in brain networks. Resting GABA in the primary motor cortex has been inversely related to the strength of the motor resting state network (Bachtiar et al., 2015; Stagg et al., 2014), as well as the functional connectivity between the left and right motor cortices (Stagg et al., 2014). In contrast, decreases in GABA have been related to increased strength of the motor network (Bachtiar et al., 2015; Sampaio-Baptista et al., 2015; Stagg et al., 2014) and interhemispheric connectivity (Stagg et al., 2014). The converging evidence suggests that resting state network strength may be driven by GABAergic modulations of oscillatory activity in network nodes (Stagg et al., 2014).

3. Perturbing inhibition

While the links between GABAergic inhibition and physiological measures have been shown across species and using a variety of techniques, its causal role in cognition can only be established by using interventions. There are three ways in which we can non-invasively perturb the balance between inhibition and excitation in the cortex: with a pharmacological challenge, with transcranial magnetic stimulation and with transcranial direct current stimulation. Below I describe how these techniques can alter inhibition levels in the cortex and what the results have shown.

a. Transcranial direct current stimulation

Early studies using electrical stimulation of the animal cortex have shown that spontaneous neuronal activity is increased with anodal and depressed with cathodal cortical electrical stimulation. DC-currents applied on the motor cortex of rats (Bindman et al., 1964) and the motor and visual cortex of cats (Creutzfeldt et al., 1962; Purpura and McMurtry, 1965) showed polarity specific effects: more neurons were found to be activated by inward currents (surface-positive, anodal) and inhibited by outward currents (surface negative, cathodal). Anodal stimulation was thus shown to increase cortical excitability by depolarising the cell membrane, while cathodal stimulation reduced activity by membrane hyperpolarisation. Electrical stimulation, therefore, modulates the likelihood of, rather than induces, neuronal discharge (Woods et al., 2016). Interestingly, the effect of the stimulation was found to be inversed in deep cortical layers (Purpura and McMurtry, 1965), suggesting its dependency on the orientation of the neurons relative to the electrical field (Stagg and Nitsche, 2011).

Recent evidence, however, has shown changes in local field potentials, rather than firing rate of single or multi-unit recordings in monkeys, following

transcranial DC stimulation (tDCs) (Krause et al., 2017). The results suggest tDCs may be modulating functional connectivity and the increase of neural excitability may be a result of modulating the timing of ongoing spiking activity, rather than controlling action potential generation. By selectively reducing coherence of suppressive low-frequency oscillations, the stimulated neurons may become more sensitive to surrounding high-frequency activity. Additional evidence from animal models suggests that earlier animal studies using invasive cortical stimulation are not capturing the same mechanism of action as non-invasive tDCs used in human studies, in the sense that the electric current reaching the cells is insufficient for depolarisation (Jackson et al., 2016).

Polarity-specific effects have also been shown following stimulation of the human motor cortex. Measuring motor evoked potentials showed amplitude to be increased following anodal, while decreased following cathodal tDCs compared to a non-stimulated condition (Nitsche and Paulus, 2000). Consistent with the effect of tDCs on the motor cortex, measuring visual evoked potentials showed amplitude to be increased following anodal, while decreased following cathodal tDCs compared to a non-stimulated condition (Antal et al., 2004a). Further, measurements of neurotransmitter concentration with MRS following tDCs of the motor cortex showed a significant GABA decrease for both anodal and cathodal stimulation, while an additional significant decrease for Glx during cathodal stimulation (Stagg et al., 2009a). In the case of anodal stimulation, increased excitability may be related to reduced GAD-67 activity (Floyer-Lea et al., 2006) that would explain the reduction in GABA synthesis. In the case of cathodal stimulation, a reduction in neuronal excitability results in reduced glutamate synthesis, as previously seen in the monkey striate cortex (Carder and Hendry, 1994). Reduced glutamate synthesis, in turn, downregulates GAD-67 activity and GABA synthesis. These results suggest that the excitatory effect of anodal stimulation may reflect a downregulation of inhibition, via reduced GABA synthesis, while the inhibitory effect of cathodal stimulation may correspond to downregulation of excitation, via reduced glutamate synthesis

(Stagg et al., 2009a). Anodal stimulation of the occipito-temporal cortex also selectively reduced GABA (but not glutamate) during stimulation, as measured by MRS (Barron et al., 2016), suggesting the effect of stimulation on neurotransmitter concentrations is not confined to the motor cortex.

Polarity-specific behavioural effects of tDCs have also been shown in the literature. In the visual cortex, cathodal stimulation of V5/MT+ has been shown to selectively improve motion detection (Antal et al., 2004b), possibly by suppressing redundant information and focusing cortical activity on the optimum motion encoding neuronal pattern. In the motor cortex, anodal stimulation has been found to facilitate motor learning (Stagg et al., 2011c; Vines et al., 2008), while cathodal stimulation has been shown to impede and slow down behavioural improvement (Stagg et al., 2011c; Vines et al., 2008). Online anodal (but not cathodal) stimulation during prism adaptation of chronic visual neglect patients has been shown to increase treatment resistance (O'Shea et al., 2017). Interestingly, if stimulation precedes training, the facilitatory effect is no longer present (O'Shea et al., 2017) or stops being polarity specific, with both types of stimulation detrimental to training (Stagg et al., 2011c). A later study has shown that anodal tDCs preceding training on a motor task gives rise to homeostatic mechanisms. These are reflected by increased synaptic $GABA_A$ activity post-stimulation, resulting in reversal of the facilitatory effect of stimulation (Amadi et al., 2015). On the contrary, improved orientation discriminability was found for offline rather than online anodal tDCs of the primary visual cortex (Pirulli et al., 2013). However, one may consider that orientation discriminability may actually benefit by the inhibitory effect of the homeostatic mechanism following anodal stimulation shown in (Amadi et al., 2015). Thus, the behavioural effect does not support offline vs online anodal tDCs, but instead provides more evidence for the reversed after-effects of stimulation. Based on the above, I chose to use *online* tDCs to perturb cortical excitability during training on visual tasks.

b. Transcranial magnetic stimulation

Rapid-rate transcranial magnetic stimulation has been shown to alter cortical excitability as measured by motor-evoked potential (MEP) amplitude (Pascual-Leone et al., 1994). Specifically, theta burst stimulation (TBS) that consists of 50-Hz trains of three TMS pulses at 5Hz has been proposed to differentially modulate inhibition: when applied intermittently vs continuously it was shown to increase vs decrease MEP amplitude (Huang et al., 2005), while continuous TBS been shown to increase MRS GABA in the motor cortex (Stagg et al., 2009b).

c. Pharmacology

The use of neurotransmitter agonists and antagonists in human and animal research has been widely employed to investigate the role of key neurotransmitters in modulating behaviour. GABAergic (ant)agonists can be used to perturb the balance between inhibition and excitation in the cortex.

GABA agonists are drugs that can boost inhibition by binding to GABAergic receptors, blocking GABA transportation or keeping neurons hyperpolarised for longer. In the animal literature, the use of GABA agonists has been shown to block long term potentiation (Trepel and Racine, 2000). In the human literature, enhancing GABAergic inhibition has been shown to reduce working memory capacity (Lozano-Soldevilla et al., 2014), the ability to detect masked targets (van Loon et al., 2012) and performance at a set of visual perceptual tasks (Pompeia et al., 2008). There are different types of GABA agonists that have been shown to target different pools of GABA. Benzodiazepines are positive allosteric modulators that act on the ion channels of $GABA_A$ receptors, increasing the efficiency of available GABA. They are believed to be targeting synaptic phasic GABA (Nutt et al., 2015). GABA reuptake inhibitors block GABA transporters (GAT-s) and prevent translocation of GABA from the

extracellular to the intracellular space. They are believed to act on synaptic GABA and increase extracellular GABA (Gonzalez-Burgos, 2010).

GABA antagonists have been mainly used in the animal literature to investigate the effects of decreased inhibition in the cortex. Bicuculline methiodide has been shown to induce long term potentiation in the rat M1 (Castro-Alamancos et al., 1995), reverse cognitive deficits in mice (Varvel et al., 2005), but also induce cognitive, behavioural and dopaminergic abnormalities that resemble schizophrenia (Enomoto et al., 2011).

Chapter III

Combined MRS-fMRI reveals differential GABAergic contributions to visual learning

1. Introduction

Understanding the structure of the world around us entails extracting and discriminating meaningful patterns from cluttered environments. Effortless as this may seem, it poses for the brain a challenging task that involves suppressing noisy and ambiguous sensory signals. As discussed in Chapter I, experience and training have been shown to facilitate perceptual judgments and optimise visual recognition processes in the brain (Fine and Jacobs, 2002; C. Gilbert et al., 2001; Goldstone, 1998). For instance, an experienced bird watcher is not only able to break the camouflage and detect a bird in a leafy tree, but also determine whether it is a carrion crow or a hooded crow. Yet, the inhibitory mechanisms that the human brain employs to suppress task-irrelevant information and optimise perceptual decisions through training remain largely unknown.

Theoretical models of perceptual learning (Dosher et al., 2013; Dosher and Lu, 1998; R. W. Li et al., 2004) posit that experience and training facilitate our ability to a) detect targets in clutter by filtering external noise, b) discriminate highly similar objects by suppressing irrelevant features and retuning task-relevant feature templates. Although considerable behavioural evidence supports this framework, its neural implementation remains uncertain. Previous fMRI studies have demonstrated changes in the overall activation of higher visual areas in the occipito-temporal cortex due to training on perceptual decision tasks (for reviews (Kourtzi, 2010; Welchman and Kourtzi, 2013a)). However, fMRI data do not allow us to discern excitatory from suppressive mechanisms of experience-

dependent plasticity, as BOLD reflects aggregate activity across large neural populations (Heeger and Ress, 2002; Logothetis, 2008).

Here I ask whether GABA, the primary inhibitory neurotransmitter in the brain, mediates our ability to improve in making perceptual decisions through training on a visual task. As discussed in Chapter I, previous work in animals has demonstrated that GABAergic inhibition is associated with learning and synaptic plasticity (Castro-Alamancos et al., 1995; Trepel and Racine, 2000). Further, GABA, as measured by MR Spectroscopy in humans, has been shown to relate to individual performance in visual perceptual tasks (Edden et al., 2009; van Loon et al., 2013; Yoon et al., 2010), homeostatic plasticity in animals (Fagiolini, 2004) and humans (Lunghi et al., 2015) as well as individual ability for motor learning (Kolasinski et al., 2017). While decreased motor cortex GABA has been associated with improved performance in the context of motor learning (Blicher et al., 2015; Floyer-Lea et al., 2006; O'Shea et al., 2017; Sampaio-Baptista et al., 2015), the link between changes in GABA and visual perceptual learning remains largely unexplored.

In the study presented in this chapter, I test the role of GABAergic inhibition in learning to make perceptual judgements under two types of uncertainty in the environment: interpreting cluttered scenes (i.e. identifying objects embedded in noise) and discriminating highly similar objects (i.e. identifying fine feature differences). I employed two tasks that rely on these processes differentially: (1) a signal-in-noise task that involves extracting shapes (radial vs. concentric Glass patterns) masked by noise versus (2) a feature-difference task that involves judging fine differences. Previous functional brain imaging studies have shown that training in these tasks results in learning-dependent changes in brain activations in the LOC (Kourtzi et al., 2005; Li et al., 2012; Mayhew et al., 2010; Zhang et al., 2010), an area known to be involved in shape processing. Yet, the BOLD signal measured by fMRI does not allow us to discern the contribution of excitatory vs. inhibitory mechanisms to learning-dependent plasticity.

Here, I combine MRS-measurements of GABA in the lateral occipital cortex before vs. after training with fMRI measurements during training to test whether changes in GABA concentration relate to learning-dependent changes in fMRI activation in the lateral occipital cortex and behavioural improvement on these tasks. To test for task-specific inhibitory mechanisms of learning-dependent plasticity, we compare measurements in two tasks: Signal-in-noise vs. Feature differences task. I hypothesise that detecting targets in clutter relies on enhanced neural gain processes, while discriminating fine features relies on enhanced tuning to the behaviourally relevant features.

My findings provide evidence for dissociable inhibitory mechanisms that shape processing in the visual cortex and mediate behavioural improvement in perceptual decision making through training. I demonstrate that decreased GABA after training relates to improved detection of targets in clutter, while increased GABA relates to enhanced sensitivity in discriminating fine feature differences. These GABAergic changes moderate the relationship between functional activation and behaviour, demonstrating the contribution of inhibitory processes to learning-dependent plasticity in visual cortex.

2. Methods

a. Participants

Forty six participants (21 female; mean age 25.04 ± 3.69 years) participated in this study Sample size was determined based on power calculations following previous studies on motor learning showing an effect size of r=0.65 or r=0.60 at 90% power for correlations of GABA with behaviour or BOLD, respectively (Stagg et al., 2011a). All participants were right-handed, had normal or corrected-to-normal vision and gave written informed consent. The study was approved by the University of Cambridge ethics committee. Three participants were excluded from the study due to incomplete data resulting from technical

failure. Of the remaining participants, twenty one participated in the Signal-in-Noise (SN) experiment and twenty two in the Feature-differences (FD) experiment. Each group was trained only on one of the two tasks (SN, FD) to avoid transfer effects across tasks that have been previously reported when the same individuals were trained sequentially on both tasks (Chang et al., 2013; Dosher and Lu, 2007). In Table 3.1 we present the number of datasets collected per task and number of datasets left after rejecting poor quality data in the different pre-processing stages.

b. Experiment Design

Participants were presented with Glass patterns, as described in chapter II, section 1. Stimuli were presented in the centre of the screen, at a visual angle of 7.9^{o} x 7.9^{o}. Participants were asked to judge whether the presented stimulus on each trial was radial or concentric and received feedback on their average performance (i.e. percent correct) every 10-15 trials as indicated by a vertical colour-bar. Participants were familiarised with this task before training in the lab and were then trained during an fMRI session. The day after the fMRI scanning, we tested participants on the trained task for 216 trials (transfer test) without feedback (Figure 3.1).

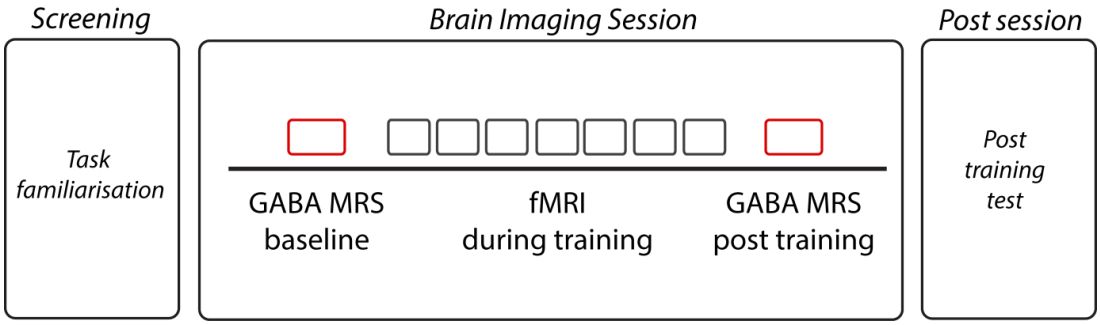


Figure 3.1: Experiment design.

Participants were familiarised with the task during a screening session at the lab. On the day of the brain imaging session, MRS measurements (45min) were taken before and after training, while fMRI measurements were taken during training (45min). The day after the imaging session, participants were tested in the lab for 216 trials without feedback.

c. Brain imaging session

All participants took part in a single brain imaging session during which they were trained on either the Signal-in-Noise or the Feature-differences task. We recorded fMRI data during training and MRS data from occipito-temporal cortex before and after training. Each session lasted approximately three hours: the MRS measurements before and after training were 45 minutes long, while the training-fMRI measurements lasted for one hour including structural and localiser scans. We avoided participant fatigue by having a short break outside the scanner before and after the training-fMRI part.

The fMRI measurements comprised 7-8 experimental runs (data were missing from several participants (n=9) for the eighth training run). Each run lasted 330s. We used an event related design and ensured that the order of trials was matched for history (two trials back) such that each trial was equally likely to be preceded by any of the conditions. The order of the trials differed across runs and participants. Two stimulus conditions (radial, concentric) and one fixation condition, with 36 trials per condition, were presented in each run. Each run comprised 110 trials (108 trials across conditions and 2 initial trials for balancing the history of the second trial) and two 9 s fixation periods at the beginning and end of the run. Participants were presented with a Glass pattern stimulus per trial and asked to judge whether the presented stimulus was radial or concentric. Participants received feedback on their average performance (i.e. percent correct) every 10-15 trials as indicated by a vertical colour bar.

For fixation trials, a fixation dot was displayed in the centre of the screen for 3s. For experimental trials, a 200ms stimulus presentation was followed by a 1300ms fixation. After this fixed delay, a response cue appeared as either a red "+" or "x". If the response cue was a red "+", participants indicated radial versus concentric by pressing the left versus the right key. If the response cue was a red "x", the opposite keys were used (e.g., radial = right key). This allowed us to dissociate the motor response (button press) from the stimulus related fMRI

activations. The response cue remained on the screen for 1000ms, followed by a fixation dot 500ms before the next trial onset. Participants were familiarized with the task before scanning (Figure 3.2).

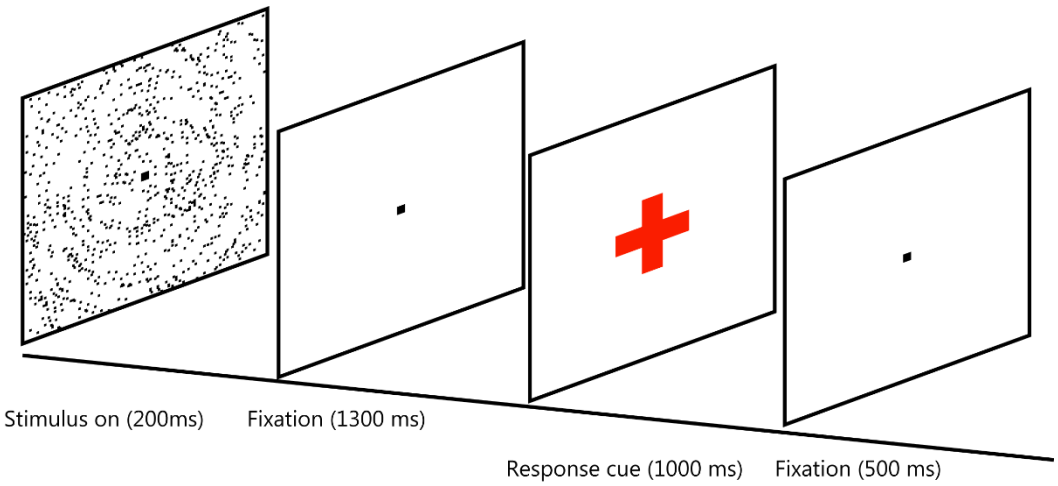


Figure 3.2: Experimental trial structure.

The stimulus was presented for 200ms. A 1300ms fixed delay was followed by a response cue that prompted participants to respond with the respective button-category combination. The response cue remained on the screen for 1000ms, followed by a fixation dot 500ms before the next trial onset.

d. Data acquisition

Functional MRI data acquisition

Experiments were conducted at the Cognition and Brain Sciences Unit, Cambridge (3T Magnetom Trio, A Tim System, Siemens). We collected T2*-weighted functional and T1-weighted anatomical (1x1x1mm) data with a 12-channel sensitivity encoding (SENSE) head coil. Echo planar imaging data (gradient echo-pulse sequences) data were acquired from 27 slices (whole-brain coverage; TR, 1500 ms; TE, 29 ms; flip-angle, 78°; resolution 2.5 x 2.5 x 4mm).

MR Spectroscopy data acquisition

We collected MRS data with a 32-channel sensitivity encoding (SENSE) head coil. We collected localizer images ([TR/TE] = 90/3.63 ms; [FOV] = 350x350x263; 5 mm slice thickness) to confirm head positioning and three-dimensional T1-weighted structural data (MP-RAGE; TR/TE/TI=2010/3.53/1100 ms; FOV=256x256x224 mm; isotropic 1 mm in-plane resolution).

We centred the voxel for both MRS measurements ($25 \times 25 \times 25$ mm^3) in the right occipito-temporal cortex (Figure 3.3), as this region has been previously shown to be involved in template representation (Chang et al., 2014) and judgements of object properties (Ellison and Cowey, 2006). We positioned the MRS voxel manually using anatomical landmarks (Superior Temporal Gyrus, Middle Occipital Gyrus) on the acquired T1 scan to ensure that the voxel placement matched between the pre- and post- training measurements and across participants. We used a 2D 1H J-PRESS sequence (Prescot and Renshaw, 2013; Schulte et al., 2006) (TR/TE = 2000/31-229 ms; ΔTE = 2 ms (100 TE steps); 4 signal averages per TE step with online averaging; 2D spectral width = 2000x500 Hz, and 2D matrix size = 1024x100). Measurements with this sequence at 3T have been previously shown to be reliable and reproducible (Prescot and Renshaw, 2013; Schmitz et al., 2017). We conducted B0 shimming within the MRS voxel combining an automated phase map with interactive manual shimming until the full-width at half-maximum measured for the real component of the unsuppressed water signal was below 20 Hz. We placed six saturation bands at least 1cm away from the cubic MRS voxel faces to suppress outer volume (OVS), using hyperbolic secant adiabatic full passage RF pulses. OVS was interleaved with water suppression via a WET scheme (Prescot and Renshaw, 2013). Water unsuppressed 2D 1H MRS data were also collected and used for eddy current correction.

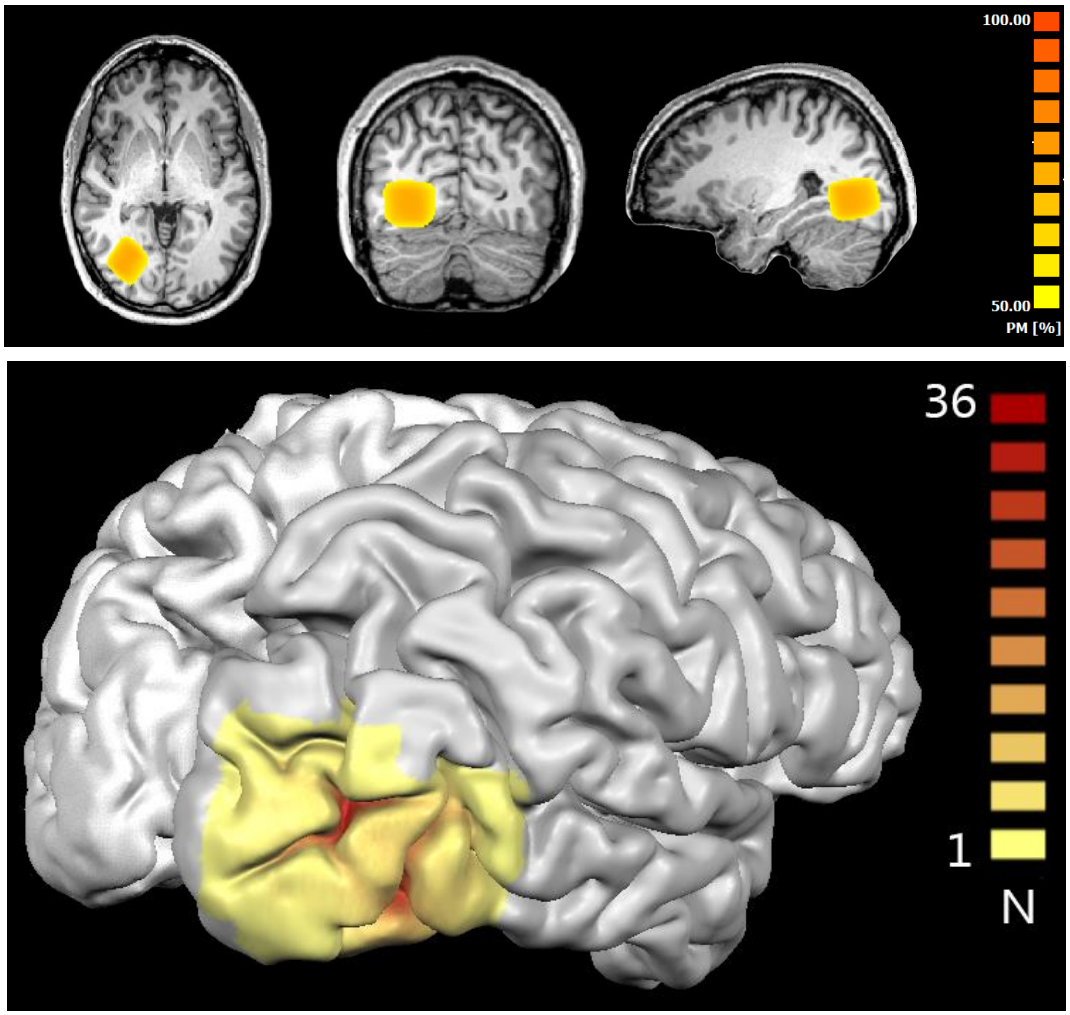


Figure 3.3: MRS voxel placement

MRS measurements were acquired from a 2.5 x 2.5 x 2.5 cm^3 volume centred on the lateral occipital cortex, using anatomical landmarks. Top: voxel position probability map, showing the voxel placement common across 50% to 100% of participants. This mask was used to constrain GLM analyses. Bottom: voxel probability map on the grey matter surface reconstruction. Red corresponds to brain voxels included in all participants' MRS voxels.

e. Data analysis

Behavioural data

We employed a single interval forced choice task, where participants were asked to choose between two stimulus classes (radial or concentric) in each trial. To quantify discriminability (Figure 3.4) between the two Glass patterns classes (radial vs. concentric), we computed d' (Stanislaw and Todorov, 1999) across trials per run, as the difference between the z-transform of each stimulus class' hit and false alarm rates. In particular, if the stimulus was radial (tR) and the participant responded "radial" (rR), this was counted as a hit for the radial class (tRrR) or a correct rejection for the concentric class. If the stimulus was radial (tR) and the participant responded "concentric" (rC), this was counted as a miss for the radial class (tRrC) or a false alarm for the concentric class. When calculating response rates, we computed hit rate for radial and concentric as follows:

Radial Hit Rate: tRrR /tR, Radial False Alarm Rate: tCrR/tC

Concentric Hit Rate: tCrC/tC, Concentric False Alarm Rate: tRrC/tR

Also:

Radial Hit Rate + Concentric False Alarm Rate = tRrR/tR + tRrC/tR = tR/tR = 1

and

Concentric Hit Rate + Radial False Alarm Rate = tCrC/tC + tCrR/tC = tC/tC = 1

d' can be computed using the Radial or Concentric Hit and False Alarm Rates as shown below:

d' = z (Radial Hit Rate) - z (Radial False Alarm Rate)

= z (1-Concentric False Alarm Rate) - z (1-Concentric Hit Rate)

= -z (Concentric False Alarm Rate) + z (Concentric Hit Rate),

where z is the inverse cumulative distribution function for a normal distribution (0,1).

To quantify behavioural improvement, we calculated: a) learning rate that indicates the rate of change in perceptual sensitivity as measured by d' per training run, b) Δd' that indicates difference in perceptual sensitivity early (first run) vs. late (last run) in training. To compute learning rate, we fitted individual participant training data with a logarithmic function: $y = k * \ln x + c$, where x is the training run, y is the run d', c is the starting d' and k corresponds to the learning rate using MATLAB 2013a (The MathWorks, Natick, MA, USA). Positive learning rate indicates that performance improved with training, whereas negative or close to zero learning rate indicates no behavioural improvement.

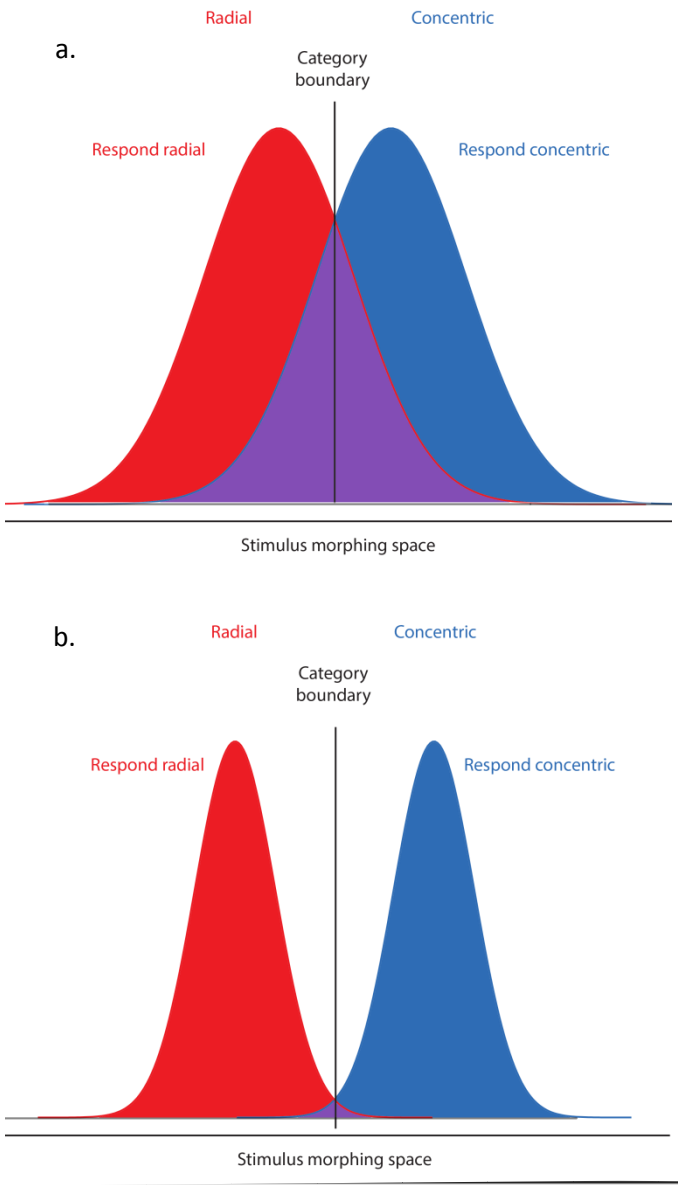


Figure 3.4: Stimulus class discriminability

a. Before training there is large overlap between the response distributions of participants, as they cannot differentiate between the two categories near the category boundary.

b. After training, participants' responses are more selective and there is small overlap between the two response distributions, as they are now able to discriminate between the two stimulus classes near the category boundary.

MRS data pre-processing

We pre-processed MRS data according to (Prescot and Renshaw, 2013), using MATLAB and the prior-knowledge fitting software ProFit (Schulte and Boesiger, 2006). ProFit provides successful two-dimensional fitting by combining maximum prior knowledge integration with LC Model (Provencher, 1993) and a succession of non-linear and linear parametrised fitting with variable projection (van der Veen et al., 1988).

We assessed the quality of the fit by means of visual inspection and calculation of the Cramer-Rao Lower Bounds (CRLB) of variance. Visual inspection is an essential step when assessing MRS data quality. While pre-processing tools and fitting toolboxes are equipped to account for multiple sources of noise, it's possible that erroneous fits with misleading fit errors may suggest a contaminated dataset is of good quality. This is often the case when the spectrum suffers from lipid contamination. Spectral peaks in the range of 1.5 to 1.75ppm correspond to macromolecules and if present, the 1.8ppm GABA peak is contaminated and erroneously fitted. Such contamination is often (but not always) coupled with high CRLB for the fit.

Only participants without fat contamination and GABA CRLB values <20% (Stagg and Rothman, 2013) for both pre and post training fitted data were included in further steps of MRS related analyses (three participants from the Feature-differences task were excluded due to this contamination, Figure 3.5, Table 3.1). Residual water was removed from each row of water suppressed 2D matrices using a Hankel singular value decomposition (HSVD) MATLAB routine (Cabanes et al., 2001; Prescot and Renshaw, 2013).

We normalised metabolite concentrations to the concentration of total Creatine (tCre). tCre has been widely used as a reference metabolite in MRS studies (Donahue et al., 2010; Sampaio-Baptista et al., 2015) and this method for normalisation has been shown to have better reproducibility compared to other methods (Bogner et al., 2010). We then subtracted pre- from post-training

concentrations to estimate GABA/tCre changes before compared to after training.

To control for potential variability of the MRS voxel placement within and between participants, we extracted the Talairach coordinates of each participant's pre- and post-training MRS acquisition voxels (Figure 3.3). The mean difference in voxel placement within participants was minimal (x=-0.60mm; y=-0.40mm; z=-0.52mm). Only for one participant (Signal-in-noise task) was the difference in the MRS voxel position before and after training larger than two standard deviations above the mean; data for this participant were excluded from further analyses (Table 3.1). The voxel position before vs. after training did not differ significantly between the two tasks ($t(34)=0.085$, $p=0.93$).

To account for the variability in tissue composition within the MRS voxel across participants, we calculated the percentage of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) in each of the MRS measurement voxels. We conducted whole brain tissue-type segmentation of the T1-weighted anatomical scan using FAST (Zhang et al., 2001), in the FMRIB Software Library (Smith et al., 2004). We then divided GABA concentration by GM/(GM+WM+CSF) and Creatine concentration by (GM+WM)/(GM+WM+CSF) (Kolasinski et al., 2017).

We used bootstrapped Pearson's correlations to measure the linear association between variables (GABA, behavioural improvement, BOLD change) as implemented in the Robust Correlation toolbox (Pernet et al., 2013). Skipped-correlations detect bivariate outliers and account for their removal when testing for correlation significance. Bivariate outliers were detected using the box-plot rule on z-scored values: the algorithm calculates orthogonal distances of all data points from the centre of the bivariate distribution and marks as outliers data points with distances that exceed the interquartile range. Where bivariate outliers were detected we reported Skipped Pearson's r and bootstrapped

confidence intervals. We used Fisher's test to compare correlation metrics between tasks and Steiger's test within task.

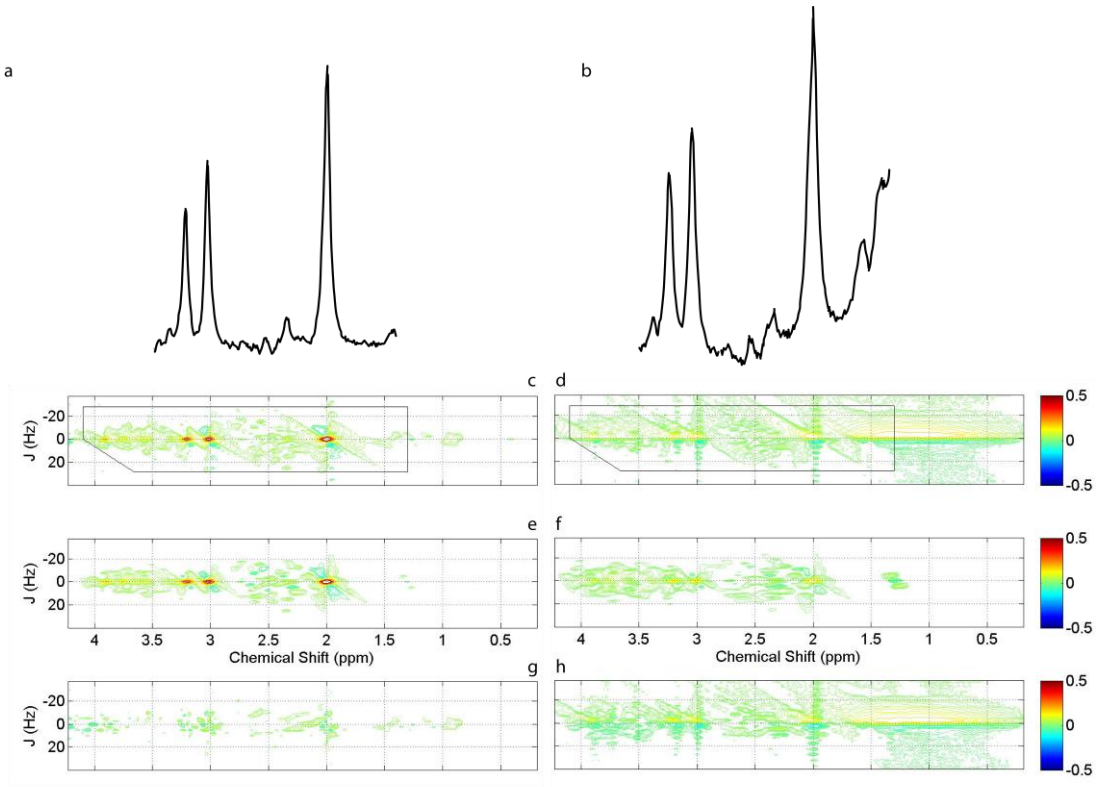


Figure 3.5: Examples of lipid contamination in MRS spectra.

In healthy tissue, fat contamination is caused by extracranial lipids when the MRS voxel is placed near the skull, compromising metabolite quantification. When preprocessing the MRS data, we estimated fat contamination after visual inspection of the two-dimensional spectra (c,d), their fits (e,f) and residual plots (g,h). We used two empirical criteria for data exclusion, following previous studies (Schmitz et al, 2017).

First, we collapsed the first frequency dimension, producing a 1D representation that resembles a typical MRS spectrum. The difference between a spectrum without (a) and with (b) fat contamination is observed between 0.9-2.8ppm, where lipids resonate. High peaks in these frequencies indicate fat contamination (b) and affect the spectrum baseline. Second, we confirmed this fat contamination by visually inspecting the two-dimensional spectral fits (e,f) and residuals (g,h). The colour-scale represents the range of chemical compound concentrations and is the same for all participants. Larger peaks, as for example NAA at 2ppm, are depicted with distinct red circles, as their concentration is a lot higher than their neighbouring metabolites (c,e). Fat contamination results in a noisy spectrum (d) with large residuals (h), indicating a poor fit. As the spectrum is affected by the presence of large peaks in resonances between 0.9-2.8ppm, we observe a reduced range of metabolite concentrations; that is, relatively large peaks (e.g NAA at 2ppm) are no longer well defined (d,f) or coloured in red (higher values). We removed data from further analyses when both criteria were met. This was the case for data from three participants in the Feature Differences task.

fMRI data pre-processing

We used BrainVoyager QX 2.8 (Brain Innovation, Maastricht, The Netherlands) for fMRI data analysis (Goebel et al., 2006). We used an automated alignment routine (rigid body transformation) together with manual adjustments to ensure precise co-registration of the functional and anatomical data. T1-weighted anatomical data were used for coregistration, and three-dimensional head motion correction, temporal high-pass filtering (Fast Fourier Transform with a cut-off of 3 cycles) and removal of linear trends. Trials with motion larger than 3mm were excluded from further analyses. Three participants with excessive motion across experimental runs were excluded from fMRI related analyses (one for the Feature-differences task and two for the Signal-in-Noise task, Table 3.1). Spatial smoothing (Gaussian filter, full-width-at-half-maximum 5mm) was used for group GLM analysis. The functional images were aligned to anatomical data under careful visual inspection, and the complete data were transformed into Talairach space (nearest-neighbor interpolation). The functional runs were co-aligned to the first functional volume of the first run of the session.

fMRI data analysis

To investigate fMRI learning-dependent changes during training, we grouped the fMRI runs into three training blocks: first two ('early training'), middle three and last two ('late training') runs. We analysed the data using a General Linear Model (GLM) with two task related regressors (stimulus vs. fixation trials) and six head movement regressors based on the motion correction parameters. We conducted whole-brain voxel-wise covariance analyses to identify voxel clusters that show significant correlations between BOLD activation change (early vs. late training blocks) and behavioural improvement. To assess the relationship between learning-dependent changes in fMRI and GABA measurements, we conducted a covariance analysis that tested for voxels that showed significant correlation between fMRI activity change (last vs. early training block) and

GABA change (post- minus pre-training GABA measurement). We used Brain Voyager's cluster-extent thresholding tool (ClusterThresh plugin) and run Monte Carlo simulations to estimate the cluster-extent threshold and confirm a family wise error threshold of p=0.05.

Table 3.1: Datasets collected and used after removing poor quality data in each modality

	Signal-in-Noise	**Feature differences**
Datasets collected	21	22
Excessive motion in fMRI	2	1
Fat contamination in MRS	0	3
Offset in MRS voxel	1	0
Total datasets used	18	18

3. Results

Learning-dependent changes in behaviour and fMRI

We tested two different groups of participants on either (1) a signal-in-noise (SN) task that involves extracting shapes (radial vs. concentric Glass patterns) from background noise or (2) a feature-difference (FD) task that involves judging fine differences induced by morphing between the two stimulus classes. For each task, participants improved during a single training session that took place during scanning (Figure 3.6), consistent with previous reports showing fast behavioural improvement early in the training (for a review see Sagi, 2011). A repeated measures ANOVA (Task (SN vs. FD) x Training (training runs) showed significantly improved performance –as measured by d'– after training

(main effect of Training: F(6,192)= 3.79, p=0.001) but no significant effect of Task (F(1,32)=0.01, p=0.91) nor Training x Task interaction (F(6,192)= 0.61, p=0.722), suggesting similar improvement in both tasks. Testing participants the following day after training (transfer test) showed that performance was significantly different from the first training run for both tasks (main effect of Session: (F(1,32)= 10.59, p=0.003; Task x Session interaction: (F(1,32)=0.89, p=0.35) but not significantly different from the last training run (main effect of Session: F(1,32)=0.95, p=0.34; Task x Session interaction: F(1,32)=0.18, p=0.68), suggesting lasting performance improvement due to training. In contrast, no significant changes in performance were observed for a no-training control group who did not receive training in between test sessions (main effect of Session: F(1,6)= 1.13, p=0.33; Task x Session interaction: F(1,6)=0.0003, p=0.99).

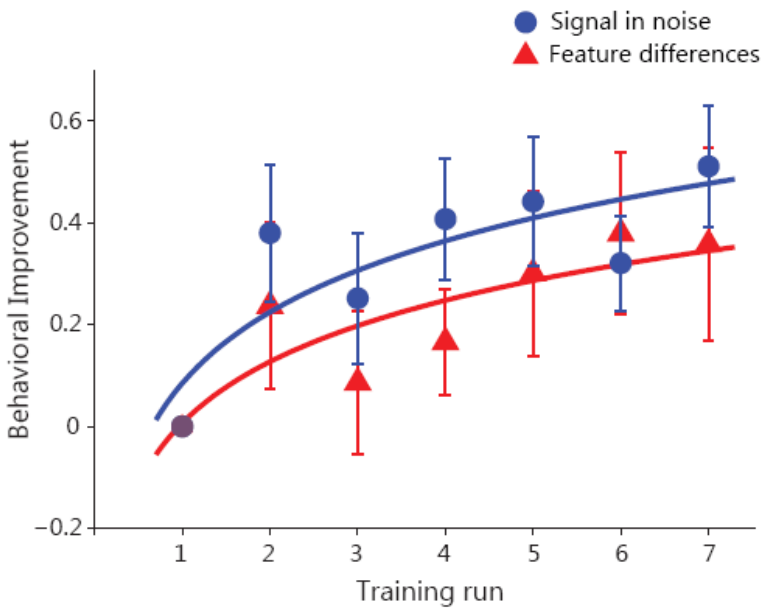


Figure 3.6: Behavioural improvement during training

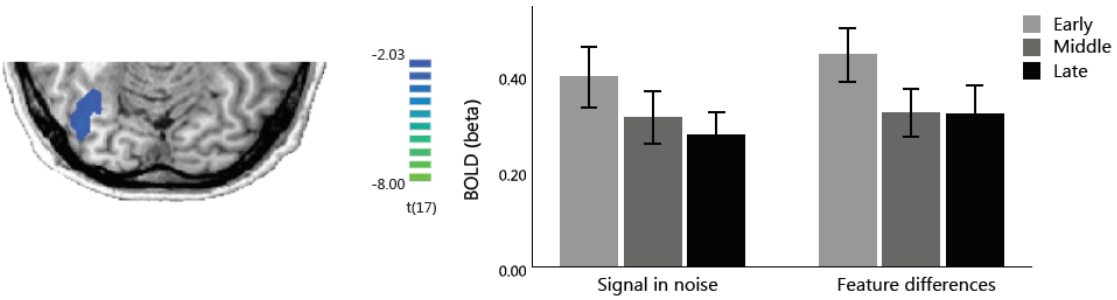
The task accuracy per run is normalised to the first run of the task and the improvement index is fitted with a logarithmic function to obtain learning rate. Behavioural improvement during training: mean d' per training run normalised to d' in the first run. Data were fitted with a logarithmic function; error bars indicate standard error of the mean across participants. The trend of higher performance in the SN than the FD task was not statistically significant; that is, there was no significant difference between tasks early in the training (i.e. first training block; t(34)=0.23, p=0.82), nor a significant main effect of Task (F(1,32)=0.01, p=0.91) nor a significant Task x run interaction (F(6,192)=0.61, p=0.72), suggesting similar performance before training and behavioural improvement after training between tasks.

To further quantify behavioural improvement, we computed two complementary measures: a) delta d prime (Δd': last training run minus first training run) that indicates difference in perceptual sensitivity early vs. late in training, b) learning rate that indicates the rate with which perceptual sensitivity (d' calculated per training run) changes during training. These measures have been previously used in perceptual learning studies to quantify the effect of training on performance (Ball and Sekuler, 1987; Chang et al., 2013; Dosher et al., 2013). Behavioural improvement was similar between tasks, as indicated by no significant differences between tasks in learning rate ($t(34)=0.03$, $p=0.974$) nor Δd' ($t(34)=0.806$, $p=0.426$).

We next tested whether behavioural improvement relates to functional brain changes with learning. First, we tested for learning-dependent changes in functional brain activations during training. GLM analysis of the fMRI data across training runs showed significant changes in occipito-temporal BOLD for both tasks (Figure 3.7a), suggesting that BOLD changes at this early stage of learning (i.e. single training session that resulted in maximum 74% mean performance) do not differ between tasks. This is consistent with previous fMRI studies showing learning-dependent changes within a single training session (Mukai et al, 2007). It is possible that the two tasks may show discriminable BOLD activations after more extensive training resulting in saturated performance, as shown by our previous studies using similar learning paradigms with multiple training sessions (Kourtzi et al., 2005; Li et al., 2012; Mayhew et al., 2010). Second, we conducted whole-brain voxel-wise covariance analyses using either learning rate or Δd' as covariates. For these analyses, we pooled the data across tasks, as changes in both behavioural performance and BOLD with training were similar between tasks. Our results showed significant correlations between BOLD change (late vs. early training runs) in the posterior occipito-temporal cortex and behavioural improvement (learning rate, Δd') across tasks (Figure 3.7b). These results provide evidence for learning-dependent changes in occipito-temporal cortex that relate to behavioural improvement, consistent with

our previous studies and the role of this region in visual learning and global shape processing (Kourtzi et al., 2005; Kuai et al., 2013; Zhang et al., 2010). Therefore, we next focused on the posterior occipito-temporal cortex and tested whether learning-dependent BOLD changes relate to changes in GABA concentration in this region.

a. BOLD changes during training



b. BOLD-behaviour covariate analysis

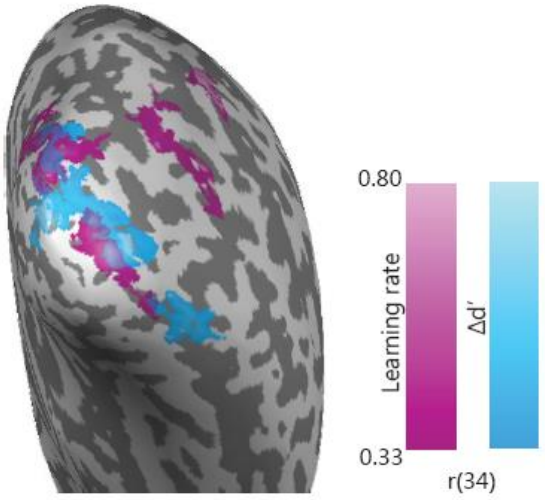


Figure 3.7: Learning-dependent changes in BOLD

a. GLM analysis of the fMRI data across all training runs showed that significant BOLD changes in occipito-temporal cortex during training for both tasks (main effect of Task: $F(1,34)= 0.20$, $p=0.66$; Task x Block interaction: $F(1.9,64.6)$, $p=0.71$). Bar-plots show BOLD signal (percent signal change) in occipito-temporal cortex across runs; mean data is plotted during training: early (first two training runs), middle (middle three training runs), late (last two training runs) for the two tasks; error bars indicate standard error of the mean across participants.

b. Whole-brain covariance analyses (cluster threshold corrected, $p<0.05$) with either learning rate (purple) or Δd' (blue) on fMRI data (first two runs vs. last two runs) that were pooled across the two tasks showed positive significant clusters in the posterior occipito-temporal cortex. Activations are shown on the cortical surface of the right hemisphere (sulci are shown in dark grey, gyri in light grey). The colour bar indicates Pearson's r correlation values.

Relating GABA to behavioural improvement

Previous MRS studies have shown that GABA concentrations in the visual cortex relate to performance in perceptual tasks (Edden et al., 2009) and homeostatic plasticity (Lunghi et al., 2015). Here, we test whether GABA-ergic suppression relates to behavioural improvement and learning-dependent functional changes in the visual cortex, by comparing MRS-measurements of GABA in the posterior occipito-temporal cortex before vs. after training.

First, we tested whether behavioural improvement –as measured by learning rate and Δd'– relates to changes in visual cortex GABA with training. We recorded GABA concentrations before and after training within a voxel centred on the posterior-occipito-temporal cortex (Figure 3.3), consistent with the fMRI analysis showing learning-dependent BOLD changes with training in this region. Correlating learning rate and Δd' with GABA changes showed dissociable effects for the two tasks (Figure 3.8, Figure 3.S1). In particular, for the Signal-in-noise task we observed a negative correlation of GABA change with learning rate (r=-0.43, CI=[-0.74, -0.07]), but no significant correlation with Δd' (r= -0.14, CI=[-0.49, 0.29]). In contrast, for the Feature-differences, task we observed a positive correlation of GABA change with Δd' (r=0.54, CI=[0.05, 0.85]), but no significant correlation with learning rate (r= 0.13, CI=[-0.38, 0.62]). Further, the significant correlations of GABA change with behavioural improvement (learning rate for SN; Δd' for FD) were significantly different between tasks (Fisher's z=2.91, p=0.004). These dissociable effects could not be simply explained by differences between tasks, as the two tasks resulted in similar behavioural improvement.

To ensure that our results were specific to GABA changes in the posterior occipito-temporal cortex due to training, we performed the following controls. First, correlation of GABA change and behavioural improvement remained significant when we corrected for a) tissue (grey matter, white matter, cerebrospinal fluid) composition (SN, correlation with learning rate: r=-0.41,

CI=[-0.70, -0.07]; FD, correlation with Δd': r=0.56, CI=[0.03, 0.83]) and b) differences in data quality (as measured by Cramer-Rao Lower Bounds – see Materials and Methods) between the two GABA measurements (SN, correlation with learning rate: r=-0.44, CI=[-0.71, -0.12]; FD, correlation with Δd': r=0.46, CI=[0.03, 0.76]). Second, correlating percentage GABA change (GABA change / pre-training GABA) with behavioural improvement to control for pre-training GABA showed significant correlations for both tasks (SN, correlation with learning rate: r= -0.45, CI=[-0.78, -0.002]; FD, correlation with Δd': r=0.58, CI=[0.18, 0.81]). These correlations were significantly different between tasks (Fisher's z=2.87, p=0.004) and remained so when we normalised GABA to NAA rather than creatine concentration (Fisher's z=2.73, p=0.01). Third, changes in Glutamate, the other major cortical neurotransmitter, did not correlate significantly with behavioural improvement (SN, correlation of Glutamate change with learning rate: r=0.33, CI=[-0.22, 0.67]; FD, correlation of Glutamate change with Δd': r=-0.30, CI=[-0.58, 0.06]). These correlations of Glutamate change with measures of behavioural improvement were significantly different from correlations of GABA change with behavioural improvement (SN, correlations with learning rate: Steiger's z=2.99, p=0.003; FD, correlations with Δd': Steiger's z=33.4, p=0.001). Finally, correlations of GABA change and behavioural improvement remained significant after accounting for Glutamate change (SN, correlation of GABA change with learning rate: r=-0.41, CI=[-0.69, -0.08]; FD, correlation of GABA change with Δd': r=0.54, CI=[0.04, 0.85]), suggesting that our results were specific to GABA and do not generalize to glutamate.

Our analyses so far showed significant correlations of changes in GABA and behaviour due to training. Yet, we did not observe significant differences in mean GABA concentration in occipito-temporal cortex before vs. after training (main effect of MRS block: F(1,34)= 0.06, p=0.81; Task x MRS block interaction: F(1,34)= 0.21, p=0.65) (Figure 3.S2). Previous studies have reported mean changes in GABA concentration in the motor cortex (Floyer-Lea et al.,

2006; Sampaio-Baptista et al., 2015) due to training and visual cortex due to changes in homeostatic plasticity (Lunghi et al., 2015). The main difference between our study and these previous reports is that participant performance increased but did not saturate during the single training session employed in our study (i.e. participant reached mean performance 74%), in contrast to previous studies that showed saturated performance after training. Thus, it is likely that mean changes in GABA concentration are more pronounced when participant performance has plateaued after training. Further, it is likely that 7T imaging (rather than 3T imaging used in our study) affords increased signal-to-noise ratio and time resolution that may benefit measurements of change in GABA concentration (Barron et al., 2016; Lunghi et al., 2015).

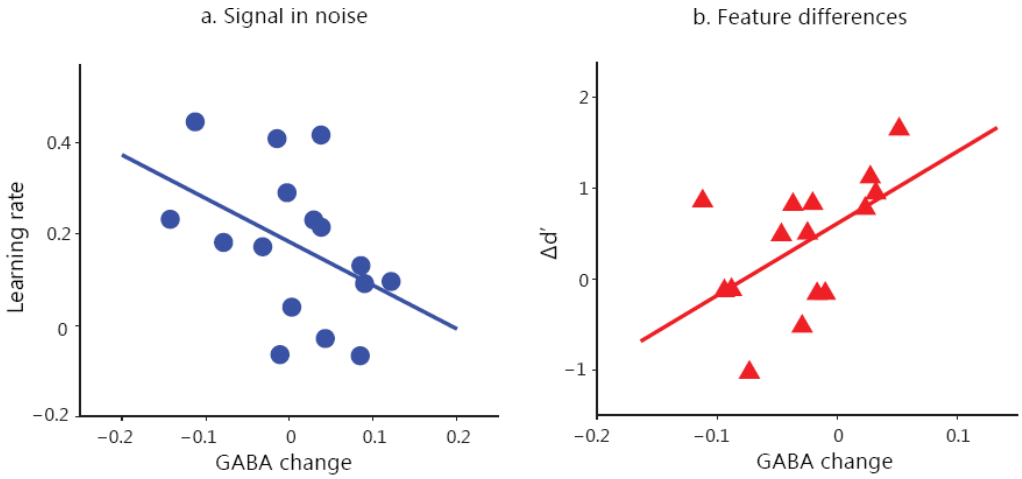


Figure 3.8: Distinct GABAergic contributions for the two tasks

Here, we show skipped Pearson's correlations indicating a significant negative correlation of GABA change in occipito-temporal cortex with learning rate for the Signal-in-noise task (r=-0.43, CI=[-0.74, -0.07]) and a significant positive correlation with Δd' for the Feature-differences task (r=0.54, CI=[0.05,0.85]). Correlations of GABA change with Δd' for the Signal-in-noise task or learning rate for the Feature-differences task were not significant (Figure 3.S1). The plots indicate that for a small number of participants the data deviated from the overall pattern of the correlation; e.g. for some participants in the SD task, GABA/tCr values were higher rather than lower compared to baseline. Our treatment of the data (i.e. behavioural improvement is expressed as percent over early performance and control analysis where GABA data is expressed as percent over baseline) accounts for potential differences across participants in performance early in training or baseline GABA before training. It is possible that this individual variability was due to the single training session employed in our study during which participant performance did not saturate (i.e. participant best reached 72% mean performance across participants). Bivariate outliers are not shown.

Relating GABA to learning-dependent BOLD change

Next, we tested whether learning-dependent changes in visual GABA (before vs. after training) relate to changes in BOLD within the posterior occipito-temporal cortex. We conducted a GLM covariance analysis to test whether BOLD changes (late vs. early training runs) relate to GABA changes in this region. This analysis showed opposite correlations between GABA and BOLD change for the two tasks: negative correlation for the Signal-in-Noise, while positive correlation for the Feature-differences task (Figure 3.9a). We corroborated this result by extracting BOLD signal from the voxel clusters in the posterior occipito-temporal that resulted from the covariance analysis of fMRI with behavioural improvement (Figure 3.7b). Correlations of change in GABA and BOLD –extracted from this independently defined region of interest (Figure 3.7b)- were opposite and significantly different between the two tasks (SN: r=-0.58 CI=[-0.82, -0.22], FD: r=0.70 CI=[0.37, 0.90], Fisher's z=4.19, p<0.0001) (Figure 3.9b). These results suggest that learning-dependent GABA changes measured with MRS relate to local BOLD changes, consistent with previous reports both in the LOC (Barron et al., 2016).

Our findings suggest that task-dependent suppression mechanisms relate to functional changes in visual cortex and behavioural improvement. To further test this hypothesis, we performed moderation analyses (Hayes, 2012) (Figure 3.10) that allowed us to test whether the influence that an independent variable (i.e. BOLD) has on the outcome (i.e. behaviour) is moderated by one or more moderator variables (i.e. GABA, task). Our results showed that this model is significant ($F(7,28)=3.77$, $p=0.01$) and the relationship between BOLD change and behavioural improvement depends multiplicatively on GABA change and task, as indicated by a significant three-way interaction between task, GABA change, and BOLD change ($F(1,28)= 7.17$, $p=0.01$; R-square change=0.13). Taken together, these moderation analyses suggest that task-dependent GABA-ergic suppression moderates the relationship between functional brain plasticity and behavioural improvement in the visual cortex.

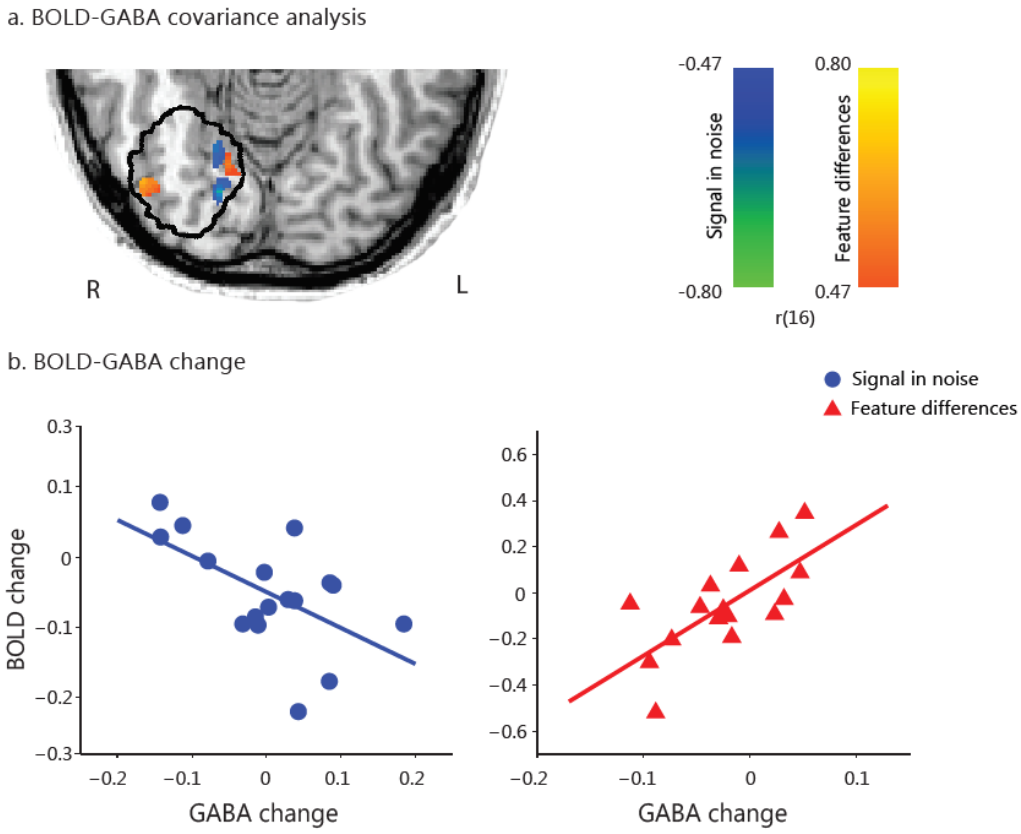


Figure 3.9: Differential GABA-BOLD correlations for the two tasks

a. GLM covariance analysis of GABA change with BOLD change within a masked region defined by the MRS voxel probability map (i.e. grey matter voxels that fall within each participant's MRS voxels with minimum 50% probability, as outlined in black). We used fMRI data (i.e. first two vs. last two fMRI runs) that were collected closer to the time when GABA was collected (before vs. after training). Activations are shown in radiological co-ordinates. GABA change correlated negatively with BOLD change for the Signal-in-Noise task (green to blue colour bar), while positively for the Feature-differences task (orange to yellow colour bar). The colour bars indicate Pearson's r.

b. Correlation of change in GABA and BOLD extracted from an independently defined region of interest; i.e. BOLD was extracted from the voxel clusters in posterior occipito-temporal cortex that resulted from the covariance analysis of fMRI with behavioral improvement (Figure 3.7b). This analysis showed opposite and significantly different correlations (SN: r=-0.58 CI=[-0.82, -0.22], FD: r=0.70 CI=[0.37, 0.90], Fisher's z=4.19, p<0.0001) and corroborated the results shown in Figure 3.9a.

Control analyses

To ensure that the dissociable correlations we observed between tasks for behaviour, GABA and BOLD were not due to differences between the two groups of participants that were each trained on a different task (SN vs FD group), we compared behavioural and imaging data between groups before training. First, our analyses did not show any significant differences in GABA concentration before training ($t(34)=0.11$, $p=0.91$) nor in behavioural performance early in training (i.e. first training run) ($t(34)=0.23$, $p=0.82$) between the two groups. Second, we compared signal-to-noise ratio (SNR) between tasks for the first MRS measurement (i.e. pre-training) and the first two fMRI runs (i.e. early in the training, as there were no fMRI measurements before training). We did not find any significant differences in MRS SNR ($t(34)=0.77$, $p=0.45$), nor fMRI temporal SNR (tSNR) between the two tasks ($t(34)=0.73$, $p=0.47$). These results suggest that the dissociable results we observed between tasks could not be simply due to differences across individuals that trained in different tasks.

Further, to ensure the learning-dependent changes we observed were not confounded by changes in the scanner environment during training, we conducted the following control analyses. First, we calculated the variation of the scanner centre frequency across training runs for each participant. We found that the mean scanner centre frequency variation across participants was very small (0.0000125 ± 0.0000019 MHz), and there was no significant interaction between Training (first two vs. last two fMRI runs) and Task ($F(1,34)=0.68$, $p=0.42$). Second, a similar analysis on tSNR across fMRI runs did not show a significant interaction between Training (first two vs. last two fMRI runs) and Task ($F(1,34)= 1.62$, $p=0.21$). Further, to control for measurement differences in the MRS before vs. after training we conducted the following analyses. First, to assess measurement quality we calculated spectral SNR for each MRS measurement. This analysis showed no significant interaction between MRS block and task ($F(1,34) = 2.37$, $p=0.13$) nor a main effect of block ($F(1,34)=$

1.60, p= 0.22). Second, to assess spectral resolution before vs. after training, we calculated peak linewidth for each MRS measurement. This analysis showed no significant interaction between MRS block and task ($F(1,34)=0.90$, $p=0.35$) nor a significant main effect of MRS block ($F(1,34)= 2.97$, $p=0.09$). These results suggest that the MRS data quality was similar before and after training for both tasks. Taken together these analyses suggest that the dissociable correlations between BOLD and GABA we observed between tasks could not be due to differences in the quality of the BOLD or GABA measurements during training.

Finally, to ensure that our results were specific to learning-dependent changes, we excluded data from participants who did not show positive improvement during the single training session employed in our study, as indicated by learning rate (n=3) or Δd' (n=8). Despite the smaller data sample, the following results remained significant: a) correlations of GABA change with behavioural improvement (SN: r=-0.52, CI=[-0.80, -0.09]; FD: r=0.72, CI=[0.29, 0.94]), b) correlations of BOLD change (early vs. late training runs) with behavioural improvement (learning rate: r=0.58, CI=[0.30, 0.77]; Δd': r=0.42, CI=[0.05, 0.67]). Further, the correlations between GABA change and BOLD change (extracted from the voxel clusters revealed by the independent covariance analysis with behavioural improvement) remained significantly different between tasks (z= 2.84, p= 0.01).

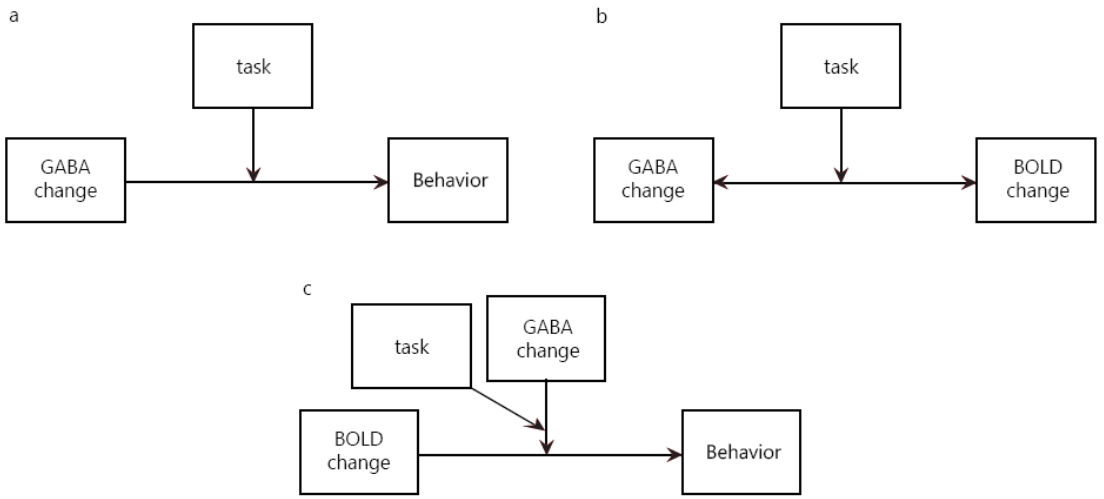


Figure 3.10: Task-dependent GABAergic plasticity moderates the relationship of functional brain plasticity and behavioural improvement.

We conducted moderation analyses to test whether task-dependent training moderates a) the influence of GABA change on behavioural improvement and b) the relationship between GABA and BOLD change. Model (a) was significant ($F(3,27)=3.06$, $p=0.04$), with a significant interaction between Task and GABA ($F(1,27)=8.56$, $p=0.01$; R-square change=0.24), indicating that task-dependent training moderates the influence of GABA change on behavioural improvement. Model (b) was significant when the outcome variable was either GABA ($F(3,29)= 6.28$, $p=0.002$; Task x BOLD interaction: $F(1,29)=16.27$, $p=0.0004$; R-square change=0.34) or BOLD ($F(3,29)=8.55$, $p=0.0003$; Task x GABA interaction: $F(1,29)=24.58$, $p=0.00003$; R-square change=0.45), indicating that task-dependent plasticity moderates the relationship between change in GABA and BOLD.

4. Discussion

In this study, we trained two groups of participants to detect shapes in clutter and to discriminate between highly similar targets. We used GABA-MRS before and after training to measure the concentration of the inhibitory neurotransmitter GABA in the occipito-temporal cortex. We used fMRI to measure whole brain activation during training and test the relationship between GABA and BOLD changes and how they relate to learning.

The lateral occipital cortex is known to be involved in shape processing (Kourtzi and Kanwisher, 2001), visual categorisation (Li et al., 2009, 2007) and integration of global forms (Ostwald et al., 2008), whereas its role in visual processing has been shown to change during training (Chang et al., 2014). Our results showed similar learning dependent changes in behavioural performance and BOLD in these tasks at early stages of learning (i.e. training for a single session). This is consistent with previous fMRI studies of perceptual learning that have shown learning-dependent changes in the overall fMRI responses in visual cortex (e.g. (Kourtzi et al., 2005; Mukai et al., 2007; Sigman et al., 2006)) or enhanced discriminability of fMRI patterns with training (Byers and Serences, 2014; Jehee et al., 2012; Kuai et al., 2013; Zhang et al., 2010).

Interrogating fMRI signals alone does not allow us to discern between the brain mechanisms that underlie these skills, as BOLD reflects the aggregate activity of excitatory and inhibitory signals at the scale of large neural populations (Heeger and Ress, 2002; Logothetis, 2008). However, combining MRS measurements of GABA with fMRI uncovers distinct suppression mechanisms that moderate the relationship between behavioural improvement and experience-dependent plasticity in visual cortex. Previous studies have investigated the relationship of baseline GABA measurements with performance in the context of visual (Edden et al., 2009) and sensory-motor tasks (Heba et al., 2016; Kolasinski et al., 2017; Stagg et al., 2011a) as well as reward-based learning (Scholl et al., 2017). Here, we test whether learning-dependent changes in GABA (i.e. GABA changes

before vs. after training) relate to changes in performance (i.e. behavioural improvement) and functional activation. Our findings provide evidence that changes in GABA-ergic suppression in the visual cortex relate to learning-dependent changes in behaviour and functional brain plasticity. In particular, for learning to see in clutter, decreased occipito-temporal GABA relates to increased BOLD and improved performance, as indicated by faster learning rate. This is consistent with previous animal work linking synaptic inhibition to reduced neuronal gain (Mitchell and Silver, 2003). In contrast, we demonstrate that for learning to discriminate fine feature differences, increased occipito-temporal GABA relates to increased BOLD and improved performance, as indicated by enhanced sensitivity in visual discrimination after training. This is consistent with studies showing that in the human occipital cortex, higher GABA baseline levels relate to increased sensitivity for orientation discrimination (Edden et al., 2009), while in the cat improved orientation selectivity is found after a GABA agonist injection (Li et al., 2008).

Finally, we investigated the coupling between co-localised GABA and BOLD changes. The negative relationship between GABA and BOLD changes observed for the Signal-in-Noise task has been proposed before in the human (Barron et al., 2016; Walter et al., 2016) and animal literature (Chen et al., 2005) and has been suggested to reflect attenuated BOLD responses as a result of GABAergic inhibition. While a positive relationship between baseline GABA measurements and BOLD changes has been previously reported (Harris et al., 2015; Lipp et al., 2015), this is the first report of a positive correlation between learning related GABA and BOLD changes. This result for the Feature-differences task suggests enhanced neuronal responses as a result of sharper tuning due to increased inhibition (Zhang et al., 2010).

Here, we focused on the link between learning-dependent GABA changes to BOLD changes in LOC. However, we found additional clusters of activation that correlate with LOC GABA changes and extend over occipito-temporal, occipito-parietal, salience and executive networks (Tables 3.S3, 3.S4). This

finding is not surprising as we expect changes in the inhibitory levels in LOC to relate to changes in activation in distant brain areas, as part of the wider brain networks involved in visual processing and learning (Grill-Spector et al., 2000; Kourtzi et al., 2005; Op de Beeck et al., 2006). Further, functional connectivity studies combined with measurements of inhibitory GABA in different brain areas during training are needed to investigate the link between GABA and BOLD changes.

Conclusion

Here I show for the first time the relationship between behavioural improvement on two distinct visual tasks and the inhibitory contributions as measured by GABA-MRS before and after training. Further, I provide evidence for the relationship between changes in GABA and BOLD responses measured from the same area during learning. Our results show that fMRI BOLD alone cannot reveal the dissociable neural mechanisms involved in learning, while differential GABAergic mechanisms moderate the link between changes in BOLD activation and behavioural improvement.

Open questions

Is the GABA-behaviour relationship specific to LOC?

Here I show dissociable GABAergic contributions to visual learning for two distinct visual tasks. I chose to measure GABA in LOC, having considered its key role in shape processing and differential function in detecting patterns in noise vs discriminating similar patterns, as suggested by fMRI studies. However, we also know from the literature that a wider network of brain areas is involved in perceptual judgements of visual stimuli (Grill-Spector et al., 2000; Kourtzi et al., 2005; Op de Beeck et al., 2006). Here I show that behavioural

improvement in the two tasks correlates with BOLD activity in LOC, as well as other brain areas that extend over fronto-parietal and motivational networks (Table 3.S1, 3.S2). It would be interesting to investigate how connectivity in these networks relates to inhibitory processing, in order to understand better the circuit mechanisms that mediate learning. In chapter IV, I measured resting functional connectivity between key nodes in these networks in the occipito-temporal and parietal cortex and relate it to resting inhibition and behavioural improvement in different visual tasks.

The functional role of LOC in visual learning of shapes, the confirmation of learning dependent BOLD changes in LOC, as well as the dissociable LOC GABA-behaviour correlations suggest our results are specific to LOC. However, the lack of GABA measurements from a control region (e.g. motor cortex) does not allow us to isolate LOC as the sole locus of learning-related inhibitory mechanisms that contribute to our visual tasks. In Chapter IV, I measured GABA from both the occipito-temporal and the posterior-parietal cortex to confirm the specificity of the findings.

What is the time-scale of the GABA change during training?

Here I measure GABA approximately 20 minutes before and after training. Currently, we do not have enough information regarding the expected timecourse of learning-related GABA changes in the visual cortex. Previous studies have reported changes in GABA due to stimulation within the range and time scales observed in our study (10-15% change observed within 20-30 min) (Barron et al., 2016; O'Shea et al., 2017; Stagg et al., 2009a). Evidence from the motor cortex suggest GABA changes of about 10-15% can be measured 10-20 minutes after training has ceased, while a trend is observed during 30 minutes of training (Floyer-Lea et al., 2006). However, we do not know how this translates in the visual cortex, especially in the context of a fast learning visual task. It is possible that fast GABA changes early on in training are required, which would need to be measured with shorter and more frequent acquisitions during training

rather than only after. In chapter IV, I used high-field MRS to measure GABA changes before and during training to test the time course of the learning-dependent GABA changes.

Is there a causal link between GABA changes and behavioural improvement?

Here I show GABAergic inhibition is linked to behavioural improvement for two visual learning tasks. However, our results support a correlational relationship between changes in GABA and improved performance. Our evidence so far cannot sustain causality and interventional methods are required to confirm GABA changes are causally linked with visual learning. In chapter V, I describe a study using transcranial direct current stimulation to investigate causal links between GABA changes in LOC and behavioural improvement in the two visual tasks.

Supplementary material

Table 3.S1: Whole brain BOLD with learning rate covariate

Cluster	Size	Hemisphere	Peak voxel	Area	r	p
1	1627	Right	32, -59, -3	Fusiform Lingual	0.54	0.0006
2	3027	Right	11, -86, 27	Cuneus	0.53	0.0009
3	1647	Bilateral	8, 25, 24	Anterior Cingulate	0.53	0.0009
4	1365	Bilateral	-7, 13, 36	Cingulate	0.49	0.0022
5	2774	Left	-10, -32, 45	Paracentral	0.60	0.0001

Table 3.S2: Whole brain BOLD with Δd' covariate

Cluster	Size	Hemisphere	Peak voxel	Area	r	p
1	2750	Right	14, -89, 9	Cuneus Middle occipital	0.54	0.000659
2	4922	Left	-25, -92, 9	Cuneus Middle occipital	0.60	0.000093
3	15484	Bilateral	-13, -35, 60	Postcentral	0.70	0.000002
4	3356	Left	-13, 20, -12	Anterior Cingulate	0.68	0.000006
5	1392	Left	-10, 7, 39	Cingulate	0.53	0.000868
6	1279	Left	-16, 55, 12	Medial Frontal	-0.60	0.000120
7	2083	Left	-40, 49, 12	Middle Frontal	-0.63	0.000034
8	1161	Left	-61, 1, 9	Precentral	0.50	0.001816

Table 3.S3: Signal-in-Noise: whole brain BOLD with GABA covariate

Cluster	Size	Hemisphere	Peak voxel	Area	r	p
1	972	Right	17, 43, 3	Ant. Cingulate	0.65	0.0037
2	891	Right	11, -2, 60	SFG	-0.66	0.0027
3	810	Right	17, -77, -6	Lingual gyrus/ Fusiform gyrus/ LOC	-0.67	0.0026
4	1026	Left	-37, -2, 45	MFG	-0.66	0.0026
5	1161	Left	-43, -65, 3	MTG / LOC	-0.70	0.0013
6	1080	Left	-46, -8, 24	Precentral gyrus	-0.69	0.0015
7	1026	Left	-49, -2, -9	STG	0.71	0.0009

Table 3.S4: Feature-differences: whole brain BOLD with GABA covariate

Cluster	Size	Hemisphere	Peak voxel	Area	r	p
1	1026	Right	57, 33, 1	IFG	-0.79	0.000107
2	36450	Right	31, -75, 5	Post. Cingulate LOC	0.87	0.000003
3	1728	Right	25, -49, 53	Postcentral gyrus / IPS	0.69	0.001642
4	1377	Right	10, -49, -13	Cerebellar Lingual	0.74	0.000450
5	945	Left	-8, -48, -11	Cerebellar Lingual	0.69	0.001639
6	3402	Left	-21, -6, 63	SFG	0.69	0.001488
7	810	Left	-8, -63, 40	Precuneus	0.70	0.001286
8	3618	Left	-38, -72, 2	MOG/LOC	0.77	0.000211
9	1890	Left	-32, 32, 21	MFG	0.70	0.001125
10	1026	Left	-32, 5, 28	Precentral gyrus	0.68	0.001749
11	1674	Left	-45, -22, 25	Insula	0.70	0.001255

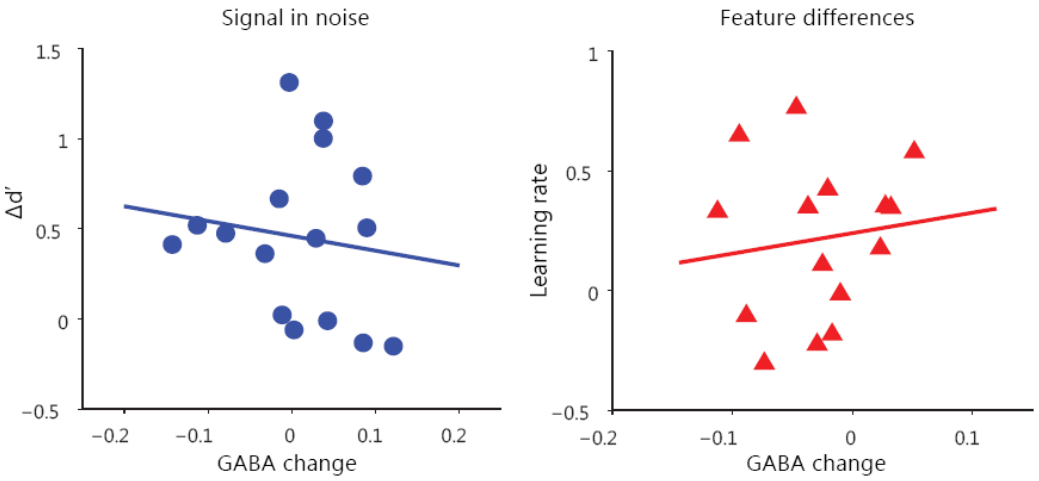


Figure 3.S1: Correlations of GABA change and behavioral improvement
For the Signal in Noise task, the correlation of GABA change with Δd' was not significant (r=-0.14, CI=[-0.49, 0.29]) and was not significantly different from the correlation of GABA change with learning rate (Steiger's z=1.25, p=0.21). For the Feature differences task, the correlation of GABA change with learning rate was not significant (r= 0.13, CI=[-0.38, 0.62]) but was significantly different from the correlation of GABA change with Δd' (Steiger's z=2.27, p=0.02).

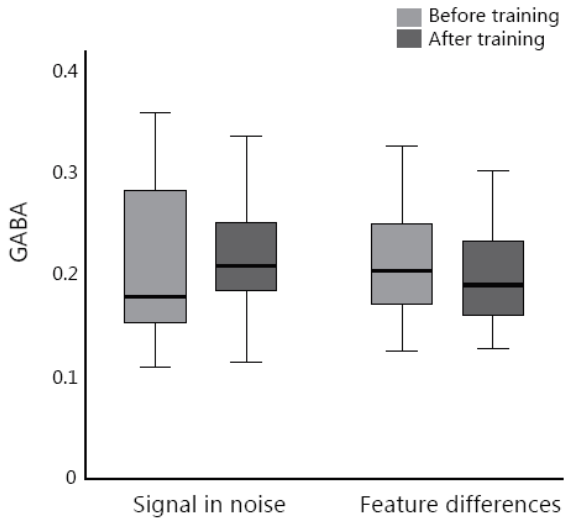


Figure 3.S2: GABA concentration before vs. after training
No significant differences were observed in GABA concentration in occipito-temporal cortex across participants before vs. after training (main effect of MRS block: F(1,34)= 0.06, p=0.81; Task x MRS block interaction: F(1,34)= 0.21, p=0.65). Boxplots denote median and interquartile ranges; indicating variability in GABA concentration across participants.

Chapter IV

Investigating the time-course of GABA changes during visual learning

1. Introduction

In Chapter III, I showed that GABAergic inhibition in the occipito-temporal (OCT) cortex moderates visual learning in dissociable ways for learning to detect patterns from clutter vs discriminating between fine features. Our results showed that during training, decreased OCT GABA, as measured by MR spectroscopy, related to improved detectability in the Signal-in-Noise task, where participants were asked to detect patterns in visual clutter, while increased OCT GABA related to improved discriminability in the Feature differences task, where participants had to discriminate highly similar patterns.

Our findings propose that differential GABAergic mechanisms are involved to meet different task demands and explain the link between changes in fMRI BOLD activation and behavioural improvement. However, the timescale of the GABA concentration changes during training is currently unknown. Here, we extend beyond standard correlational approaches that relate single measurements of GABA at baseline (i.e. when participants are at rest) to behaviour to test whether training changes GABA, as measured by MRS in the human brain. In particular, we test whether changes in GABAergic inhibition during task-specific training relate to improvement in perceptual decisions by measuring longitudinal changes in GABA during training (i.e. while the participants were trained on a task) rather than only GABA levels at baseline. To achieve this, we took advantage of the high spectral resolution afforded by ultra-high field (7T) MR Spectroscopy (MRS) to reliably resolve GABA (Puts

and Edden, 2012; Tkác et al., 2009) and take fast and reliable repeated measurements of functional GABA during training.

Further, we know that brain-wide networks are involved in perceptual judgments (Grill-Spector et al., 2000; Kourtzi et al., 2005; Op de Beeck et al., 2006), showing differential patterns of activation during training (Li et al., 2012; Mayhew et al., 2010) and playing an important role at different stages of training (Chang et al., 2014). Previous human fMRI studies have demonstrated learning-dependent changes in functional activation (i.e. increased or decreased activation) in both decision-related (i.e. posterior parietal) and sensory (i.e. visual) areas due to training on perceptual tasks (for reviews (Kourtzi, 2010; Welchman and Kourtzi, 2013b)). To test the role of inhibitory processing in learning for both visual and posterior parietal cortex, we implemented an imaging protocol that measured GABA in two voxels (one in occipito-temporal, one in posterior parietal cortex) in alternating order and allowed us to track longitudinal changes in GABA in both areas during training. Interestingly, previous studies have proposed that perceptual learning is implemented by top-down influences from decision-related areas that re-weight processing in sensory areas (Law & Gold 2010; Ahissar & Hochstein 2004). To test whether learning involves local processing within visual cortex vs. suppressive interactions between decision-related and sensory areas, we combined GABA measurements in occipito-temporal and posterior parietal cortex with functional brain connectivity– as measured by resting state fMRI. In particular, we tested the hypothesis that learning is implemented by local inhibitory processing in visual cortex that is gated by functional interactions between sensory and decision-related areas. Specifically, we tested whether learning-dependent changes in visual cortex GABA relate to functional connectivity between visual and posterior parietal cortex.

Our results provide evidence for distinct GABAergic inhibition mechanisms in a cortical network that is known to be involved in perceptual decisions. In particular, increased parietal GABA with training suggests suppression of task-

irrelevant information. In contrast, changes in occipito-temporal GABA with training relate to enhanced target detection and discriminability, suggesting learning-dependent changes in the processing of task-relevant features. Further, analysis of functional brain connectivity at rest reveals interactions within this network that relate to GABA changes and behavioural improvement during training. Learning to detect targets from clutter is implemented by local connectivity and disinhibition of the visual cortex. In contrast, learning feature differences is implemented by interactions between parietal and visual areas that relate to increased GABAergic inhibition in visual cortex. Our results provide evidence that learning improves perceptual decisions through suppressive interactions within decision-related circuits in the human brain.

2. Methods

a. Participants

Forty seven participants (31 female; mean age 25.6 ± 3.3 years) participated in this study. Sample size (minimum 40 participants) was determined based on power calculations following previous studies on motor learning showing an effect size of r=0.65 at 90% power for correlations of GABA with behaviour (Stagg et al., 2011a). We collected up to 25 participants in each task to account for data rejection due to motion artefacts. All participants were right-handed, had normal or corrected-to-normal vision and gave written informed consent. The study was approved by the University of Oxford ethics committee.

b. Experiment Design

All participants took part in a single brain imaging session during which they were randomly assigned and trained on either the Signal-in-Noise or the Feature Differences task. We recorded whole brain resting-state functional MRI (rs-fMRI) data before training while participants fixated on a cross at the centre for the screen. Following the rs-fMRI scan, we recorded MRS GABA before and

during training (Figure 4.1). We collected MRS GABA from one baseline block before training and three blocks during task training. Each block comprised two MRS acquisitions: one from occipito-temporal (OCT) and one from posterior parietal (PPC) cortex. The order of the voxels within each block was counterbalanced across participants. During each block participants were presented with Glass patterns, as described in Chapter II, for 400 trials (200 trials per MRS voxel acquisition). During the baseline block (400 trials) participants engaged in a task with similar stimuli as those presented during the training; that is, participants viewed random dot patterns (0% signal dipoles) and were asked to respond (button press) as soon as a pattern appeared. This ensured that differences in GABA between blocks could not be simply attributed to differences in overall alertness. During the MRS training blocks (3 blocks, 400 trials each), participants were presented with Glass patterns and were asked to judge and indicate by button press whether the presented stimulus in each trial was radial or concentric. Two stimulus conditions (radial vs. concentric Glass patterns; 200 trials per condition), were presented for each training block. For each trial, a stimulus was presented for 300ms and was followed by fixation (i.e. blank screen with a central fixation dot) while waiting for the participant's response (self-paced training paradigm). Trial -by-trial feedback was provided by means of a visual cue (green tick for correct, red 'x' for incorrect) followed by a fixation dot for 500ms before the onset of the next trial (Figure 4.2). Each MRS acquisition lasted for 5 minutes and 56 seconds, and each training block (400 trials) for 11 minutes and 11 seconds ± 63 seconds. Thus, in most cases training was completed within the duration of the training block (i.e. 2 MRS acquisitions x 5min 56 s). In the event that the training took longer than the MRS block, the next MRS acquisition was delayed until completion of the previous training block. Each session lasted approximately two hours.

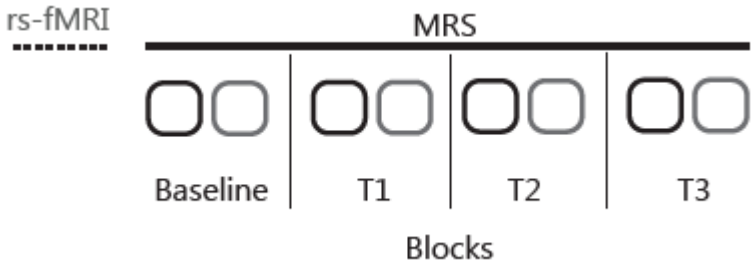


Figure 4.1: Experiment design.

Each participant took part in a single session during which we acquired rs-fMRI and MRS data. We collected MRS GABA during one block before (Baseline) and three blocks during training (T1, T2, T3). Each block comprised two MRS acquisitions: one from occipito-temporal (OCT voxel – black squares) and one from posterior-parietal (PPC voxel – grey squares) cortex. The order of the voxels within each block was counterbalanced across participants. During each block participants were presented with stimuli for 400 trials (200 trials per MRS voxel acquisition).

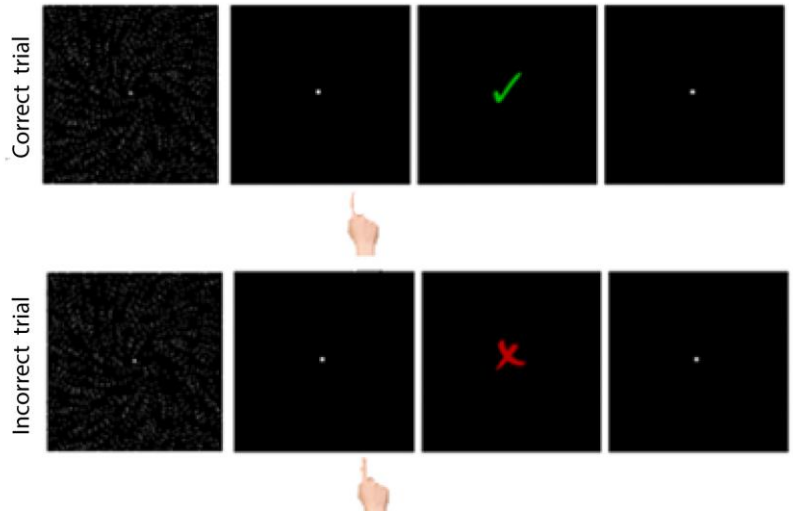


Figure 4.2: Experimental trial structure.

Stimuli were on for 300ms, followed by fixation while the participants made their judgements. Delayed (100ms) feedback in the form of a green tick for correct and red cross for incorrect was given for 200ms and a 500ms fixation followed before the next trial.

c. Data acquisition

Experiments were conducted at the Wellcome Centre for Integrative Neuroimaging, using a Siemens 7T Magnetom (Siemens, Erlangen) with a 32-channel head coil.

We acquired structural data (MPRAGE; TR 2200 ms; TE 2.82 ms; slice thickness 1.0 mm; in-plane resolution 1.0 x 1.0 mm^2; GRAPPA factor = 4) and echo planar imaging data (gradient echo-pulse sequences) from 40 slices (TR 2250 ms; TE 28 ms; slice thickness 3.0mm; in-plane resolution 3.0 x 3.0 mm^2; GRAPPA factor = 2, 140 volumes).

We acquired MRS data using a semi-localization by adiabatic selective refocusing (semi-LASER) sequence (Scheenen et al., 2008) (64 averages, TR 5010ms, TE 36ms). We chose to utilize a short-echo, a full signal intensity semi-LASER sequence to achieve lower apparent T2 relaxation, minimal J-coupling evolution and smaller chemical shift displacement errors relative to the PRESS and STEAM sequences (Öz and Tkáč, 2011). In addition, the adiabatic refocusing pulses in the semi-LASER provided minimal signal loss, high B1+ insensitivity and localization against the varying destructive interferences throughout the brain at ultra-high field. Alternatively, the long-echo GABA editing sequence may result in potential T2* weighting of BOLD signal on the spectra. To minimize this, we used the full signal intensity of the semi-LASER sequence to achieve lower apparent T2 relaxation. This MRS sequence has been extensively tested and resulted in high quality spectra across high and ultra-high field magnetic fields at different MRI centres (C Lemke et al., 2015; Lunghi et al., 2015; Öz and Tkáč, 2011; Terpstra et al., 2016; van de Bank et al., 2015). We used VAPOR (Tkáč et al., 1999) water suppression and outer volume suppression (van de Bank et al., 2015). We measured two MRS voxels (2 x 2 x 2 cm^3 isotropic), one in the left occipito-temporal cortex (OCT voxel) and one in the left posterior parietal cortex (PPC voxel) (Figure 4.3), avoiding contact with the dura to minimize macromolecule contamination. We focused on the left hemisphere as previous fMRI (Mevorach et al., 2009a) and TMS (Chang et al.,

2014; Mevorach et al., 2006) studies have shown that the left posterior parietal cortex is involved in suppressing distracting signals. To cover both areas with the same dielectric pad, we placed both MRS voxels on the left hemisphere. We positioned the MRS voxels manually using anatomical landmarks (Superior Temporal Gyrus and Middle Occipital Gyrus for OCT and the Intraparietal Sulcus for PPC) on the acquired T1 scan to ensure that voxel placement was consistent across participants. This was further confirmed by post-processing analysis of the position for each MRS voxel. In particular, we extracted the MNI coordinates of the centre of gravity for each MRS voxel per participant. We computed pairwise Euclidean distances between the coordinates of each participant and the mean coordinates of the group (OCT: x=-38.8±4.2mm, y=-67.8±4.7mm, z=2.3±4.2mm; PPC: x=-31.5±4.5mm, y=-50.4±6.4mm, z=41.0±5.2mm). The average distance from the mean coordinates across participants was 6.8±3.2mm for OCT and 8.3±4.4mm for PPC and did not differ between tasks (Voxel x Task interaction: $F(1,45)= 0.37$, $p=0.55$; main effect of Task: $F(1,45)=1.43$, $p=0.24$).

A dielectric pad (BaTiO3, 14.5 x 12.5 cm^2) was placed over the left occipito-parietal cortex to increase B1 efficiency in the regions where the MRS voxels were placed (Clark Lemke et al., 2015). First and second order shims were adjusted for each voxel separately using FASTMAP (fast, automatic shimming technique by mapping along projections) with echo-planar imaging readout (Gruetter and Tkáč, 2000). Acquisition parameters were optimised for each voxel by determining the appropriate transmit voltage (flip angle calibration) and flip angle (VAPOR calibration), to maximize readout and water suppression respectively (Clark Lemke et al., 2015). For each MRS acquisition we collected unsuppressed-water spectra for eddy-current correction, reconstruction of the phased array spectra and metabolite quantification.

d. Data analysis

Behavioural data

We quantified behavioural improvement during training as the difference in mean performance (i.e. mean accuracy per 200 trials) between the first training block and each subsequent training block, divided by performance in the first training block (equation 1). For each training block *t*:

$$Improvement_{\ t=2,3,4,5,6} = \frac{Performance_{\ t=2,3,4,5,6} - Performance_{\ t=1}}{Performance_{\ t=1}} \quad (1)$$

To take into account individual variability in performance, we estimated behavioural improvement as the difference in mean performance between the first training block and the block with best performance per participant (85% of the participants achieved best performance in the two last training blocks per MRS voxel), divided by performance in the first training block.

MRS data pre-processing

Eddy-current correction and reconstruction of the phased array spectra was applied using in-house scripts. Water residual signal was removed using a Hankel singular value decomposition (HLSVD) MATLAB routine (Cabanes et al., 2001). LC-Model (Provencher, 2001) was used to quantify metabolite concentrations in the range of 0.5 to 4.2 ppm using optimal initialization parameters. We referenced metabolite concentrations to the sum of the concentrations of Creatine (Cr) and Phosphocreatine (PCr), that is total Creatine (tCr). In particular, for each MRS voxel, we normalised GABA/tCr in each training block to GABA/tCr in the baseline block (Figure 4.5a). We computed GABA/tCr change for each participant as the difference between GABA/tCr in the training block with best performance and GABA/tCr in the baseline block. tCr has been widely used as a reference metabolite in MRS studies (Donahue et al., 2010; Sampaio-Baptista et al., 2015) and this normalization method has been shown to have better reproducibility compared to other methods (Bogner et al., 2010).

Only data without lipid contamination, GABA CRLB values smaller than one standard deviation above the mean and GABA values per block within two standard deviations from the mean across participants were included in further steps of MRS related analyses. That is, OCT data for 6 participants (2 for SN, 4 for FD) and PPC data for 6 participants (4 for SN, 2 for FD) were excluded due to high CRLB values. Thus, data from 18 participants were included for further analysis for the SN and 22 participants for the FD task. To account for variability in tissue composition within the MRS voxel across participants, we conducted whole brain tissue-type segmentation of the T1-weighted anatomical scan using SPM12.2 (SPM segment) and calculated percentage of grey matter (GM) voxels in each of the MRS voxels. The mean GM tissue fraction was 44±8% for OCT and 46±7% for PPC and GM tissue content did not differ significantly between the two MRS Voxels ($t(81)=1.17$, $p=0.24$). We accounted for the percentage of GM voxels in the MRS Voxel in a linear regression model that described the dissociable links between changes in OCT GABA and behavioural improvement, confirming that our results were not driven by variability in tissue composition across participants.

rs-fMRI data pre-processing

We pre-processed the resting-state fMRI (rs-fMRI) data using SPM12.2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) following the optimised pipeline described in recent work (Vergara et al., 2017). Data were excluded from one participant with incomplete data acquisition. We first processed the T1-weighted anatomical images by applying brain extraction and segmentation (SPM segment). From the segmented T1 we created a white matter (WM) mask and a cerebrospinal fluid (CSF) mask. For each participant, we corrected the EPI data for slice scan timing (i.e. to remove time shifts in slice acquisition, SPM slice timing), motion (least squares correction) and susceptibility distortions (applying fieldmap correction, SPM realign & unwarp). We then co-registered the EPI data to the T1 image (rigid body) per participant and calculated the mean CSF and WM signal per volume (SPM coregister & reslice). We

subsequently aligned the T1 image to the MNI space (affine) and applied the same transformation to the EPI data and the MRS voxels (OCT, PPC) (SPM normalise). We resliced the aligned EPI data to native resolution (3 x 3 x 3 mm^3) and applied spatial smoothing with a 5mm isotropic FWHM Gaussian kernel (SPM smooth). Finally, we despiked any secondary motion artefacts using the Brain Wavelet Toolbox v1.1 (Patel et al., 2014).

We modelled the pre-processed data in a first-level analysis model (SPM first-level analysis) using an autoregressive AR(1) model to treat for serial correlations and regressing out the signal from CSF, WM, the motion parameters (translation, rotation and their squares and derivatives) and the signal from noise components (i.e. components overlapping with ventricles or brainstem) (Griffanti et al., 2014).

Functional connectivity analysis

We computed functional connectivity measures (connectivity between MRS voxels, temporal coherence within each MRS voxel) based on the following method. We computed the overlap across participant MRS voxels for OCT and PPC separately and created a group MRS mask that included grey matter voxels present in at least 50% of the participants' MRS voxels. For each participant, we extracted the average time course of the grey matter voxels within each MRS mask. We then applied a 5th order Butterworth band-pass filter, between 0.01 and 0.08 Hz, to remove effects of scanner noise and physiological signals (respiration, heart beat) (Murphy et al., 2013).

We computed the functional connectivity between the OCT and the PPC MRS voxels as the Pearson correlation between the average time course from each of the MRS masks. We then applied Fisher z-transform to the correlation coefficient and derived an OCT-PPC connectivity value per participant. To confirm the specificity of the OCT-PPC connectivity, we computed the functional connectivity between OCT and two control areas (V1, M1). We defined masks of equal size to the MRS masks based on anatomical co-

ordinates: primary visual cortex (V1, MNI coordinates [3, -85, 5]) and left M1 (MNI coordinates [-39, -22, 53]).

We assessed the temporal coherence within each MRS mask (OCT, PPC) by correlating the time course of each voxel within the mask with the MRS mask's average time course. This method was first described by Van Dijk et al (Van Dijk et al., 2010) and has been widely used in recent studies (Bachtiar et al., 2015; Campbell et al., 2016; Sherman et al., 2014; Stagg et al., 2014). We then applied Fisher z-transform to the correlation matrix and averaged the z-values across voxels, resulting in one connectivity value per participant and MRS voxel.

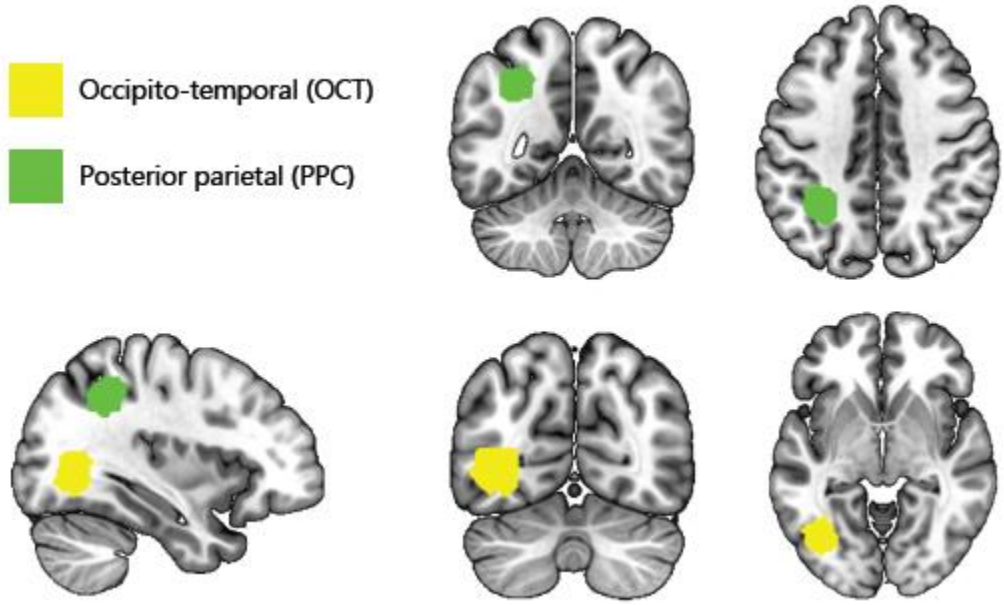


Figure 4.3: MRS voxel placement.

We positioned the MRS voxels using anatomical landmarks (Superior Temporal Gyrus and Middle Occipital Gyrus for OCT and the Intraparietal Sulcus for PPC) on the acquired T1 scan to ensure that voxel placement was consistent across participants. The average distance of individual MRS voxels from the mean coordinates (x=-38.8±4.2mm, y=-67.8±4.7mm, z=2.3±4.2mm for OCT; x=-31.5±4.5mm, y=-50.4±6.4mm, z=41.0±5.2mm for PPC) across participants was 6.8±3.2mm for OCT and 8.3±4.4mm for PPC and did not differ between the two tasks (Voxel x Task interaction: $F(1,45)=0.37$, $p=0.55$; main effect of Task: $F(1,45)=1.43$, $p=0.24$). We computed the overlap across participant MRS voxels for OCT (yellow) and PPC (green) separately. We illustrate a group MRS mask (sagittal, coronal, axial view) that includes grey matter voxels present in at least 50% of the participants' MRS voxels.

To compare changes in behavioural performance and neurotransmitter concentrations across blocks, tasks and MRS voxels we used a linear mixed effects approach. LME models are appropriate for modelling longitudinal data and can account for missing values. This way we were able to make comparisons including data from all participants. In each of the models we tested both for random (Participants) and fixed (MRS Block, Voxel and Task) effects. To relate behavioural improvement to GABA changes and rs-fMRI connectivity, we computed Pearson skipped correlations using the Robust Correlation Toolbox (Pernet et al., 2013). This method accounts for potential bivariate outliers and determines statistical significance using bootstrapped confidence intervals (CI) for 1000 permutations. To directly compare the relationship of GABA change and rs-fMRI connectivity with behavioural improvement between the two tasks we used linear regression models with interaction terms. Data distribution assumptions of normality and heteroscedasticity of variance were verified using Shapiro-Wilk and Levene's tests respectively.

Table 4.1: Datasets collected and datasets used after removing poor quality data for each modality.

	Signal-in-Noise	Feature differences
Participants	22	25
MRS data for OCT	17	19
MRS data for PPC	18	22
rs-fMRI	21	25

3. Results

Training improves behavioural performance

We tested two groups of participants on a) a Signal-in-Noise (SN) task that involves extracting shapes (radial vs. concentric Glass patterns) masked by noise or b) a Feature Differences (FD) task that involves judging fine differences induced by morphing between two stimulus classes. Participants were asked to judge the identity of the stimulus presented per trial (i.e. radial vs. concentric). Our results showed that participants improved in their judgments within a single training session during scanning (Figure 4.4), consistent with previous reports showing fast behavioural improvement early in the training (for a review see Sagi, 2011). A linear mixed effects (LME) model with Task and MRS Block (6 blocks, 200 trials per block) as fixed effects showed significantly improved performance during training for both tasks (main effect of Block: $F(1,249)= 9.35$, $p=0.002$). No significant interaction between Task x block ($F(1,249)=0.10$, $p=0.75$) was observed, suggesting similar improvement for both tasks (Figure 4.4).

To quantify behavioural improvement due to training in individual participants (i.e. when participants achieved highest accuracy), we compared performance at the beginning of training (i.e. first training block) to the best performance achieved by each participant during training (Figure 4.4). We chose this measure to capture individual variability across participants that may be more pronounced in our data, as participants were trained only for a single training session in contrast to our previous studies that have shown that participant performance saturates after multiple training sessions on similar perceptual tasks (Kourtzi et al., 2005; Li et al., 2012; Mayhew et al., 2010). A repeated measures 2-way ANOVA (Task x block) showed significantly improved performance during training for both tasks (main effect of block: $F(1,45)=59.88$, $p<0.0001$) but no significant interaction between Task x block ($F(1,45)=1.07$, $p=0.31$).

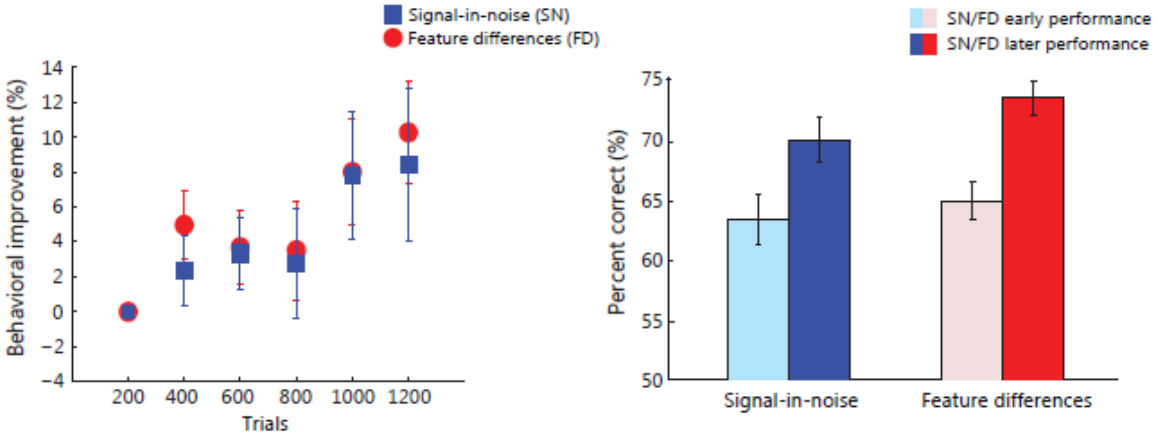


Figure 4.4: Behavioural improvement during training.

We calculated behavioural improvement during training as the difference in mean performance (i.e. mean accuracy) during each block (200 trials) from the first training block (200 trials), divided by performance in the first training block. Further, we compared individual participant accuracy early in training (first 200 trials) to the best accuracy achieved per participant during training (200 trials). Note that most participants (85 %) showed best performance in the last two MRS blocks. Error bars indicate standard error of the mean across participants.

Learning-dependent changes in occipito-temporal and posterior parietal GABA

To test whether GABAergic inhibition in the visual or posterior parietal cortex changes with training, we measured GABA before (baseline block) and during training. We tested two MRS voxels – one centred on the occipito-temporal cortex (OCT voxel) and the other on the posterior parietal cortex (PPC voxel) (Figure 4.3)– following previous studies showing that these areas are involved in learning using the same tasks and stimuli as in our study (Li et al., 2009; Mayhew et al., 2010). Each block comprised one MRS acquisition per voxel and the order of the voxels within each block was counterbalanced across participants.

Figure 4.5a shows that OCT GABA changed in the two tasks during training in opposite directions. In contrast, PPC GABA changed in the same direction (i.e. increased during training) for both tasks. These effects were supported by an LME analysis (Task, Voxel and MRS Blocks as fixed effects) that modelled GABA before (baseline block) and during (three training blocks) training in both OCT and PPC. This analysis showed that learning-dependent changes in GABA levels differed between tasks and regions (Task x Voxel x Block: $F(1,264)=6.24$, $p=0.01$). In particular, GABA levels in the occipito-temporal cortex (OCT GABA) was higher in the FD than the SN task during training (LME model for OCT GABA with Task and MRS Block as fixed effects; Task x Block: $F(1,119)=10.77$, $p=0.001$) (Figure 4.5a). In contrast, GABA in the posterior parietal cortex (PPC GABA) increased with training (LME model for PPC GABA with Task and MRS Block as fixed effects; main effect of Block: $F(1,145)=6.44$, $p=0.01$) but did not differ significantly between tasks (Task x Block: $F(1,145)=0.18$, $p=0.68$) (Figure 4.5a).

Further, for the FD task GABA significantly increased in OCT and PPC (LME model for FD task GABA with Voxel and MRS Block as fixed effects; main effect of Block: $F(1,143)=5.26$, $p=0.02$). In contrast, for the SN task GABA changes during training differed in the two regions (LME model for SN task with Voxel and MRS Block as fixed effects; Task x Block: $F(1,121)=13.06$, $p=0.0004$). That is, we found a significant decrease for OCT GABA (LME model for SN task with MRS Block as fixed effect; main effect of Block: $F(1,58)=16.65$, $p=0.0001$), but a non-significant increasing trend for PPC GABA (LME model for SN task with MRS Block as fixed effect; main effect of Block: $F(1,63)=3.26$, $p=0.08$).

We conducted the following control analyses that corroborated our results. First, we demonstrated that the learning-dependent changes we observed in GABA levels could not be simply due to the order with which the MRS voxels were acquired during training. For OCT GABA, the Task x Block interaction

remained significant (Task x Block: F(1,115)=13.51, p=0.0004) when we included the order of voxel acquisition in the LME model . Further, there was no significant effect of MRS acquisition order (LME model for OCT GABA with Acquisition Order, Task and MRS Block as fixed effects; main effect of Order: F(1,115)=0.08, p=0.78; Order x Task x Block: F(1,115)=1.58, p=0.21).

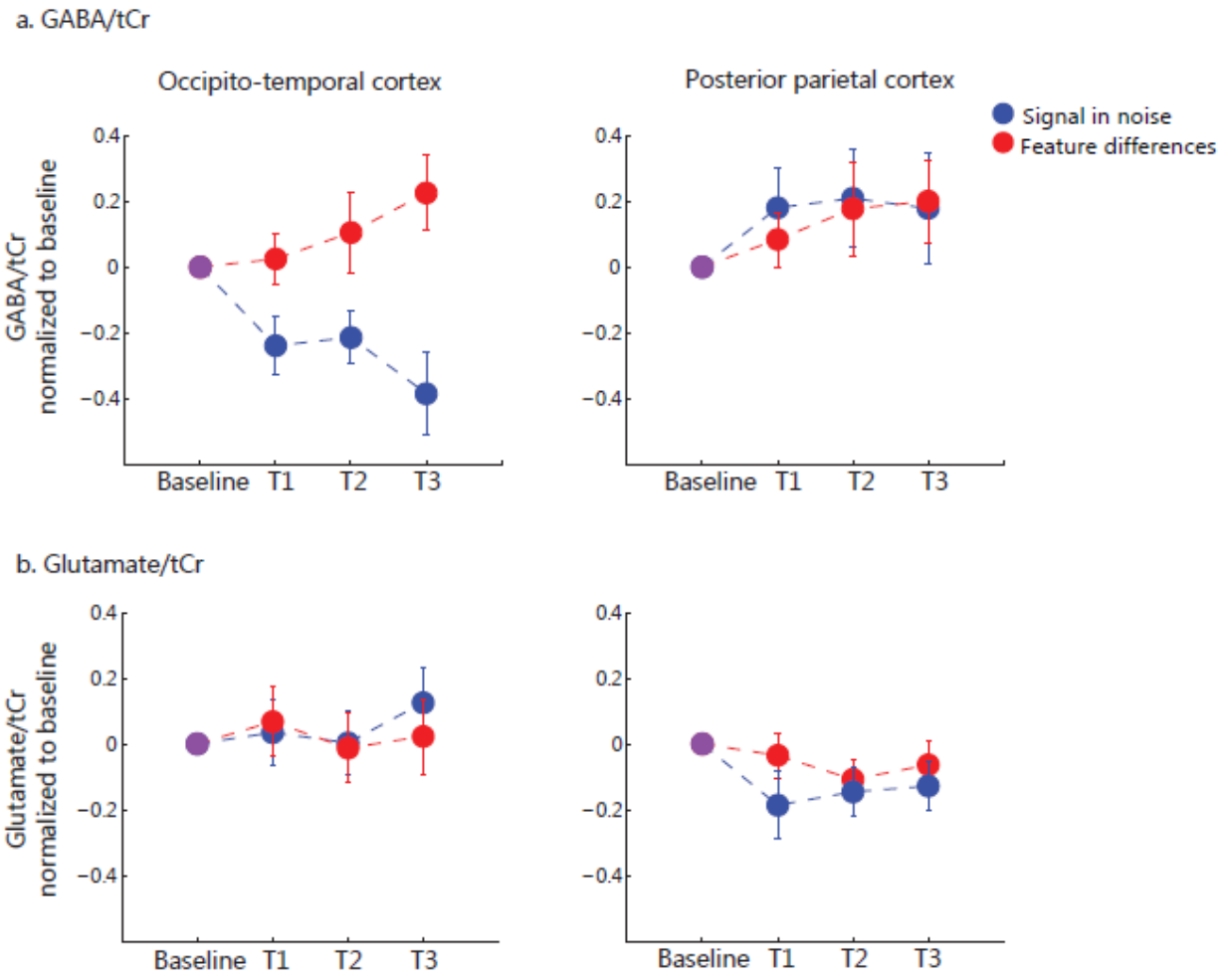


Figure 4.5: OCT and PPC GABA timecourse during training on Signal-in-Noise vs Feature differences

a. MRS-measured GABA over time is shown from two voxels (occipito-temporal, posterior parietal cortex) per task (Signal in noise, Feature differences). For each MRS-voxel, we normalised GABA/tCr per training block (T1, T2, T3) to GABA/tCr recorded during the baseline block; that is, we computed GABA/tCr change subtracting GABA/tCr measurements in each of the three training blocks from the baseline block. b. MRS-measured Glutamate over time is shown from two voxels (occipito-temporal, posterior parietal cortex) per task (Signal-in-noise, Feature differences). For each MRS-voxel, we normalised Glutamate/tCr per training block (T1, T2, T3) to Glutamate /tCr recorded during the baseline block; that is, we computed Glutamate/tCr change subtracting Glutamate/tCr measurements in each of the three training blocks from the baseline block.

Similarly, for PPC GABA, the main effect of block remained significant ($F(1,141)=7.72$, $p=0.01$) and there was no significant effect of MRS acquisition order (LME model for OCT GABA with Acquisition Order, Task and MRS Block as fixed effects; main effect of Order: $F(1,141)=1.51$, $p=0.22$; Order x Task x Block: $F(1,141)=1.50$, $p=0.22$).

Second, we tested whether the learning-dependent changes we observed in GABA, were simply due to differences in data quality across multiple MRS measurements. In particular, we tested whether the learning-dependent changes we observed in GABA, were due to differences in signal-to-noise ratio (SNR) across multiple MRS measurements potentially due to artefacts (e.g. head movement, gradient heating). For OCT GABA, there was no significant differences in SNR across blocks, nor a significant interaction between Task x block (LME model for OCT SNR with Task and MRS Block as fixed effects; main effect of Block: $F(1,118)=0.12$, $p=0.73$; Task x Block: $F(1,118)=0.45$, $p=0.50$). Similarly for PPC GABA, there was no significant effect of block, nor a significant interaction of Task x block (LME model for PPC SNR with Task and MRS Block as fixed effects; main effect of Block: $F(1,145)=0.52$, $p=0.47$; Task x Block: $F(1,145)=0.90$, $p=0.34$), suggesting that our results could not be explained simply by differences in MRS SNR over time. Further, we tested whether the learning-dependent changes we observed in GABA, were simply due to BOLD effects. BOLD effects on MRS spectra are presented as narrowing of the linewidth (Bednařík et al., 2015). To control for potential effects from BOLD on the GABA measurements, we compared spectral full width at half maximum (FWHM) across MRS blocks for the two regions. For OCT GABA, there was no significant differences in FWHM across blocks, nor a significant interaction between Task x block (LME model for OCT FWHM with Task and MRS Block as fixed effects; main effect of Block: $F(1,118)=1.28$, $p=0.26$; Task x Block: $F(1,118)=2.31$, $p=0.13$). Similarly for PPC GABA, there was no significant effect of block, nor a significant interaction of Task x block (LME model for PPC FWHM with Task and MRS Block as fixed effects; main effect of Block: $F(1,145)=2.77$, $p=0.10$; Task x Block: $F(1,145)=0.89$, $p=0.35$),

suggesting that our results could not be explained simply by differences in peak linewidth over time.

Third, a no-training control experiment on an independent group of participants (n=8) ensured that there were no significant differences ($t(7)=1.56$, $p=0.16$) between two measurements of OCT GABA over time (45 apart, that is comparable to the our main study) when participants were simply exposed to similar Glass patterns stimuli but performed a fixation task rather than the SN or FD tasks.

Fourth, we tested whether the learning-dependent changes we observed in GABA/tCr were driven by changes in tCr concentration during training. For OCT GABA, there were no significant differences in tCr across blocks, nor a significant interaction between Task x block (LME model for OCT tCr with Task and MRS Block as fixed effects; main effect of Block: $F(1,119)=0.11$, $p=0.74$; Task x Block: $F(1,119)=0.02$, $p=0.90$). Similarly for PPC GABA, there was no significant effect of block, nor a significant interaction of Task x block (LME model for PPC tCr with Task and MRS Block as fixed effects; main effect of Block: $F(1,137)=0.59$, $p=0.45$; Task x Block: $F(1,137)=0.42$, $p=0.52$), suggesting that our results could not be explained simply by changes in tCr concentration over time.

Fifth, our results remained significant when we referenced GABA to water rather than tCr concentration (Figure 4.6), suggesting that our results replicate across referencing methods. That is, we observed a significant Task x block interaction for OCT (LME model for OCT GABA with Task and MRS Block as fixed effects; Task x Block: $F(1,119)=10.68$, $p=0.001$) and a significant main effect of block for PPC (LME model for PPC GABA with Task and MRS Block as fixed effects main effect of Block: $F(1,137)=7.08$, $p=0.01$).

Sixth, to ensure our results were not simply driven by GABA measurements at baseline, we tested a linear mixed effects model on the training blocks only (i.e. excluding the baseline block; LME model for OCT GABA with Task and training MRS Block as fixed effects). This analysis showed a significant

interaction between Task and MRS blocks (Task x Block: F(1,83)=4.97, p=0.03) and a significant main effect of MRS block (F(1,83)=4.06, p=0.05). Further, post-hoc pairwise comparisons for OCT GABA showed a significant difference between the 1st and 3rd training block for the SN task (p=0.047) and between the 2nd and 3rd training block for the FD task (p=0.03). These analyses suggest that the learning-dependent GABA changes we observed were due to training rather than simply differences in GABA between the training blocks and the baseline.

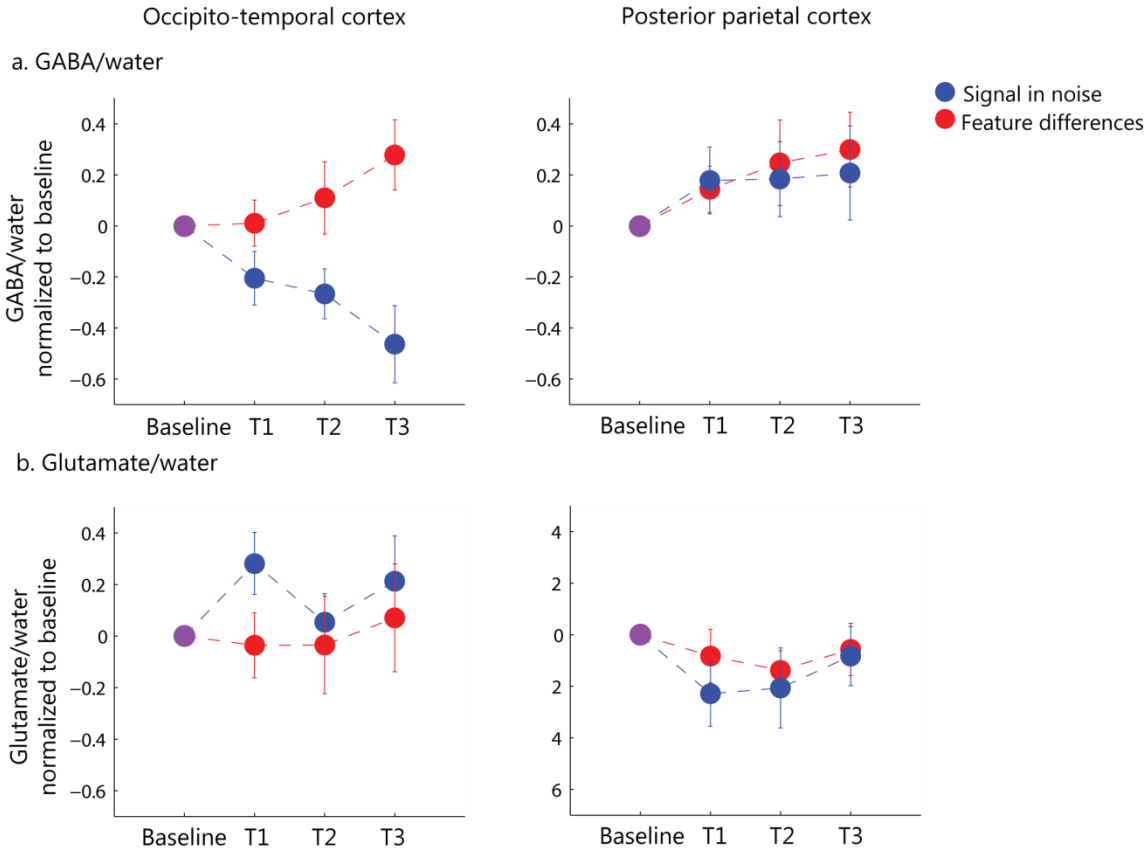


Figure 4.6: Measurements of GABA/Water and Glutamate/Water during training

a. For each MRS-voxel, we normalised GABA/Water per training block (T1, T2, T3) to GABA/Water recorded during the baseline block; that is, we computed GABA/Water change subtracting GABA/Water measurements in each of the three training blocks from the baseline block. b. For each MRS-voxel, we normalised Glutamate/Water per training block (T1, T2, T3) to Glutamate/Water recorded during the baseline block; that is, we computed Glutamate/Water change subtracting Glutamate/Water measurements in each of the three training blocks from the baseline block.

Finally, to investigate the neurochemical specificity of our results, we tested for changes in Glutamate, the other major cortical neurotransmitter, during training (Figure 4.5b). We found no significant differences in Glutamate changes between tasks (LME model for OCT Glutamate with Task and MRS Block as fixed effects Task x Block: $F(1,119)=0.53$, $p=0.47$; for PPC Glutamate: main effect of Block: $F(1,137)=2.65$, $p=0.11$), suggesting that our results were specific to GABA and do not generalize to Glutamate.

Learning-dependent changes in GABA relate to behavioural improvement

In chapter III, I reported that changes in occipito-temporal GABA levels after training on visual learning tasks relate differentially to behavioural improvement in the Signal-in-Noise vs Feature differences tasks. I showed that decreased vs increased occipito-temporal GABA after training relates to increased sensitivity to noise vs increased discriminability of fine features, respectively. Here, we test the link between behavioural improvement and learning-dependent changes in GABA for both OCT and PPC. For each region (i.e. MRS voxel) we calculated GABA change by subtracting GABA concentration in the best performance block from the baseline block for each participant and related GABA change to behavioural improvement.

Correlating change in OCT GABA to behavioural improvement showed differences between tasks. Specifically, we observed a significant negative correlation of OCT GABA change with behavioural improvement ($r=-0.43$, $CI=[-0.75, -0.02]$) for the SN task, while a significant positive correlation ($r=0.55$, $CI=[0.10, 0.78]$) for the FD task (Figure 4.7). A linear regression analysis confirmed this dissociation (OCT GABA change x Task Interaction: $F(1,29)=9.03$, $p=0.005$), suggesting that lower vs. higher occipito-temporal GABA after training relates to improved performance when learning to detect targets vs. discriminate feature differences, respectively. This dissociable result between tasks cannot be simply explained by differences in task difficulty, as

participants showed similar behavioural improvement across tasks. Further, to account for variability in tissue composition across participants and MRS voxels, we calculated the percentage of grey matter (GM) in each of the MRS voxels. The interaction between OCT GABA change and Task in the linear regression model remained significant when we accounted for the percentage of GM voxels in the MRS Voxel ($F(1,29)=5.33$, $p=0.03$) suggesting that our results could not be due to variability in tissue composition across participants. Finally, the same interaction remained significant ($F(1,29)=7.41$, $p=0.01$), when we used percentage GABA change (GABA change / baseline GABA), suggesting that our results could not be due to differences in baseline GABA.

In contrast to these correlations of OCT GABA change with behavioural improvement, we did not observe any significant correlations between PPC GABA change and behavioural improvement for either task (SN: $r=-0.23$, CI=[-0.61, 0.19]; FD: $r=0.05$, CI=[-0.37, 0.43]) nor a significant PPC GABA change x Task interaction ($F(1,34)=0.57$, $p=0.45$). Following previous work on the role of the posterior parietal cortex early rather than later in training (Chang et al., 2014), we next tested the link between PPC GABA and performance early in training for the two tasks. A linear regression model with baseline PPC GABA and Task as predictors of behavioural performance (first training block) showed a significant effect of PPC GABA ($F(1,37)=4.69$, $p=0.04$), but no interaction between PPC GABA and Task ($F(1,37)=0.03$, $p=0.85$). This result was confirmed by a significant positive correlation between baseline PPC GABA and performance in the first training block ($r=0.34$, CI=[0.06, 0.59]), suggesting GABAergic inhibition in the parietal cortex before training relates to suppression of task-irrelevant information early in the training for both tasks. We did not find a significant effect of OCT GABA ($F(1,33)=2.14$, $p=0.15$) nor an interaction between OCT GABA and Task ($F(1,33)=2.33$, $p=0.14$), suggesting that this result linking GABA to performance early in training was specific to parietal cortex.

Taken together, these results suggest distinct suppression mechanisms for visual learning in occipito-temporal vs. posterior parietal cortex. In particular, PPC GABA increased with training for both tasks, suggesting suppressive processing of irrelevant information (i.e. background clutter for the SN task; task-irrelevant features for the FD task). Interestingly, higher PPC GABA related to better performance early in training, suggesting that suppressive processing in the posterior parietal cortex contributes to early rather than later stages of learning. In contrast, GABAergic inhibition in occipito-temporal cortex differed between tasks: decreased vs. increased OCT GABA for the SN vs. FD task respectively related to enhanced behavioural improvement. These results suggest distinct suppressive mechanisms in visual cortex that may: a) increase excitation of large neuronal populations and enhance target detectability from clutter through decreased GABAergic inhibition (SN task), b) facilitate retuning of feature templates and perceptual discriminability for fine discriminations through increased GABAergic inhibition (FD task).

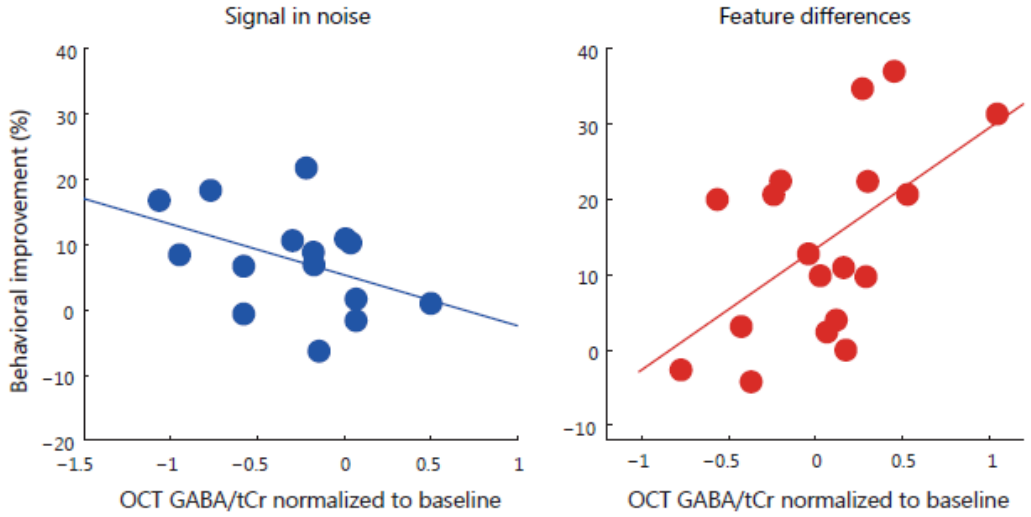


Figure 4.7: Correlating OCT GABA change with behavioural improvement.

Skipped Pearson's correlations showing a significant negative correlation of GABA/tCr normalised to baseline in occipito-temporal cortex with behavioural improvement for the Signal-in-noise task (n=16, r=-0.43, CI=[-0.75, -0.002]), while a significant positive correlation for the Feature Differences task (n=19, r=0.55, CI=[0.10, 0.78]). The plots indicate that for a small number of participants the data deviated from the overall pattern of the correlation; e.g. for some participants in the SD task, GABA/tCr values were higher rather than lower compared to baseline. Our treatment of the data (i.e. behavioural improvement is expressed as percent over early performance and control analysis where GABA data is expressed as percent over baseline) accounts for potential differences across participants in performance early in training or baseline GABA before training. It is possible that this individual variability was due to the single training session employed in our study during which participant performance did not saturate (i.e. participant best reached 72% mean performance across participants).

Functional connectivity at rest relates to learning-dependent changes in behaviour and GABA

Previous studies have shown that functional connectivity in motor (Sampaio-Baptista et al., 2015) and visual (Baldassarre et al., 2012) networks relates to behavioural improvement and learning-dependent plasticity. Further, functional connectivity as measured by resting-state fMRI has been shown to relate to MRS-assessed GABA (Bachtiar et al., 2015; Kapogiannis et al., 2013; Stagg et al., 2014), suggesting that GABAergic inhibition relates to local neural dynamics. Here we test whether functional connectivity between the occipito-temporal and posterior parietal cortex relates to behavioural improvement and GABA changes during training to detect targets in clutter vs. discriminate fine features.

First, we tested whether functional connectivity between these regions relates to behavioural improvement in each of the two learning tasks. We extracted the rs-fMRI time course from the grey matter voxels within the occipito-temporal MRS voxel and the posterior parietal MRS voxel. We measured functional connectivity by correlating the rs-fMRI time courses at rest between these two regions (OCT-PPC connectivity). We observed a significant positive correlation between OCT-PPC connectivity and behavioural improvement for the FD task ($r=0.37$, CI=[0.03, 0.69]), while a significant negative correlation for the SN task ($r=-0.72$, CI=[-0.90, -0.29]) (Figure 4.8). This dissociation in the relationship of OCT-PPC connectivity and behavioural improvement between tasks was confirmed by a linear regression showing a significant interaction between OCT-PPC connectivity and Task ($F(1,39)=10.72$, $p=0.002$), suggesting that higher connectivity between parietal and visual cortex facilitates learning of fine feature differences. To test whether this link between functional connectivity and behavioural improvement is specific to interactions between parietal and visual areas, we extracted rs-fMRI for two additional control regions: early visual cortex and motor cortex. We did not observe any significant results for correlations of behavioural improvement and rs-fMRI

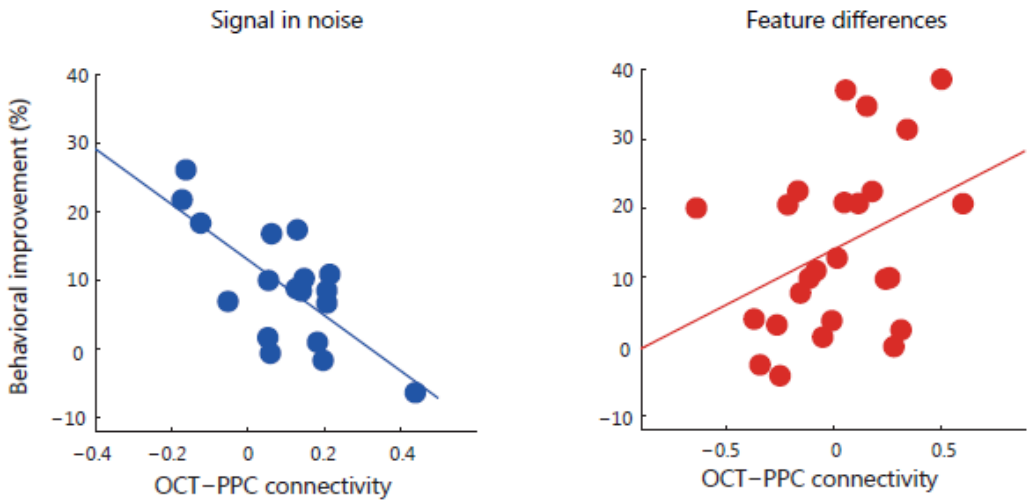


Figure 4.8: Correlating OCT-PCC functional connectivity with behavioural improvement

Skipped Pearson's correlations showing a significant negative correlation of OCT-PCC connectivity with behavioural improvement for the Signal-in-noise task (n=21, r=-0.72, CI=[-0.90, -0.29]), while a significant positive correlation for the Feature Differences task (n=25, r=0.37, CI=[0.03, 0.69]).

connectivity between the occipito-temporal cortex and a) early visual cortex (SN: r=0.37, CI=[-0.17, 0.70]; FD: r=-0.22, CI=[-0.56, 0.15]) or b) motor cortex (SN: r=-0.26, CI=[-0.61, 0.1 2]; FD: r=-0.06, CI=[-0.39, 0.42]).

Second, we tested whether OCT-PPC functional connectivity relates to changes in visual cortex GABA during training. Our results showed a significant positive correlation between functional connectivity and OCT GABA change for the FD task (r=0.55, CI=[0.13, 0.87]), but not the SN task (r=0.27, CI=[-0.08, 0.60]) (Figure 4.9a). These correlations were specific to GABA changes in occipito-temporal cortex. That is, there were no significant correlations between OCT-PPC connectivity and PPC GABA change for the FD task (r=0.29, CI=[-0.37, 0.69]) nor the SN task (r=0.20, CI=[-0.31, 0.60]). A multivariate linear regression showed that OCT-PPC connectivity had a significant effect on GABA change in OCT (F(1,14)=20.74, p=0.0005), but not PPC (F(1,14)=0.46, p=0.51) for the FD task. The correlation between functional connectivity and OCT GABA change for the FD task remained significant (r=0.49, CI=[0.10, 0.82]), when we tested for percentage GABA change (GABA change / baseline GABA), suggesting that our results could not be due to differences in baseline

GABA. These results demonstrate that higher parietal-visual cortex connectivity impacts on GABAergic inhibition in visual cortex, suggesting top-down influences to suppressive processing in visual cortex for learning fine feature differences.

Interestingly, for the Signal-in-Noise task we observed a significantly negative correlation between connectivity within the occipito-temporal cortex (i.e. connectivity measured as rs-fMRI correlations across voxels within the OCT MRS voxel) and OCT GABA change (r=-0.55, CI=[-0.86, -0.01]) (Figure 4.9b). That is, higher connectivity within visual cortex related to decreased OCT GABA with training, suggesting that learning to detect targets in clutter is supported by local connectivity and decreased suppression within visual cortex. Correlations of functional connectivity and GABA change in the occipito-temporal cortex were not significant for the FD task (r=0.12, CI=[-0.29, 0.51]) and were significantly different from correlations for the SN task (Z=2.33, p=0.02). The correlation between connectivity within the occipito-temporal cortex and OCT GABA change for the SN task remained significant (r=-0.55, CI=[-0.88, -0.003]), when we used percentage GABA change (GABA change / baseline GABA), suggesting that our results could not be due to differences in baseline GABA. Correlating rs-fMRI connectivity within the occipito-temporal cortex with PPC GABA change did not show any significant results (r=0.18, CI=[-0.39, 0.60]), suggesting that local connectivity relates specifically to GABA changes in visual cortex.

Taken together, our results suggest that learning fine discriminations involves interactions between suppressive processes that may facilitate suppression of task-irrelevant signals in posterior parietal cortex while retuning of task-relevant features in visual cortex. In contrast, learning to detect targets from clutter involves local interactions and disinhibition of visual cortex that facilitates target detection. To further test this proposal, we performed moderation analyses (Hayes, 2012) that allowed us to test whether the influence that an independent variable (i.e. GABA change) has on the outcome (i.e. behavioural improvement)

is moderated by a moderator variables (i.e. Connectivity). Our results showed that OCT-PPC connectivity moderates the relationship between OCT GABA and behaviour for the FD task ($F(1,15)=6.19$, $p=0.03$) but not the SN task ($F(1,12)=0.74$, $p=0.41$). In contrast, local connectivity within OCT moderates the relationship between GABA change and behaviour for the SN task ($F(1,12)=7.65$, $p=0.02$) but not the FD task ($F(1,15)=2.80$, $p=0.11$). These moderation analyses suggest that the relationship between learning-dependent changes in GABA and behaviour is moderated by functional connectivity; that is, interactions between visual and parietal circuits for the FD task, while local interactions within visual cortex for the SN task.

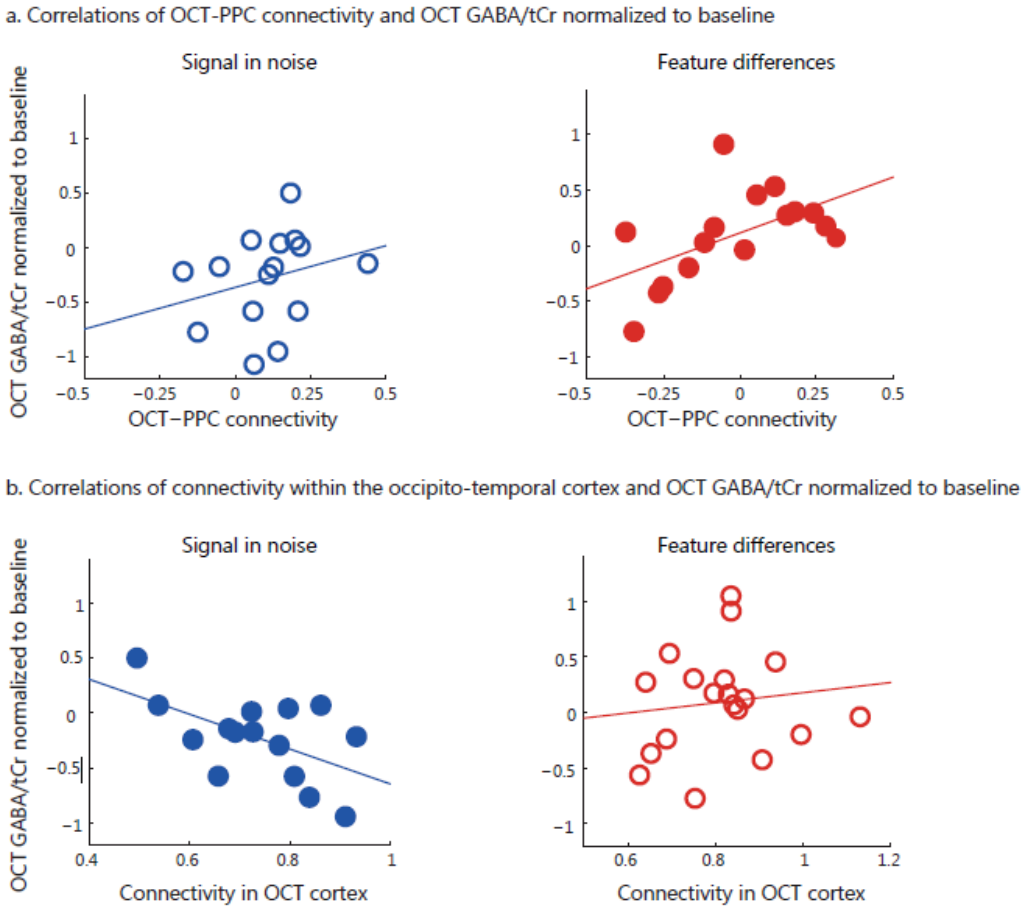


Figure 4.9: Correlating functional connectivity with OCT GABA/tCr normalised to baseline

a. Skipped Pearson's correlations showing a significant positive correlation of OCT-PCC connectivity with OCT GABA/tCr normalised to baseline for the Feature Differences task (n=19, r=0.55, CI=[0.13, 0.87]), but not the Signal-in-noise task (n=16, r=0.27, CI=[-0.08, 0.60]). b. Skipped Pearson's correlations showing a significant negative correlation of functional connectivity within the occipito-temporal cortex with OCT GABA/tCr normalised to baseline for the Signal-in-noise task (n=16, r=-0.55, CI=[-0.86, -0.01]), but not the Feature Differences task (n=19, r=0.12, CI=[-0.29, 0.51]). Significant correlations are indicated by closed symbols; non-significant correlations by open symbols.

4. Discussion

In this study, we investigated the time-course of inhibitory processes involved in visual learning. We trained two groups of participants to detect shapes in clutter and to discriminate between highly similar targets. We used GABA-MRS before and during training to measure the concentration of the inhibitory neurotransmitter GABA in the occipito-temporal (OCT) and parietal cortex (PPC). We used resting state fMRI to measure connectivity between OCT and PPC and test the relationship between OCT-PPC connectivity and resting GABA levels. We investigated the link between baseline GABA levels, GABA changes, resting connectivity and behavioural improvement during training. Our findings advance our understanding of the inhibitory mechanisms for visual plasticity in the following main respects.

First, we demonstrate dissociable GABAergic inhibition mechanisms for learning in a posterior cortical network (i.e. occipito-temporal and posterior parietal cortex) known to be involved in perceptual decisions. In particular, increased GABAergic inhibition in the posterior parietal cortex with training suggests suppressive processing of task-irrelevant information. In contrast, changes in occipito-temporal GABA with training relate to enhanced target detection and discriminability, suggesting learning-dependent changes in the processing of behaviourally-relevant features. Second, we provide evidence that interactions within this network, as measured by functional brain connectivity at rest, gate suppressive processing of sensory signals. Learning to detect targets from clutter involves local interactions and disinhibition in visual cortex, while learning feature differences involves suppressive interactions between decision-related (parietal) and visual areas.

In chapter III, I showed that OCT plays a key role in learning to detect targets in clutter and discriminate between highly similar targets. Specifically, I showed decreased OCT GABA after training relates to improved sensitivity to noise in the Signal-in-Noise task, while increased OCT GABA after training relates to

improved discriminability in the Feature differences task. Here we replicate this finding and show that maximum behavioural improvement relates to decreased OCT GABA for the Signal-in-Noise task and increased OCT GABA for the Feature differences task. As discussed in chapter III, decreased GABA after training in the Signal-in-Noise task may suggest plastic gain changes in large neuronal populations that are engaged to improve detectability of noisy patterns. Increased OCT GABA after training in the Feature differences task may suggest tuned pattern representations in the occipito-temporal cortex.

Our findings provide evidence for distinct suppression mechanisms in posterior parietal vs. occipito-temporal cortex for learning. In particular, we demonstrate a common mechanism across tasks in the posterior parietal cortex. That is, GABA increases during training may facilitate suppression of task-irrelevant information (i.e. background clutter when learning to detect targets, task-irrelevant features when learning fine differences). It is unlikely that changes in GABA levels with training reflect reduced attention to the task, as the task remained sufficiently demanding (i.e. mean best performance was 72%) during the single training session employed in our study. Our findings are consistent with the known role of parietal cortex in perceptual decision-making and attentional selection. In particular, the posterior parietal cortex has been implicated in detecting low-saliency targets by suppressing distractors (Mevorach et al., 2009a), providing a salient representation of the environment and top-down attentional feedback (Gottlieb, 2007), accumulating sensory information (Mazurek et al., 2003) and directing attention to task-relevant features (Freedman and Ibos, 2018; Gottlieb, 2007). Interestingly, we show that parietal cortex GABA before training relates to performance early in training across tasks. This is in contrast with visual cortex changes in GABA that relate to improvement in behavioural performance during training. This finding suggests that suppression of task-irrelevant information in the posterior parietal cortex may precede suppression in the visual cortex related to the processing of task relevant features. This is consistent with previous studies showing that

TMS in the parietal cortex disrupts performance in visual discrimination tasks early in the training compared to TMS in the visual cortex that disrupts performance after training (Chang et al., 2014).

Finally, investigating functional connectivity within this posterior cortical network revealed suppressive interactions that relate to our ability to improve perceptual judgments through training. Here, we demonstrate that higher connectivity between parietal and visual cortex at rest relates to increased GABA levels in visual cortex during training and behavioural improvement in fine feature discrimination. This finding suggests top-down influences from the parietal cortex on suppressive processing in the visual cortex for retuning feature templates. Previous theoretical investigations have proposed that sensory selectivity is enhanced by suppressive feedback mechanisms that change recurrent processing in visual cortex (Moldakarimov et al., 2014) and relate to attention-guided selection of behaviourally relevant information (Roelfsema and Ooyen, 2005). Our findings suggest that suppression of task irrelevant information in the parietal cortex enhances tuning of task-relevant features in visual cortex through top-down feedback, consistent with previous proposals that perceptual learning re-weights sensory processing (Ahissar and Hochstein, 2004b; Law and Gold, 2008; Raiguel et al., 2006; Yang and Maunsell, 2004b).

In contrast, we show that higher connectivity within the visual cortex (rather than connectivity between visual and parietal cortex) relates to decreased GABA in visual cortex and behavioural improvement in detecting targets from clutter. Lateral interactions within the early visual cortex are shown to support contextual processing and shape integration (Gilbert and Li, 2012; Stettler et al., 2002). Further, recurrent processing has been implicated in robust representations of ambiguous stimuli in higher visual cortex (O'Reilly et al., 2013), suggesting that local connectivity in occipito-temporal cortex facilitates visual processing under uncertainty.

In sum, our findings provide evidence for distinct suppression mechanisms that support our ability to optimise perceptual decisions through training. We

propose that local suppressive processing within visual cortex enhances target detection, while top-down suppression from decision-related areas (i.e. posterior parietal cortex) enhances re-tuning of task-relevant features for fine discrimination in the visual cortex. Decision-making models have proposed suppressive mechanisms that resolve competition between neuronal ensembles that represent behavioural choices (Bogacz et al., 2006). Our findings provide novel insights in understanding how these suppressive mechanisms are implemented in decision-related and sensory areas in the human brain and optimise our ability for perceptual decisions through training.

Chapter V

Polarity-specific modulation of cortical excitability facilitates visual learning

1. Introduction

So far, I have presented evidence for the involvement of GABA in visual learning. In chapter III, I used MRS to measure GABA before and after training on two dissociable visual learning tasks: detecting visual patterns from clutter (Signal-in-Noise task) and discriminating between highly similar patterns (Feature Differences). I found that GABA changes in the occipito-temporal cortex, an area known to be involved in shape processing, relate to behavioural improvement in a differential manner: decreased occipito-temporal GABA after training correlates with faster learning rate for the Signal-in-Noise task, while increased occipito-temporal GABA after training correlates with improved discriminability between radial and concentric patterns for the Feature differences task. In chapter IV, I investigated the time course of GABA changes during training on the two tasks, from the occipito-temporal (OCT) and the posterior parietal cortex (PPC), an area known to be involved in enhancing target salience by suppressing irrelevant information (Mevorach et al., 2010, 2009a, 2009b). This study replicated the results for occipito-temporal GABA, showing decreased occipito-temporal GABA during training on the Signal-in-Noise task, while increased occipito-temporal GABA during training on the Feature differences task.

The results so far provide evidence for dissociable inhibitory processes in the occipito-temporal cortex for detecting targets in clutter vs discriminating highly similar stimuli. To extend beyond correlative evidence, I sought to perturb cortical excitability using transcranial direct current stimulation (tDCs) that has

been previously shown to alter overall responsivity of the visual cortex (i.e. modulate visual evoked potentials) (Antal et al., 2004a). My findings on the relationship of GABA change and behavioural improvement lead to opposite predictions for the effect of tDCs on the two learning tasks. In particular, anodal tDCs is known to be excitatory (Nitsche and Paulus, 2000) and has been shown to result in local GABA reduction in visual (Barron et al., 2016) and motor cortex (Stagg et al., 2009a). Further, anodal tDCs has been shown to facilitate learning in motor (O'Shea et al., 2017; Stagg et al., 2011c) and perceptual tasks (Fertonani et al., 2011; Pirulli et al., 2013; Sczesny-Kaiser et al., 2016). My results for the Signal-in-Noise task showed that GABA change correlated negatively with behavioural improvement, suggesting that decreased GABA relates to higher behavioural improvement. Therefore, I hypothesised that excitatory anodal tDCs would enhance performance during training on this task. If occipito-temporal GABA decrease is not simply sufficient, but necessary for improving on this task, cathodal (inhibitory) tDCs should impede behavioural improvement, while sham tDCs should provide no benefit. In contrast, cathodal stimulation is thought to be inhibitory; that is, it has been shown to reduce cortical excitability (Nitsche and Paulus, 2000) by decreasing glutamatergic transmission (Stagg et al., 2009a). Further, cathodal tDCs on the occipital cortex has been shown to facilitate performance in perceptual judgments by suppressing incorrect sensory input (Antal et al., 2004b). My results for the Feature differences task showed that GABA change correlated positively with behavioural improvement, suggesting that increased GABA relates to higher behavioural improvement. Therefore, I hypothesised that the inhibitory cathodal –rather than the excitatory anodal– stimulation would enhance performance during training on this task. While cathodal tDCs does not increase GABA levels, its effect on glutamate and GABA results in an inhibitory balance (see chapter II). If occipito-temporal GABA increase is necessary for improving on this task, then anodal (excitatory) tDCs should impede behavioural improvement, while sham tDCs should provide no benefit.

I found enhanced improvement compared to sham for the anodal group in the Signal-in-Noise task and for the cathodal group in the Feature differences task. There was no significant performance decline for cathodal stimulation in the Signal-in-Noise or for anodal stimulation for the Feature differences task. This study concludes the thesis by establishing causal relationships between GABAergic mechanisms and visual learning that are task specific.

2. Methods

a. Participants

Eighty four participants (45 female; mean age 23.8 ± 3.4 years) took part in this single-blinded study. All participants were right-handed, had normal or corrected-to-normal vision and gave written informed consent. The study was approved by the University of Cambridge ethics committee. We randomly assigned participants into six groups of 14 participants: training on the Signal-in-Noise task during online anodal, cathodal or sham stimulation vs. training on the Feature-differences task during online anodal, cathodal or sham stimulation.

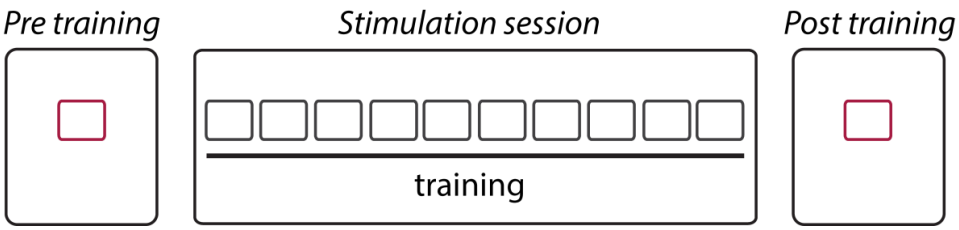


Figure 5.1: Experiment design.

Participants took part in three sessions. In the pre training session their baseline performance was measured in a 90-trial block without feedback. In the stimulation session, they were trained with error-feedback while receiving online tDCs (35 minutes). In the post training session, participants were tested in a 90-trial block without feedback.

b. Experiment Design

Participants were presented with Glass patterns, as described in chapter II. Stimuli (size=7.9^{o} x 7.9^{o}), were presented on the non-stimulated hemifield (11.6^{o} arc min from fixation). Participants were asked to judge whether the presented stimulus on each trial was radial or concentric. The study consisted of three experimental sessions (Figure 5.1). In the first session (pre-training) we measured participants' task performance in one block of 90 trials without feedback, which corresponds to their baseline performance. In the second session (stimulation session), all participants were trained with error feedback for 5 experimental blocks (200 trials per block). On each trial a 300ms stimulus presentation was followed by a fixation dot. After the participant's response, delayed (100ms) error feedback was presented for 200ms, followed by a fixation dot 500ms before the next trial onset. In the third session, we measured task performance in one block of 90 trials without feedback (Figure 5.2).

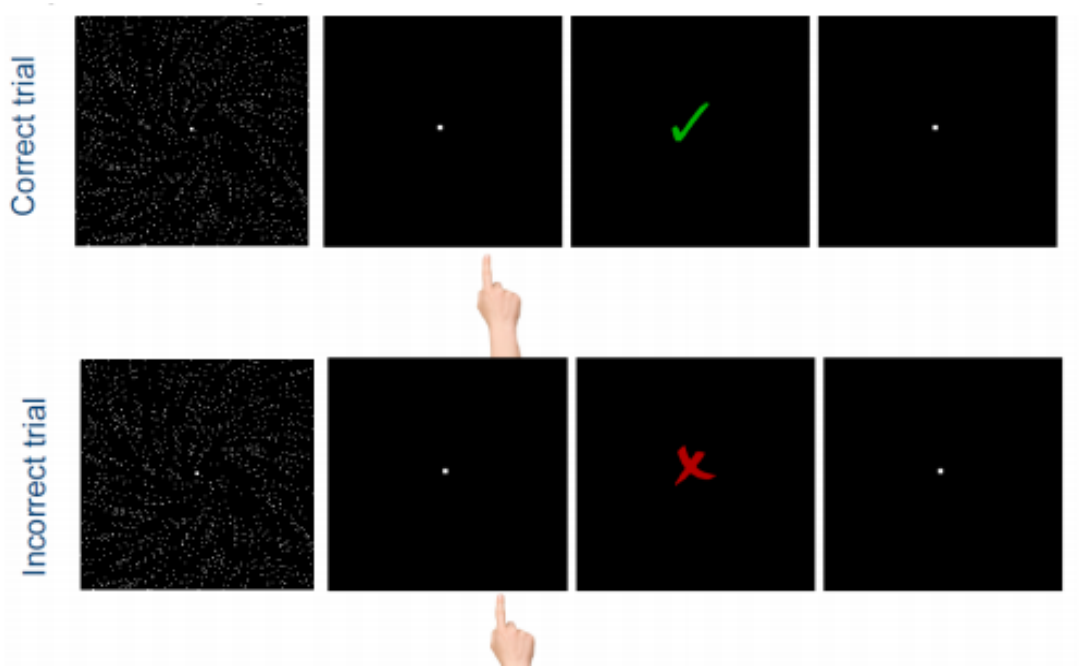


Figure 5.2: Experimental trial structure.

On each trial a 300ms stimulus presentation was followed by a fixation dot. After the participant's response, delayed (100ms) error feedback was presented for 200ms, followed by a fixation dot 500ms before the next trial onset.

c. Data acquisition

We used a multi-channel transcranial electrical stimulator (neuroConn DC-STIMULATOR MC, Ilmenau, Germany) to deliver anodal, cathodal or sham stimulation. We used a pair of rubber electrodes (3×3 cm^2 stimulating electrode, 5×5 cm^2 reference electrode), placed in square sponges that had soaked in saline. Using small stimulating electrode vs large reference electrode results in increased current density and stimulation efficiency for the stimulating contact, while decreased current density and a smaller effect on the cortex under the reference. Using different-size electrodes has been proposed as a way of increasing the focality of tDCs (Nitsche et al., 2008). In the anodal and cathodal conditions, 1mA current was ramped up over 10s, was held at 1mA for 35min and was subsequently ramped down over 10s. In the sham condition, the current ramped up (10s) and down (10s) in the beginning of the session. We used online stimulation (i.e. stimulation during training), as this protocol has been previously shown to enhance the lasting effect of training (O'Shea et al., 2017). This facilitatory effect is not present or polarity specific when stimulation precedes training, and both types of stimulation (anodal vs. cathodal) impede learning (Stagg et al., 2011c).

Based on the results of our previous studies presented in chapters III and IV, we chose the target area for the stimulation to be the (right) occipito-temporal cortex. In order to define the location of the stimulating electrode on the scalp, we applied the following procedure. We used functional anatomical scans to identify the functional area of posterior occipito-temporal cortex in the right hemisphere of a subset of participants as a volume of interest. Using neuronavigation (Brainsight 2, Montreal, Canada), we located the closest point to the centre of mass of the volume on the surface of the participant's scalp. We then mapped the points on a 10-20 EEG system diagram using triangulation to identify EEG electrode positions that we could use as landmarks for positioning (Figure 5.3a). To achieve consistent electrode placement across participants, we placed the bottom right corner of the square stimulating electrode on T6, using a

10-20 system EEG cap, maintaining the same orientation across participants, parallel to the line connecting T6 and O2 (Figure 5.3b). The reference electrode was placed on Cz. We used FreeSurfer (Dale et al., 1999) to reconstruct head models from anatomical scans and SimNIBS 2.0.1 (Thielscher et al., 2015) to simulate electric field density resulting from stimulation over the grey matter surface (Figure 5.3c). This analysis showed that the current density was largely unilaterally localized, the peak of the electric field density was observed under the anode electrode around the posterior occipito-temporal cortex.

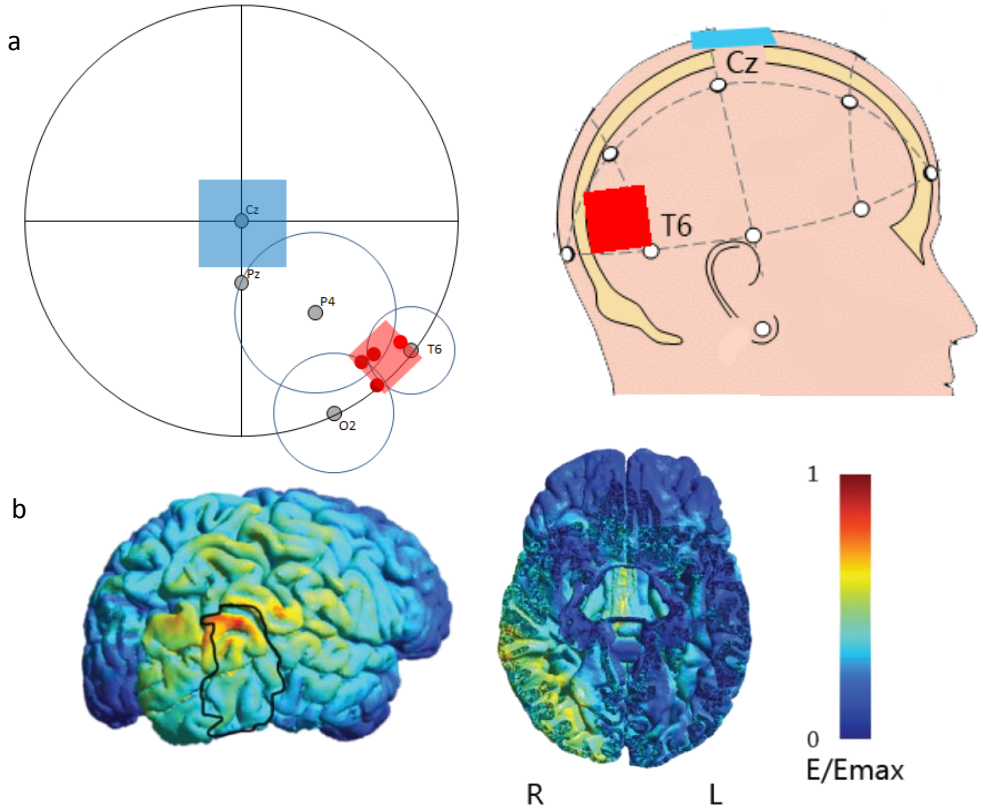


Figure 5.3: Definition of stimulation target and simulation of resulting electric field

a. We used functional anatomical scans to identify the functional area of posterior occipito-temporal cortex in the right hemisphere of a subset of participants as a volume of interest. We then mapped the centres of mass on a 10-20 EEG system diagram using triangulation. b. We place the bottom right corner of the stimulating electrode on T6, maintaining the same orientation parallel to the line connecting T6 and O2, while the reference electrode was centred on Cz. c. Electrical field density simulation (shown on the cortical surface and a representative axial slice) showed that the current density was largely unilaterally localized, the peak of the electric field density was observed under the anode electrode around the posterior occipito-temporal cortex) and the stimulation reached the occipito-temporal region where the MRS voxel was placed in Chapter III. Heatmap indicates electric field strength from 0 (blue) to maximum (red). The black outline indicates mean activation across participants (n=33) for an independent functional localizer scan (i.e. activation for intact vs. scrambled images of objects) that has been extensively used to identify regions in the posterior occipito-temporal cortex that are involved in shape processing (Kourtzi and Kanwisher, 2001). The figure illustrates substantial overlap between the tDCs electric field and regions in the posterior occipito-temporal cortex involved in shape processing.

Electric field density simulations have been established as a visualisation method that can be used when planning a tDCs study. As explained in chapter II, the effect of electric stimulation on the brain heavily depends on the orientation of the neurons to the electric field. This means that cortical areas with small differences in cortical folding will not be affected in the same way by tDCs. Therefore, ideally, electric field simulations should be applied on the individual brains of the participants who are stimulated and placement could be adjusted to result in the same electric field density maps across participants. Since this was not feasible in our study, we can utilise the simulation to confirm: a) the focality of the field in the approximate area of the occipito-temporal cortex, b) the lateralised field that extends between the two electrode contacts, c) the weak electric field density elsewhere in the brain. We can further compare the electric field density map on the grey matter surface with the MRS voxel placement used in the imaging studies in chapters III (Figure 3.3) and IV (Figure 4.3). We find good correspondence between the GABA-MRS voxels and stimulation target used in this study.

d. Data analysis

To quantify behavioural performance (discriminability between the two Glass patterns classes) during training we computed d' as described in Chapter III.

To compare behavioural performance between the different tDCs groups for the two tasks, we run a repeated-measures ANOVA, with training-block and stimulation as factors, using SPSS (IBM Corporation, Armonk, NY, USA). To directly compare the two tasks, we normalised behavioural performance (d') in the active stimulation groups (anodal, cathodal) to the sham stimulation group. For each block, we computed the average d' across participants in the sham group. We subtracted this mean d' per block from each participant's data in the anodal and cathodal groups. We then calculated the group mean d' for each block normalised to sham and conducted a repeated-measures ANOVA on the data from the active stimulation groups (anodal, cathodal) normalised to the sham group, with task, stimulation and training-block as factors. We used

Greenhouse-Geisser (for epsilon less than 0.75) and Huynh-Feldt (for epsilon greater than 0.75) corrections of significance.

3. Results

We trained participants to discriminate Glass patterns that were either embedded in background noise (Signal-in-Noise task) or were highly similar to each other (Feature-differences task) due to morphing between the two stimulus classes (concentric vs. radial patterns). We assigned participants in six different groups that received anodal, cathodal or sham stimulation during training on the Signal-in-Noise or the Feature-differences task. Our results (Figure 5.4) showed significant improvement in behavioural performance, as measured by d', for anodal compared to sham stimulation for the Signal-in-Noise (Training block x Stimulation: $F(2,52.5)= 3.99$, $p=0.02$) but not the Feature-differences task (Training block x Stimulation: $F(2.2,57.9)=0.45$, $p=0.66$). In contrast, we observed improved performance during cathodal compared to sham tDCs for the Feature-differences task (main effect of stimulation: $F(1,26)= 6.13$, $p=0.02$) but not the Signal-in-Noise task (main effect of stimulation: $F(1,26)= 0.001$, $p=0.98$). To compare behavioural improvement between tasks, we normalised performance during tDCs (anodal or cathodal) to performance during sham stimulation (Figure 5.4a). A repeated-measures ANOVA showed a significant Task, Stimulation x Training block interaction ($F(2.5, 130)=3.19$, $p=0.03$).

Together, these results demonstrate dissociable effects of tDCs stimulation on behavioural improvement between tasks, suggesting that GABA-ergic suppression alters learning and experience-dependent plasticity in the posterior occipito-temporal cortex. In particular, we demonstrate that excitatory stimulation enhances performance during training to detect targets from noise, while inhibitory stimulation enhances fine feature discriminability. These results are consistent with the opposite correlations of change in occipito-temporal GABA and behavioural improvement that we observed in Chapters III and IV across tasks. Taken together, our findings suggest that GABA-ergic processing

in visual cortex optimises noise filtering for target detection, while retuning of feature templates for fine discrimination.

We next conducted the following control analyses to ensure that our results relate to learning-dependent changes in behavioural performance rather than differences in task difficulty across stimulation groups. Comparing performance across participants before training (i.e. pre-training block with no feedback or stimulation) showed no significant effect of task ($F(1,78)=0.05$, $p=0.82$), nor a significant interaction between task and stimulation group ($F(2,78)=0.92$, $p=0.40$), suggesting that the tDCs-induced learning effects were not due to differences in difficulty across tasks (Figure 5.4). This was further supported by a significant effect of session ($F(1,78)=86.99$, $p<0.0001$) across stimulation groups suggesting that all participants (including the sham stimulation groups) were able to learn the task (Figure 5.4b). Further, the double dissociation we observed between task and stimulation site makes it unlikely that stimulation could produce a non-specific effect on general behavioural performance (e.g., through distraction caused by skin irritation). In contrast, comparing performance on consecutive days in a no-training control group (participants were tested twice but without training on the task) showed no significant effect of session ($F(1,17)= 0.78$, $p=0.39$) nor a significant interaction between task and session ($F(1,17)=0.30$, $p=0.59$), suggesting that the learning effects we observed were training-specific. Finally, to test whether behavioural improvement was maintained after training, we compared performance in the last training block (feedback, stimulation) vs. a post-training test that was conducted on the day following training (no feedback, no stimulation). A repeated-measures ANOVA showed no significant interaction between Session x Stimulation x Task ($F(2,78)=1.09$, $p=0.34$) suggesting that improved performance was maintained across all groups when participants were tested without tDCs stimulation.

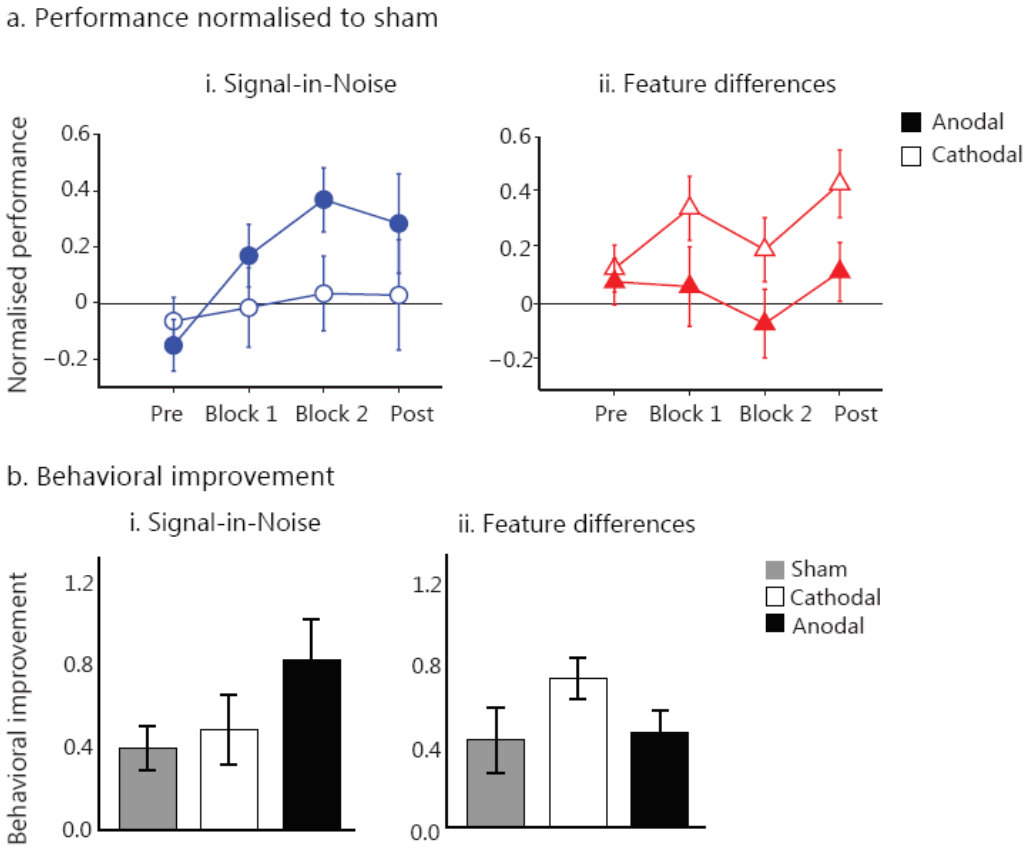


Figure 5.4: tDCs intervention facilitates visual learning.

a. Task performance (d') for the active stimulation groups (anodal, cathodal tDCS on posterior occipito-temporal cortex normalised to the sham group for the pre- and post- training blocks (no feedback, no stimulation) and the two training blocks (Block 1, Block 2; 500 trials per block). Performance (d') was significantly enhanced for anodal (but not cathodal) stimulation in the Signal-in-Noise task, while for cathodal (but not anodal) stimulation in the Feature differences task.

b. Behavioural improvement (d' post- minus pre-training) was enhanced for the anodal stimulation group in the Signal-in-Noise task and the cathodal stimulation group for the Feature differences task. Error bars indicate standard error of the mean across participants.

4. Discussion

In this study, we investigated the role of GABAergic inhibition in visual learning under a brain stimulation intervention protocol. Our previous work on training in these two tasks has shown dissociable GABAergic mechanisms for learning to detect patterns in clutter vs discriminate similar shapes (chapters III and IV). Specifically, we have shown decreased occipito-temporal GABA after training relates to improved sensitivity to noise in the Signal-in-Noise task, while increased occipito-temporal GABA after training relates to improved discriminability in the Feature differences task. Here we tested the role of GABA in these two tasks using tDCs to perturb cortical excitability in the occipito-temporal cortex. Our results demonstrate a double dissociation in learning-dependent mechanisms of visual plasticity. Excitatory anodal (rather than cathodal) stimulation enhanced learning to detect targets in clutter, consistent with the negative correlation between GABA and behaviour (i.e. decreased GABA relates to enhanced behavioural improvement). In contrast, inhibitory cathodal (rather than anodal) stimulation enhanced learning to discriminate fine features, consistent with the positive correlation between GABA and behaviour (i.e. increased GABA relates to enhanced sensitivity in fine discrimination). These findings demonstrate a direct link between GABA-ergic processing in visual cortex and enhanced visual learning.

Our findings suggest that for the Signal-in-Noise task a downregulation of inhibition, as a result of anodal stimulation on the occipito-temporal cortex, may facilitate behavioural improvement. Anodal stimulation has been previously shown to reduce MRS GABA in the motor cortex (Stagg et al., 2009a), as well as in the on the occipito-temporal cortex (Barron et al., 2016). Here we confirm the link between decreased GABAergic inhibition in the occipito-temporal cortex and improved sensitivity to noise by showing enhanced behavioural improvement when perturbing cortical excitability with anodal but not cathodal stimulation. For the Feature differences task, downregulation of excitation, as a result of cathodal stimulation on the occipito-temporal cortex, may support

neuronal tuning and increase discriminability. Cathodal stimulation has been shown to reduce cortical excitability in the motor cortex (Nitsche and Paulus, 2000), while it decreases both MRS GABA and glutamate. Since our previous work has shown a link between *increased* GABA after training and learning on the Feature differences task, we do not expect the benefit of cathodal stimulation to be as strong as the anodal vs sham for the Signal-in-Noise task (Figure 5.4). However, we still see enhanced behavioural improvement for cathodal stimulation in the Feature differences task, suggesting an increase in the concentration of on the occipito-temporal GABA may not be necessary, while an inhibitory balance between GABA and glutamate can support tuning.

If increasing vs decreasing cortical excitability facilitates learning for the Signal-in-Noise vs the Feature differences task, then we would expect a performance decline for decreased vs increased cortical excitability for the Signal-in-Noise vs the Feature differences task. In particular, we would expect cathodal stimulation to impede learning in the Signal-in-Noise task, while anodal stimulation to impair performance in the Feature differences task. Here we find that the two groups do not differ from sham stimulation. There are different reasons why this may be happening: for the Signal-in-Noise task we know cathodal stimulation decreases both MRS GABA and glutamate, however we do not know what the timescale of that change is; it is possible that the decrease in GABA resulting from cathodal stimulation is enough to support some improvement on the task early in training, while preventing a later decline in performance. It is possible participants in the cathodal group improve on the Signal-in-Noise task early on but once cortical excitability is decreased, participants stop improving thus resembling the performance of the sham group. For the Feature differences task, I showed in chapter IV that during training an increase in on the occipito-temporal GABA is coupled with an increase in PPC GABA. It is possible that cathodal stimulation of the occipito-temporal cortex may not suffice for increasing behavioural improvement to the same degree as anodal stimulation for the Signal-in-Noise task. Further, we should consider the

effect of the reference electrode placed on Cz during anodal stimulation. We know that the electric field induced by tDCs stimulation extends between the stimulating and reference electrodes. While we have chosen a large reference electrode to reduce its current density, we cannot ignore the effect it will have on cortical excitability around Cz. It is possible that during anodal stimulation in the Feature differences task, the inhibitory effect of the reference electrode extends to parietal cortex, an effect that may prevent performance decline during training.

Finally, the exact mechanism by which tDCs alters cortical excitability on the human brain is still largely unknown (see discussion in chapter II). tDCs has been suggested to selectively modulate $GABA_A$ activity (Nitsche et al., 2005; Stagg and Nitsche, 2011) and specifically synaptic rather than extra-synaptic $GABA_A$ activity (Amadi et al., 2015). However, our previous studies measuring MRS GABA changes are more sensitive to extra-synaptic GABA. It is possible that, while synaptic and extra-synaptic GABA concentrations are directly linked (see chapter II), we are not targeting the exact same GABAergic pool with MRS and tDCs.

Conclusion

Here I show for the first time polarity specific events of tDCs on two visual tasks for which dissociable GABAergic mechanisms have been suggested. I propose causal links between changes in cortical excitability and behavioural improvement on learning to detect targets in visual clutter vs discriminating highly similar targets. Our findings support targeted training interventions for visual learning.

Chapter VI

Discussion

1. Summary of thesis findings

In my thesis I investigated the role of inhibition in visual learning. I employed a learning paradigm that juxtaposes two processes: learning to detect patterns from clutter (Signal-in-Noise task) and learning to discriminate between highly similar patterns (Feature differences task). I propose that these tasks relate to differential brain mechanisms: detecting targets from noise relies on neuronal gain control, while discriminating between highly similar targets on neuronal tuning. Investigating the role of inhibition in these two kinds of visual learning, I am able to draw conclusions about the dissociable mechanisms involved.

My studies sought to answer three main questions: (a) Does GABAergic inhibition, as measured by MRS GABA, change during visual learning and, if so, are these changes relevant to behavioural improvement? (b) What is the time course of GABA changes during visual learning and does it vary between different brain areas? (c) Does GABAergic inhibition contribute causally to visual learning and, if so, can we use interventions to facilitate learning? Here I discuss the interpretation of our findings and potential future directions.

GABAergic contributions during visual learning are task specific

My findings in chapter III (that were replicated in chapter IV) showed dissociable GABAergic contributions in the occipito-temporal cortex for learning to detect patterns in clutter (Signal-in-Noise task) vs discriminate fine features (Feature differences task). This task-dependent relationship between occipito-temporal GABA change and behavioural improvement suggests that GABAergic inhibition in occipito-temporal is involved in visual learning in a task-dependent manner and may mediate changes in both neuronal gain and neuronal tuning.

Modelling work has related synaptic inhibition to reduced neuronal gain (Mitchell and Silver, 2003), while pharmacological blockade of $GABA_A$ receptors has been shown to increase gain in the rat cerebellum (Hamann et al., 2002). It is possible that learning to detect targets in clutter is implemented by decreased local suppression that facilitates recurrent processing for noise filtering and target detection (Gilbert and Li, 2012; Poort et al., 2016). Further, single-cell recordings in rats have shown that balanced inhibition, precisely following excitatory input, underlies auditory cortex tuning (Wehr and Zador, 2003). Pharmacological interventions using GABA agonists resulted in enhanced orientation selectivity in the visual cortex (Leventhal et al., 2003; Li et al., 2008), while blocking GABA-ergic suppression results in broader neural tuning (Leventhal et al., 2003; Sillito, 1979). Further, human studies have linked GABA levels in the visual cortex with orientation discrimination (Edden et al., 2009; Rokem et al., 2011). Thus, it's possible that feature template retuning is facilitated by an increase in occipito-temporal GABA during training.

In chapter III, my results showed an intriguing dissociation between tasks; that is, learning rate (but not Δd') correlated significantly with GABA change for the Signal-in-Noise task, while Δd' (but not learning rate) correlated significantly with GABA change for the Feature differences task. Recent studies characterizing the role of different populations of interneurons in visual learning

may shed light into this task-dependent GABA-ergic plasticity. In particular somatostatin-positive (SOM) interneurons have been implicated in spatial summation (Adesnik et al., 2012) and have been shown to gate plasticity during training by providing contextual information (van Versendaal and Levelt, 2016). In contrast, parvalbumin-positive (PV) interneurons have been implicated in selective inhibition (Rokem et al., 2011) that sharpens feature representations after training (Khan et al., 2018). It is therefore possible, that the dissociable correlations we observed between tasks for GABA change and behavioural improvement may reflect differential involvement of SOM vs. PV interneurons in the two tasks. Specifically, SOM interneurons involved in spatial integration may support learning to detect targets from clutter (SN task) through noise filtering. In contrast, PV interneurons involved in selective inhibition may support learning fine differences (FD task) through re-tuning of feature templates. Further, SOM vs. PV interneurons are shown to be involved at different stages during the time course of learning. In particular, SOM cells have been shown to gate learning-dependent plasticity during training (Chen et al., 2015), while PV cells form stimulus-specific ensembles with pyramidal cells after training on a visual discrimination task (Khan et al., 2018). Thus, it is possible that different behavioural measures capture the function of SOM vs. PV interneurons, consistent with the dissociation we observed between tasks for the correlations of GABA change and behavioural improvement. In particular, learning rate (i.e. the rate with which perceptual sensitivity changes during training) may capture best the function of SOM interneurons that act during learning to support noise filtering throughout the course of training. In contrast, Δd' (i.e. change in perceptual sensitivity after training) may capture best the function of PV interneurons that are shown to support tuning of stimulus-specific representations after training.

Task-specific changes in GABAergic inhibition moderate the link between changes in BOLD activation and behavioural improvement

In chapter III, I showed that behavioural improvement correlates positively with changes in occipito-temporal BOLD activations during training for both the Signal-in-Noise and the Feature differences tasks. I showed that changes in occipito-temporal GABA moderate the link between BOLD changes in the occipito-temporal cortex and behavioural improvement.

In the past 20 years, functional MRI has been a valuable tool for understanding human brain function. Its high spatial specificity and its sensitivity in revealing functional specialisation of the cortex make it a unique method for non-invasively measuring brain activation during tasks. However, fMRI BOLD cannot differentiate between excitatory and inhibitory contributions to brain activation. While fMRI BOLD is believed to increase with neural spiking, with or without net excitation, it is unclear what the effect of net inhibition is. Changes in excitation and inhibition are related to changes in metabolic activity and therefore modulation of cerebral blood flow that alters the BOLD signal. Specifically, regional inhibition can have three possible outcomes: reduced recurrent excitation (where a decrease in BOLD is expected), increased synaptic inhibition or shunting of the cortical output (where an increase in BOLD may reflect increased local metabolism), resulting in diverse associations between inhibition and BOLD signal (Logothetis, 2008).

Interestingly, studies comparing visual stimulation to rest blocks have shown increases in V1 glutamate, but no changes in GABA with functional MRS (Schaller et al., 2013) and combined fMRI-MRS found strong links between BOLD-responses to visual stimulation and glutamate concentration (Ip et al., 2017). These results suggest that BOLD responses may reflect changes in metabolism mainly captured by glutamate rather than GABA.

In order, therefore, to investigate inhibitory processes that are involved in visual plasticity, I measured the concentration of GABA before, during and after training on visual tasks, as an estimate of local cortical inhibition. In chapter III, I showed that, indeed, BOLD signal does not allow us to differentiate between inhibitory contributions to visual learning. Instead, GABA changes moderate the link between changes in BOLD and behavioural improvement. My findings suggest GABA MRS can reveal inhibitory contributions that we are unable to measure with fMRI.

Resting state connectivity relates to cortical inhibition and behavioural improvement

Recent studies have shown that functional connectivity in networks known to be involved in motor (Sampaio-Baptista et al., 2015) and visual (Baldassarre et al., 2012) learning relates to behavioural improvement, linking functional connectivity to learning-dependent plasticity. It is possible that decreased OCT-PPC connectivity at rest predicts behavioural improvement for the Signal-in-Noise task by supporting independent modulation of GABA in the two areas. In contrast, increased task-free OCT-PPC connectivity predicts improved discriminability in the Feature differences task by supporting coupled modulation of GABA in the two areas.

GABAergic inhibition has been suggested to shape network connectivity (Stagg et al., 2014). In particular, extra-synaptic GABA has been shown to relate to local oscillatory activity in the high gamma frequency range (Towers et al., 2004) and to inter-regional functional connectivity (Shmuel and Leopold, 2008). In addition, GABAergic interneurons have been shown to support intra-cortical and long-range connections (Rutishauser et al., 2012) and function as neuronal hubs that orchestrate spontaneous network synchronization (Bonifazi et al.,

2009). In the human brain, MRS-assessed GABA has been linked to functional connectivity as measured by resting-state fMRI (Bachtiar et al., 2015; Kapogiannis et al., 2013; Stagg et al., 2014), suggesting that GABAergic inhibition may relate to local neural dynamics (Cabral et al., 2011).

Studies have shown that GABA levels at a node of brain network relate to network-level intrinsic connectivity, with lower GABA levels in the primary motor cortex correlating with higher motor network strength (Bachtiar et al., 2015; Stagg et al., 2014). Further, lower GABA levels in the left M1 were shown to correlate with higher M1-M1 resting connectivity (Stagg et al., 2014). Finally, training (Sampaio-Baptista et al., 2015), as well as anodal tDCs on M1 (Bachtiar et al., 2015) have been shown to increase motor network strength. While these studies relate local inhibition to network-level connectivity, here I find evidence of local GABAergic inhibition contributions to regional connectivity. I showed that learning dependent changes in occipito-temporal GABA levels relate to resting state connectivity between occipito-temporal and parietal cortex for fine feature discrimination, while connectivity within the occipito-temporal cortex for detecting targets from clutter. This is the first report of functional connectivity between two areas relating to changes in GABA levels during training. This result suggests that in order to maintain a strong connection between occipito-temporal and parietal cortex, it's possible that connections to other brain areas need to be suppressed, driven by changes in cortical inhibition. On the contrary, decreased local inhibition may enhance lateral connections within the occipito-temporal cortex, similar to previous studies showing lower GABA levels correlating with higher network strength in the motor cortex (Bachtiar et al., 2015; Stagg et al., 2014).

GABAergic mechanisms relate causally to human visual plasticity

In chapter V, I showed that the GABAergic contributions to learning suggested in chapters III and IV are causally linked with visual plasticity. I showed that anodal (excitatory) tDCs on the occipito-temporal cortex facilitates learning to see in clutter, while cathodal (inhibitory) tDCs facilitates learning to discriminate fine features. The effects shown here are polarity specific for both tasks and confirm a causal relationship between changes in the occipito-temporal GABAergic inhibition and visual plasticity.

Excitatory and inhibitory tDCs has been employed to facilitate motor learning and reveal causal link between perturbation of motor cortical excitability and motor plasticity (Amadi et al., 2015; Stagg et al., 2011c; Vines et al., 2008). However, this is the first evidence of a double dissociation between increases vs decreases in cortical excitability and behavioural improvement in two distinct visual tasks: learning to detect patterns in visual noise vs discriminate between highly similar patterns.

Previous studies have linked GABAergic inhibition to homeostatic visual plasticity. Decreased GABA in the primary visual cortex has been shown to reactivate ocular dominance plasticity in rats (Harauzov et al., 2010), while GABA decrease following monocular deprivation has been related to the perceptual boost of the deprived eye, as measured by binocular rivalry in humans (Lunghi et al., 2015). Further, GABA has been shown to control critical periods for plasticity during development of the sensory cortical areas (Fagiolini and Hensch, 2000; Huang et al., 1999). Here I propose a causal role for GABA in visual plasticity in perceptual learning, rather than a homeostatic or developmental mechanism. I propose that excitatory anodal stimulation of the occipito-temporal cortex facilitates enhanced improvement in noise sensitivity, by supporting gain changes. In contrast, inhibitory cathodal stimulation of the occipito-temporal cortex facilitates increased feature selectivity, by supporting changes in tuning.

2. Methodological considerations

While great care was taken when planning and executing the studies described in this thesis, there are limitations and possible concerns that should be taken into account when discussing the findings.

Behavioural paradigm

Here I use Glass patterns and train participants to detect shapes in visual noise or discriminate shapes that are highly similar. These tasks have been shown to engage the occipito-temporal cortex (Ostwald et al., 2008) and training changes BOLD responses in this area (Li et al., 2012; Mayhew et al., 2010; Zhang et al., 2010). I discussed in chapter II the reasons why Glass patterns are an appropriate stimulus for studying distinct processing mechanisms, while controlling for local feature statistics. It is important to note, however, that literature on neuronal gain and tuning – the two basic mechanisms proposed here to mediate learning – largely refers to the primary visual cortex. Nonetheless, electrophysiology work has shown tuning properties (Yamane et al., 2008; Yang and Maunsell, 2004a), as well as gain-control mechanisms (Reynolds et al., 2000; Salinas and Abbott, 1997; Sundberg et al., 2009) in monkey higher visual areas. Further, changes in BOLD response gain and activation pattern selectivity have been shown in the human brain for detecting low-salience targets and discriminating highly similar high-salience targets (Kourtzi et al., 2005; Zhang et al., 2010). These studies suggest analogous inhibitory mechanisms are involved in occipito-temporal plasticity, however further studies are needed to confirm to what extent neuronal inhibition plays a role similar to the one in V1 or in animal visual systems.

The behavioural paradigm used consisted of a single training session, targeting fast learning (Poggio et al., 1992; Sagi, 2011). Changes in brain activation have been shown in a single training session (Mukai et al., 2007), however it's worth mentioning that previous imaging studies using Glass patterns involved multiple

training sessions and measured brain activation before and after training (Li et al., 2012; Mayhew et al., 2010; Zhang et al., 2010). Here I investigated the role of inhibition in early training, motivated by animal studies suggesting changes in inhibition are necessary for plasticity induction (Castro-Alamancos et al., 1995; Trepel and Racine, 2000). For this reason I restricted training to one -instead of multiple- training session and ensured that participants could still learn by reducing the number of stimulus conditions and running behavioural pilots to determine the level of difficulty used. Thus, the GABAergic contributions reported here may not reflect precisely the mechanisms described by other fMRI studies (Li et al., 2012; Mayhew et al., 2010; Zhang et al., 2010) with a different experimental design.

Further, when making links between the changes in GABAergic inhibition measured here and studies showing changes in BOLD activation, one should take into account the following: a) In Chapter III, resting GABA measurements were taken before and after a single fast-training session. b) In Chapter IV, baseline-GABA was measured during viewing of noisy stimuli, doing a reaction time task, while task-GABA was measured during training on the task. c) Here, early training is targeted, that has been shown to be mediated by distinct inhibitory mechanisms, different to stabilisation (Shibata et al., 2017).

Using MR spectroscopy to measure inhibition in the brain

GABA-MRS is an experimental technique that is has received attention in the latest years, resulting in significant development of the acquisition and analysis pipelines. While it is being increasingly used in the field of Cognitive Neuroscience, there are concerns regarding the sensitivity of the measurements, as GABA has an elusive spectral signature and the data can often be contaminated with nuisance signals (see chapter II). However, the sequences used in this thesis have been shown to produce reliable and reproducible results. Specifically, GABA measurements with edited sequences like the 2D-J PRESS

at 3 Tesla have been shown to be reliable and reproducible (Prescot and Renshaw, 2013; Schmitz et al., 2017). Likewise, semi-LASER at 7 Tesla has been shown to measure GABA reliably in the cortex (Barron et al., 2016; Kolasinski et al., 2017; C Lemke et al., 2015; Lunghi et al., 2015; van de Bank et al., 2015).

As discussed in chapter II, we interpret these links between MRS-assessed GABA, behaviour and possible neural mechanisms with caution, as the precise mechanisms that underlie changes in GABA as measured by MRS remain under investigation. In particular, it is unclear whether MRS-assessed GABA represents the entire pool of GABA available in a voxel. It is possible that the individual GABA pools are not equally visible using MRS (Floyer-Lea et al. 2006; Stagg et al. 2009). Another possibility is that MRS-assessed GABA reflects the exchange between the intra-cellular and synaptic pools of GABA (Ashton and Ushkaryov, 2005; Waagepetersen et al., 2001). A number of studies using paired-pulse transcranial magnetic stimulation (Dyke et al., 2017; Stagg et al., 2011b; Tremblay et al., 2013) have shown that MRS-assessed GABA does not relate to $GABA_A$ or $GABA_B$ activity and therefore it is unlikely to reflect synaptic transmission of GABA. It is more likely that MRS-assessed GABA reflects ambient extracellular GABA that contributes to tonic GABAergic activity (for reviews: (Johnstone et al., 2017; Rae, 2014; Stagg, 2014)). This is consistent with animal studies showing that GABA synthesis (Mason et al. 2001) is associated with GAD_{67} that is predominantly found throughout the cell, rather than GAD_{65} that is found in axon terminals (Kaufman et al., 1991). Finally, we did not observe changes in glutamate simultaneously with the change in GABA observed during training. Thus, it is unlikely that the changes in GABA reflect overall changes in the metabolite cycling. GABA undergoes rapid turnover in the mammalian cortex, and GAD activity has been shown to be rapidly modulated in a variety of physiological processes (Garraghty et al., 1991) in both human (Shen et al., 1999) and animal (Manor et al., 1996) studies. Future studies combining invasive investigations in animals

(e.g. two photon imaging of interneurons) with non-invasive MRS are necessary to shed more light on the basis MRS-assesed GABA.

Using transcranial direct current stimulation to perturb cortical excitability

Here I relate GABAergic inhibition, as measured with MR spectroscopy, with behavioural improvement and then perturb cortical excitability using tDCs. As discussed in chapter II, the mechanisms underlying the effects of tDCs on the human brain are still debated. While it is believed that tDCs targets $GABA_A$ activity (Nitsche et al., 2005; Stagg and Nitsche, 2011), it has been proposed that tDCs affects synaptic rather than extra-synaptic $GABA_A$ activity (Amadi et al., 2015). However, MRS is believed to be more sensitive to concentrations of extra-synaptic GABA (Mason et al., 2001). Therefore, discrepancies between the findings may result from differences in the GABAergic pool measured vs targeted with the two techniques.

The stimulation duration used in chapter V is longer than the 15-20min used in a large number of tDCs studies (Nitsche et al., 2008). Studies have shown that when tDCs is applied for longer periods of time, its effects on cortical excitability may be reversed. Specifically, anodal tDCs applied for 26min has been shown to induce cortical inhibition for 2h following stimulation (Monte-Silva et al., 2013). A neuronal mechanism that prevents cortical over-excitation has been proposed to mediate this reversal of the tDCs effect (Monte-Silva et al., 2013). Here tDCs duration was adapted to the duration of the training paradigm (35minutes). While it is not possible to know how the longer stimulation duration will alter cortical excitability in this study, it is important to mention that the protocol used in chapter V differs from the Monte-Silva et al, 2013 study in two ways. First, stimulation here is applied while participants are performing a visual task. It's been shown that the effect of anodal tDCs is different when stimulation is applied at rest vs during a task (Amadi et al., 2015). Second, Monte-Silva et al show this reversal of the anodal excitatory

effect directly after stimulation stops, which in our case would be after training had already finished. It's been shown that following anodal tDCs, homeostatic mechanisms result in increased synaptic $GABA_A$ activity (Amadi et al., 2015) that could account for the decreased excitability following stimulation in Monte-Silva et al, 2013. Even if a reversal of the effect on cortical excitability happens for the stimulation protocol in chapter V, behavioural enhancement in the Signal-in-Noise vs Feature differences for anodal vs cathodal stimulation would suggest that changes in GABAergic inhibition are required early in training, but may not be necessary to maintain increased performance.

Left or right?

When planning an MRS study, one is inevitably faced with a key question: in which hemisphere should the MRS voxel be placed? In chapter III, I measured GABA from the right occipito-temporal cortex, in chapter IV, I measured GABA from the left posterior parietal and left occipito-temporal cortex and in chapter V, I stimulated the right occipito-temporal cortex. Brain imaging studies have shown consistent bilateral activations in occipito-temporal cortex for shape processing (Kourtzi and Kanwisher, 2001; Ostwald et al., 2008), while TMS has shown comparable disruptive effects on left and right occipito-temporal cortex (Chang et al., 2014). Here I show that decreased GABA in both right (chapter III) and left (chapter IV) occipito-temporal cortex relates to behavioural improvement. However, fMRI (Mevorach et al., 2009a) and TMS (Mevorach et al., 2006) studies have shown that left posterior parietal cortex is selectively involved in low-salience stimuli selection. Thus, in chapter IV, the voxels were placed in the left hemisphere and it would be of interest to see whether right posterior parietal cortex shows differential GABAergic contributions, consistent with the previous fMRI and TMS studies.

GABA levels: state or trait?

Whether GABA levels reflect a brain state or a trait of the cortex is currently under investigation. Human GABA-MRS studies have linked resting GABA levels to behavioural traits, such as motor reaction times (Stagg et al., 2011a), tactile acuity (Kolasinski et al, 2017), orientation discrimination ability (Edden et al, 2009), larger orientation illusion magnitude (Song et al., 2017) and bistable perception (van Loon et al., 2013). Studies have also shown GABA level differences between patients and controls in schizophrenia (Yoon et al., 2010), as well as differences between GABAergic processes in autistic and healthy populations (Robertson et al., 2015). Near et al (Near et al., 2014) showed that GABA concentrations in the visual cortex remain stable over a period of about 7 months, suggesting GABA levels reflect an individual trait rather than a state.

However, cortical excitability (De Gennaro et al., 2007; Kreuzer et al., 2011) and GABA levels have been shown to vary with wakefulness and sleep deprivation ((Plante et al., 2012; Winkelman et al., 2008), but also (Morgan et al., 2012) for a review see (Duncan et al., 2014)). Further, normal circadian rhythm effects have been shown to alter cortical excitability in the motor cortex (Koski et al., 2005; Tamm et al., 2009) by modulating GABAergic inhibition (Lang et al., 2011). Two-photon imaging studies have shown that PV interneurons are selectively involved in controlling cortical activity during REM sleep (Niethard et al., 2016). Therefore, GABA plays a key role in shaping cortical activity across a variety of different brain states (Lee and Dan, 2012).

With MRS we measure the aggregate concentration of the neurotransmitter, neuromodulator and metabolic pools of GABA. It is, thus, plausible that MRS assessed GABA reflects state dependent functional fluctuations around the individual levels of inhibition. In this thesis, I investigated the learning dependent changes in GABAergic inhibition. I measured GABA before, during and after training on a fast-learning paradigm where measurements were between 5 and 45 minutes apart. I expressed GABA change as a difference and

as a percent change from baseline, ensuring that the results are not driven by variability in resting GABA concentration before training. Following this method, I was able to directly test whether functional fluctuations of GABAergic inhibition are linked to visual plasticity.

From motor to visual cortex

The majority of the studies we rely on as evidence for MRS-assessed GABAergic inhibition come from motor cortex literature. One may pose the question as to what extent these translate to the visual cortex: do GABA levels in the visual cortex have a behavioural relevance? Studies so far have measured GABA successfully in both cortices and have linked the basic functions of both motor and visual cortex to MRS-assessed GABA: lower GABA levels in the motor cortex relate to faster reaction times (Stagg et al., 2011a), while higher GABA levels in the sensorimotor cortex relate to better tactile acuity (Kolasinski et al, 2017). Higher GABA levels in the visual cortex relate to better orientation discrimination (Edden et al, 2009; Rokem et al, 2011). These results suggest that the relationship between MRS-assessed GABAergic inhibition and behaviour is not specific to the motor cortex and depends both on the cortical function in question and the local cytoarchitecture and circuitry (Stagg, 2014).

MRS-assessed GABA changes have been measured in the motor cortex during training and have been found to decrease for motor plasticity (Floyer-Lea et al, 2006). During motor learning, cortical recruitment and remapping may benefit from decreased cortical inhibition. Similarly, I show here that cortical recruitment for noise filtering and target detection relates to decreased MRS-GABA in the occipito-temporal cortex. On the contrary, I observed that enhanced retuning relates to increased MRS-GABA in the occipito-temporal cortex during training. An analogous mechanism has not been shown in the motor cortex. Following the positive relationship between GABA levels in somatosensory cortex and tactile acuity shown in (Kolasinski et al, 2017), it

remains to be investigated whether enhancement of tactile acuity during training would relate to increased or decreased MRS-GABA in the motor cortex.

A large proportion of the studies providing supporting evidence for the mechanism of action of tDCs are on the motor cortex. Do these findings extrapolate to the visual cortex and do we expect the same results when we stimulate different cortical areas? Anatomical differences between the motor and visual cortex may alter the effects of stimulation: large pyramidal cells, which are more easily polarised due to their lower neuronal threshold and larger membrane surface, comprise the motor cortex but are absent from the visual cortex. Further, the visual cortex has great morphological variability and cortical thinness (Fertonani et al., 2011). Therefore it's possible that cortical excitability is more easily altered in the motor than in the visual cortex. However, studies measuring visual evoked potentials (VEPs) replicated in the visual cortex the effects of tDCs previously shown in the motor cortex: VEP amplitude was found to be increased following anodal and decreased following cathodal tDCs compared to a non-stimulated condition (Antal et al., 2004a), suggesting a similar mechanism of action for tDCs on the visual cortex. Anodal stimulation of the occipito-temporal cortex selectively reduced GABA (but not glutamate) during stimulation, as measured by MRS (Barron et al., 2016), similar to the findings of anodal tDCs on the motor cortex (Stagg et al, 2009a), suggesting the effect of stimulation on neurotransmitter concentrations is not confined to the motor cortex. In this thesis I used tDCs to perturb cortical excitability, following studies showing that tDCs in the occipito-temporal cortex alters the concentration of GABA (Barron et al, 2016) and tDCs in the primary visual cortex (Pirulli et al., 2013), as well as MT (Antal et al., 2004b), facilitates behavioural improvement in visual tasks. Further interventional studies and animal work is required to elucidate the mechanism of action of tDCs on the visual cortex.

3. Future work

In this chapter, I have presented a series of questions that have been answered with my studies. The findings provide us with novel insights into the inhibitory processes involved in visual learning and ignite new questions to be investigated in the future.

Does OCT-PPC connectivity change during training?

Here I show that resting OCT-PPC connectivity predicts behavioural improvement in visual learning and relates to cortical inhibition in the occipito-temporal cortex. However, visual perceptual learning has been shown to alter regional connectivity (Lewis et al., 2009). Further, rs-fMRI has been shown to predict individual variability in cognitive task BOLD activation maps (Cole et al., 2016; Tavor et al., 2016), while resting state connectivity between brain areas has been related to coupled task-related BOLD changes (Mennes et al., 2010). A future study employing fMRI-MRS (Ip et al., 2017) may measure BOLD signal while measuring GABA during training and will answer two questions: how does OCT-PPC connectivity change during training and whether changes in connectivity relate to changes in GABA in the two areas. Based on the relationships shown here between resting functional connectivity and behavioural improvement in the two visual tasks, I predict that during training on the Signal-in-Noise task, OCT-PPC connectivity will decrease and connectivity within OCT will increase to facilitate cortical recruitment and integration of contextual information. On the contrary, during training on the Feature differences task, I predict that OCT-PPC connectivity will increase to support top-down interactions for template retuning in the visual and optimisation of decision making in the parietal cortex. Understanding the pathways involved in different types of visual plasticity will shed light on how feedforward and feedback processing are implemented during visual learning.

Is OCT-PPC functional connectivity a result of a structural connection?

Here I show resting OCT-PPC connectivity differentially predicts behavioural improvement in two visual tasks. However, diffusion tensor imaging (DTI) has shown cortical projections from the vertical occipital fasciculus (VOF) -that connects dorsal and ventral regions of the occipital cortex- to PPC (Takemura et al., 2016). A future study may combine resting state fMRI and DTI before training, together with task fMRI and post-training DTI, to investigate: a) the structural and functional connection between OCT and PPC, b) whether they predict behavioural improvement in visual learning, c) if combined with pre and post training GABA measurements, how inhibition shapes functional and structural plasticity.

Which GABAergic pool is involved in visual learning?

Here I show distinct GABAergic contributions for learning to see in clutter vs discriminate fine features. However, using MRS we are unable to differentiate between the different GABAergic pools (cytoplasmic, synaptic, extra-synaptic). While MRS is believed to be more sensitive to extra-synaptic $GABA_A$ activity (Mason et al., 2001), plastic changes are not constrained to extra-synaptic GABA (Stagg, 2014). A future study may employ GABA agonists that target synaptic GABA, such as benzodiazepines (Nutt et al., 2015) vs GABA reuptake inhibitors, such as Tiagabine (Gonzalez-Burgos, 2010), to selectively increase synaptic vs extracellular GABA and dissociate the behavioural effects in the two tasks. In the same study, MRS measurements before vs. after the pharmacological interventions will show which GABAergic pool is mainly represented in the GABA-MRS signal. A study measuring GABA levels in the occipital cortex and basal ganglia found no differences following a Tiagabine intervention (Myers et al., 2014). However, this study would need to be repeated on a larger sample and including blood drug concentration measurements. Understanding which GABAergic pool is a) involved in visual learning and b) mainly represented in the MRS signal will allow us to investigate specific and

interpretable mechanisms of plasticity, as well as design targeted pharmacological interventions to facilitate learning.

Are different GABAergic interneurons involved in different tasks?

Our findings provide novel evidence for the role of GABA-ergic processing in learning, shedding light on the neural implementation of theoretical models of perceptual learning. Future animal studies may probe the micro-circuits that give rise to learning by noise filtering vs. feature template retuning. Recent work has begun to classify cortical interneurons into distinct classes based on morphology, connectivity, and physiology (Kepecs and Fishell, 2014) and link them to distinct cortical computations (see for example (El-Boustani and Sur, 2014; Kerlin et al., 2010; Wilson et al., 2012). These distinct interneuron types may differentially contribute to learning by noise filtering vs. feature template retuning by changing the gain vs. feature selectivity of pyramidal cells. Thus, our findings propose testable hypotheses linking theoretical models of perceptual learning to the micro-circuits that mediate adaptive behaviour and underlie the macroscopic learning-dependent plasticity as measured by human brain imaging.

What about consolidation?

Here I show GABAergic contributions to fast visual learning, suggesting GABA is related to plasticity early in training. However, it has been shown that the balance between excitation and inhibition may change between early training and stabilisation of learning (Shibata et al., 2017). A future study may measure GABA before and after multiple training sessions, to reveal GABAergic contributions to learning when performance reaches a plateau. Understanding the long-term time-course of GABAergic inhibition during training will inform learning interventions: if different stages of learning (i.e. early training vs

consolidation) are supported by differential GABAergic mechanisms, we would need to design closed-loop interventions that monitor behavioural improvement and adjust the intervention steps accordingly.

Inhibitory alpha oscillations and learning

Here I show evidence for the involvement of inhibitory mechanisms in visual learning, as measured by GABA MRS. However, GABAergic interneurons are involved in generation of gamma and alpha oscillations in the cortex, while modulations of inhibitory alpha power have been shown to relate to levels of GABAergic inhibition (Lozano-Soldevilla et al., 2014). A future study may employ EEG/MEG to measure gamma and alpha oscillatory power during training, as well as GABA concentration before and after training and investigate links between GABAergic inhibition and brain oscillations.

Is tDCs the optimal way to perturb cortical excitability for visual plasticity?

Brain stimulation interventions other than tDCs have been suggested to enhance visual learning. Specifically, V1 transcranial random noise stimulation (tRNs) has been shown to facilitate improvement on an orientation discrimination task (Fertonani et al., 2011; Pirulli et al., 2013). However, the mechanism by which tRNs affects cortical excitability is not well understood. It is hypothesised that tRNs results in temporal neural summation of the stimulated neurons, selectively enhancing the activity of task-relevant neurons (Fertonani et al., 2011). It's been also suggested to enhance performance by introducing stochastic resonance in the stimulated area (Moss et al., 2004). A future study could investigate the use of tRNs during training on the Signal-in-Noise task, as stochastic resonance has been suggested to facilitate signal detection (Faisal et al., 2008; Stacey and Durand, 2000).

Alternatively, theta burst TMS (TBS) has been shown to increase vs decrease cortical excitability when applied intermittently (iTBS) vs continuously (cTBS) (Huang et al., 2005). Further, cTBS has been shown to increase MRS GABA in the motor cortex (Stagg et al., 2009b). A future study could employ iTBS vs cTBS to facilitate learning on the Signal-in-Noise vs Feature differences task. Interestingly, inhibitory cathodal tDCs results in a Glx decrease, with a highly correlated GABA decrease (Stagg et al., 2009a). It is likely that the Feature differences task will be further enhanced by cTBS than cathodal tDCs, due to the increase in GABA concentration, consistent with GABA-MRS evidence in chapters III and IV.

Can we intervene to facilitate visual learning in patient populations?

Studies have shown that patients suffering from certain neuropsychiatric disorders where the GABAergic system is compromised (e.g. schizophrenia, autism) also show visual deficits. In particular, patients with schizophrenia have been shown to have reduced GABA levels in the visual cortex compared to healthy controls (Yoon et al., 2010) and visual impairments in schizophrenics have been linked to GABAergic dysfunctions (Butler et al., 2008; Rokem et al., 2011). Further, studies on children with autism presented with Glass patterns have found deficits in visual form processing (Spencer and O'Brien, 2006), while binocular rivalry deficits in autistic populations have been linked to reduced GABAergic signalling (Robertson et al., 2015). Using the findings of this thesis, we can design brain stimulation intervention protocols to alter cortical excitability in the visual cortex and facilitate visual learning in autistic and schizophrenic populations; similar interventions using tDCs on the sensorimotor cortex of patients with spatial neglect have shown long-lasting effects (O'Shea et al., 2017) that persisted well beyond training. A future application of my findings can employ cathodal tDCs on the visual cortex to support visual functions that are compromised in autism and schizophrenia. First steps in this direction would be a) establishing a behavioural effect (e.g. deficits

in global form perception and feature discrimination), b) conducting a double-blind tDCs experiment with anodal, cathodal and sham groups and finally, c) confirming the cortical excitability changes on the pathological cortex using combined tDCs and MRS during training.

4. Thesis contribution

In my thesis I show for the first time evidence for dissociable inhibitory mechanisms that mediate visual plasticity for perceptual decisions. Further, I propose a causal link between cortical excitability and behavioural improvement in visual perceptual learning.

The findings of my thesis on the causal relationship between GABAergic inhibition and behavioural improvement can be used to support targeted training interventions. For example, brain stimulation has recently received increased attention as a tool to facilitate cognitive training and rehabilitation following stroke. Establishing links between changes in cortical excitability and behavioural improvement on visual perceptual tasks can inform such therapeutic interventions.

Further, the novel insights on the GABAergic mechanisms involved in visual learning may inform translational research in psychiatric disorders. Inhibitory mechanisms are known to be compromised in disorders such as autism and psychosis. Understanding the link between these mechanisms and behaviour will be beneficial for designing interventions (pharmacological and cognitive training programmes) that enhance the capacity for learning and flexible behaviour in disease.

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