What are we measuring with GABA magnetic resonance spectroscopy?

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A number of recent papers[1-3] have demonstrated a relationship between in vivo concentration of GABA, as assessed using Magnetic Resonance Spectroscopy (MRS), and an individual's task performance, giving a unique insight into the relationship between physiology and behavior. However, interpretation of the functional significance of the MRS GABA measure is not straightforward. Here we discuss some of the outstanding questions as to how total concentration of GABA within a cortical region relates to phasic and tonic GABA activity within the cortical volume studied.

Over recent years there has been an upsurge in interest in Magnetic Resonance Spectroscopy (MRS) as a non-invasive method to quantify neurotransmitter concentrations in discrete regions of the human brain. In a typical experiment, a voxel is placed within a region of interest, such as the primary motor cortex (M1) and the concentration of neurochemicals within that region can be assessed with a temporal resolution sufficient to allow detection of phasic changes.

There has been particular interest in using this technique to quantify changes in GABA, the major inhibitory neurotransmitter. Initial studies demonstrated the sensitivity of MRS to detect decreases in GABA within M1 after interventions such as learning,[4] ischaemic nerve block[5] and transcranial stimulation techniques such as transcranial direct current stimulation (tDCS).[6,7] These interventions are all known to increase cortical excitability, and are thought to induce Long-Term Potentiation (LTP)-like plasticity.

In addition to investigating group mean changes in GABA following plasticity-induction paradigms, MRS has more recently been applied to probe the neurochemical factors underlying inter-individual differences in behavioral performance. In a number of distinct regions of the brain a subject's resting GABA concentration is closely correlated with that person's ability to perform a task dependent on that region.[1-3] This relationship between GABA concentration and behavior is not observed for control brain regions not thought to be critical for task performance.

As well as shedding light on associations between GABA and steady state behaviors, MRS can also be used to investigate inter-individual differences in subjects' ability to perform dynamic tasks such as learning a novel sequence of motor movements. In our recent study[3] we demonstrated a close relationship between the degree to which tDCS decreases GABA in an individual on one day and that subjects' ability to learn a novel task, such that subjects with a more responsive GABA system (i.e., those who showed the greatest decrease in GABA with tDCS) were also subjects who showed the greatest ability to change their behavior through learning. This finding suggests that the ability to decrease GABA within the cortex is important for the early-stages of motor learning to occur in humans.

However, despite these promising results, it is not yet clear how directly MRS measures of GABA relate to synaptic activity; nor how accurately we can assess the relative contributions of GABA and glutamate using MRS.

The Roles of GABA in the CNS

GABA is found in two major pools within neurons and is thought to have a number of roles within the brain. Cytoplasmic GABA, primarily produced from glutamate via the tonically active 67 kD form of glutamic acid decarboxylase (GAD), is found throughout the neuron and is therefore hypothesised to have a role in metabolism.[8] Vesicular GABA is found in high concentrations within the pre-synaptic boutons; its concentration is controlled in the main via the phasically active 65 kD GAD and it plays a role in inhibitory synaptic neurotransmission.[8] In addition to these two well-reported roles, GABA also appears to have a more tonic, neuromodulatory role in cortical inhibition, via free extracellular GABA acting on extrasynaptic $GABA_A$ receptors.[9]

MRS is only capable of detecting a total concentration of a neurochemical within a localised region (typically in the order of 2 x 2 x 2 cm) of tissue; it cannot distinguish between these separate functional pools of GABA. An added complication is that some of these pools may be more tightly bound to macromolecules than others, rendering them less "visible" to MRS.[4,7]

Given the relationships demonstrated between behavior and MRS-derived measures of local GABA concentration, it seems likely that the MRS measure of GABA is, at the least, correlated with the neurotransmitter and neuromodulator pools of GABA within the cortex. Although it is difficult to test this hypothesis directly, we will discuss below the potential for complementary electrophysiological techniques, that specifically probe particular GABA receptor types, to help to determine what is being measured with MRS.

Relationship between MRS and Synaptic GABA Activity

There are two major subtypes of GABA receptor within the cortex—$GABA_A$ receptors, a family of ligand-gated chloride channels and $GABA_B$ receptors, metabotropic receptors linked to potassium channels. These two subtypes have very different speeds of response, allowing investigation of each subtype independently by varying the interval between two pulses of transcranial magnetic stimulation (TMS). Such "paired-pulse" TMS approaches involve giving a low intensity pulse (the conditioning stimulus), set at an intensity which stimulates the smaller cortical interneurons but not the pyramidal neurons, followed by a higher intensity pulse (the test stimulus) which will stimulate all neurons in the targeted region. With an interval of 2–4 ms between the conditioning and test stimuli, the test stimulus falls as the pyramidal neurons are under the influence the GABAergic interneurons, and the magnitude of the response is therefore smaller than the response to a test stimulus of the same intensity with no preceding conditioning stimulus. This phenomenon, known as Short Interval Intracortical Inhibition (SICI), has been shown to accurately reflect $GABA_A$ activity within the cortex.[10] Inter-pulse intervals of 50–200 ms are also associated with inhibition, termed Long Interval Intracortical Inhibition (LICI), and thought to reflect $GABA_B$ activity.[11]

The relationship between MRS-assessed GABA and GABA measures derived using TMS is currently unclear. Decreases in both MRS-assessed GABA concentration and SICI have been observed after motor learning[4,12] and after anodal (facilitatory) tDCS.[7,13] However, continuous theta burst stimulation (cTBS), a TMS protocol known to decrease cortical excitability, increases GABA concentration within the stimulated cortex,[6] but decreases SICI.[14]

The discrepancy in MRS and SICI measures after cTBS suggests that while there may be a relationship between synaptic $GABA_A$ activity and GABA MRS measures this may not be a simple one, especially in the context of dynamic changes. To our knowledge, there is as yet no evidence on the relationship between cTBS-induced dynamic changes in $GABA_B$ activity measured using LICI. A study directly investigating the relationship between MRS and TMS measures of GABA has yet to be performed.

GABA and Glutamate
A Finely Tuned Balancing Act

GABA and glutamate are closely linked in the human brain both biochemically, as the vast majority of GABA is metabolised from glutamate, and functionally, due to tight control of the inhibitory/excitability balance in health. This close relationship has been confirmed by MRS studies demonstrating a tight correlation between glutamate and GABA.[3,7]

However, it is likely that MRS gives a less accurate reflection of glutamatergic activity than GABAergic activity. The GABA-edited MEGA-PRESS sequence most frequently used for GABA MRS acquisitions does not allow us to separate resonances from glutamate and glutamine, meaning that most studies report this composite measure of Glx. In addition, in our experience, there are a number of technical reasons why the Glx resonance is often less reliably acquired than the GABA resonance. Finally, much fine-tuning of glutamatergic activity occurs via modulation of the NMDA receptors, changes which are invisible to MRS.

In our recent study,[3] we demonstrated correlations between behavior and GABA, over and above those found between behavior and glutamate concentration, and there is extensive evidence from the animal literature that change in GABA modulation is an early and necessary step in synaptic plasticity within the primary motor cortex.[15] However, given the technical challenges in quantifying glutamate via MRS as discussed above, it may be that we are less sensitive to modulation in glutamatergic signalling and the potential role of glutamate should not be overlooked when reporting correlations between neurotransmitter concentrations and behavior.

Conclusions

MRS provides a method that allows us to non-invasively quantify neurotransmitter concentrations in vivo, at a temporal resolution that is sufficient to detect biologically relevant changes in GABA concentrations. The technique allows us, for the first time, to investigate the role of neurochemicals in inter-individual behavioral differences. As acquisition techniques continue to improve, and with the increasing use of ultra-high field MR in humans, the promise of MRS as a neuroscience tool can only increase. However, until more is

understood about the specifics of what we are measuring with MRS, care needs to be employed when interpreting the results.

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