

Current Biology

Reduced GABAergic Action in the Autistic Brain

Highlights

- Behavioral marker of inhibitory/excitatory neurotransmission is perturbed in autism
- Marker predicts higher-order autistic symptom severity
- Inhibitory and excitatory neurotransmitters measured in the brain predict behavior
- Action of the inhibitory neurotransmitter, GABA, is reduced in the autistic brain

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In Brief

An imbalance in inhibitory/excitatory neurotransmission is proposed to affect the autistic brain, but empirical evidence in humans is lacking. Robertson et al. report a link between a robust autistic perceptual symptom and reduced action of the inhibitory neurotransmitter, γ -aminobutyric acid (GABA), in the brains of autistic individuals.

Reduced GABAergic Action in the Autistic Brain

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SUMMARY

An imbalance between excitatory/inhibitory neurotransmission has been posited as a central characteristic of the neurobiology of autism [1], inspired in part by the striking prevalence of seizures among individuals with the disorder [2]. Evidence supporting this hypothesis has specifically implicated the signaling pathway of the inhibitory neurotransmitter, γ -aminobutyric acid (GABA), in this putative imbalance: GABA receptor genes have been associated with autism in linkage and copy number variation studies [3–7], fewer GABA receptor subunits have been observed in the post-mortem tissue of autistic individuals [8, 9], and GABAergic signaling is disrupted across heterogeneous mouse models of autism [10]. Yet, empirical evidence supporting this hypothesis in humans is lacking, leaving a gulf between animal and human studies of the condition. Here, we present a direct link between GABA signaling and autistic perceptual symptomatology. We first demonstrate a robust, replicated autistic deficit in binocular rivalry [11], a basic visual function that is thought to rely on the balance of excitation/inhibition in visual cortex [12–15]. Then, using magnetic resonance spectroscopy, we demonstrate a tight linkage between binocular rivalry dynamics in typical participants and both GABA and glutamate levels in the visual cortex. Finally, we show that the link between GABA and binocular rivalry dynamics is completely and specifically absent in autism. These results suggest a disruption in inhibitory signaling in the autistic brain and forge a translational path between animal and human models of the condition.

RESULTS AND DISCUSSION

During binocular rivalry, two images, one presented to each eye, vie for perceptual dominance as neuronal populations that are selective for each eye's input suppress each other in alternation [16, 17]. The strength of perceptual suppression during rivalry is thought to depend on the balance of inhibitory and excitatory cortical dynamics [12–15] and may serve as a non-invasive

perceptual marker of the putative perturbation in inhibitory signaling thought to characterize the autistic brain.

We therefore measured the dynamics of binocular rivalry in individuals with and without a diagnosis of autism (41 individuals, 20 with autism). As predicted, individuals with autism demonstrated a slower rate of binocular rivalry (switches per trial: controls = 8.68, autism = 4.19; $F(1,37) = 16.52$, $\eta_p^2 = 0.311$, $p = 0.001$; Figure 1A), which was marked by reduced periods of perceptual suppression (proportion of each trial spent viewing a dominant percept, (dominant percept durations)/(dominant + mixed percept durations): controls = 0.69; autism = 0.55; $F(1,36) = 7.27$, $\eta_p^2 = 0.172$, $p = 0.011$; Figure 1B). The strength of perceptual suppression inversely predicted clinical measures of autistic symptomatology (Autism Diagnostic Observation Schedule [ADOS]: $R_s = -0.39$, $p = 0.027$; Figure 1) and showed high test-retest reliability in a control experiment ($R = 0.94$, $p < 0.001$; see Supplemental Experimental Procedures and also [18]). These results replicate our previous findings in an independent sample of autistic individuals [11] and confirm rivalry disruptions as a robust behavioral marker of autism.

To test whether altered binocular rivalry dynamics in autism are linked to the reduced action of inhibitory (γ -aminobutyric acid [GABA]) or excitatory (glutamate [Glx]) neurotransmitters in the brain, we measured the concentration of these neurotransmitters in visual cortex using magnetic resonance spectroscopy (MRS). Spectra were acquired from an occipital voxel ($2.5 \times 2.5 \times 3$ cm), centered bilaterally on the calcarine sulcus (Figure 2A).

GABA and glutamate are predicted to contribute to different aspects of binocular rivalry dynamics: mutual inhibition between (GABA) and recurrent excitation within (glutamate) populations of neurons coding for the two oscillating percepts [14]. Specifically, in classic models of binocular rivalry, a period of perceptual suppression is maintained through excitation within the neuronal population selective for the dominant image, as well as cross-inhibition of the neuronal population selective for the non-dominant image [12, 19, 20]. Critically, reducing either mutual inhibition or recurrent excitation is predicted to reduce the strength of perceptual suppression during rivalry in one implementation of this model [14], mirroring the dynamics we observed in autism. We therefore considered each neurotransmitter separately to test whether inhibitory or excitatory signaling was selectively disrupted in the autistic brain.

Given prior evidence from genetic, animal, and post-mortem studies, we hypothesized that inhibitory signaling may be affected in the autistic brain. This disruption could take the form of reduced levels of GABA or glutamate. However, reports

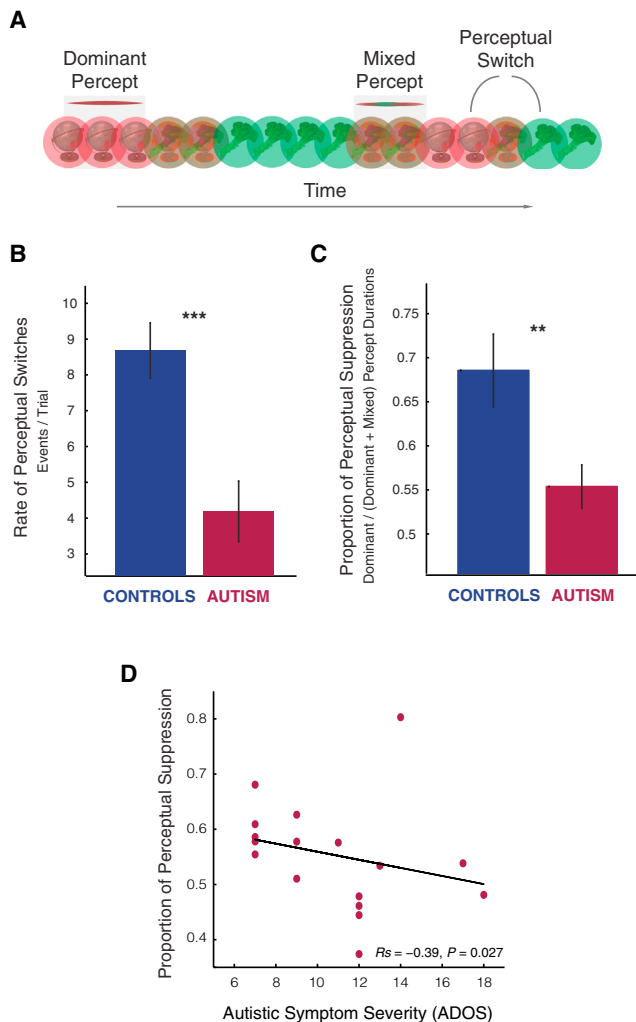


Figure 1. Atypical Dynamics of Binocular Rivalry in Autism

(A) Schematic of perceptual experience during binocular rivalry. Two images, one presented to each of an individual's eyes, oscillate back and forth in perceptual awareness as each is suppressed in turn. Throughout a run, participants were instructed to continuously report their perceived image (red, green, mixed) through button press (right, left, up).

(B) Slower rate of binocular rivalry alternations in autism. Individuals with autism demonstrated fewer perceptual switches between the inputs to their left and right eyes, compared with control participants ($\eta_p^2 = 0.311$, $p = 0.001$). (C) Reduced proportion of perceptual suppression in autism. Individuals with autism demonstrated a reduced strength of perceptual suppression, periods of time during which one image is fully suppressed from visual awareness ($\eta_p^2 = 0.172$, $p = 0.01$).

(D) The strength perceptual suppression during binocular rivalry inversely predicted autistic symptom severity, measured using the ADOS, a clinical measure of autistic symptomatology.

In all plots, error bars represent 1 SEM. ** $p \leq 0.01$, *** $p \leq 0.001$ difference between the two groups. See also Table S1.

of perturbations of key components of the GABA signaling pathway, such as receptors [3–9] and inhibitory neuronal density [10], suggest that GABA levels themselves may not be altered in autism but instead may be less predictive of rivalry dynamics.

As predicted by models of binocular rivalry, GABA concentrations in visual cortex strongly predicted rivalry dynamics in con-

trols, where more GABA corresponded to longer periods of perceptual suppression ($R_s = 0.62$, $p = 0.002$; Figure 2B). However, this relationship was strikingly absent in individuals with autism ($R_s = 0.02$, $p = 0.473$; Figure 2B). The difference between the two correlations was significant ($\eta_p^2 = 0.167$, $p = 0.013$; Figure 2C), indicating a reduced impact of GABA on perceptual suppression in the autistic brain.

Importantly, this finding was specific to GABA: glutamate strongly predicted the dynamics of binocular rivalry in autism ($R_s = 0.60$, $p = 0.004$; Figure 2B), to the same degree as that found in controls ($R_s = 0.40$, $p = 0.031$ in controls; $p = 0.71$ for the difference of this effect between autism and controls; Figure 2C). The absence of a group difference in the concentrations of GABA and Glx (both $p > 0.32$; Figure S1; Table S2) indicate that GABA levels themselves are not altered in the autistic visual cortex [21], despite a specific reduction in the effect of GABA on autistic visual behavior. These findings suggest that alterations in the GABAergic signaling pathway may characterize autistic neurobiology. Consistent with prior evidence from animal and post-mortem studies, such dysfunction may arise from perturbations in key components of the GABAergic pathway beyond GABA levels, such as receptors [3–9] and inhibitory neuronal density [10].

These results demonstrate reduced GABAergic, but conserved glutamatergic, action in the autistic visual system. No other metabolite measured predicted rivalry dynamics in either group (all $p > 0.11$ for TNAA, TCho, TCr, and Ins), and GABA was the only metabolite to show a markedly reduced impact on the dynamics of binocular rivalry in autism (all other effects between autism and controls: $p > 0.29$; Figure 2C). Furthermore, this relationship was specific to GABA measured in the visual cortex: binocular rivalry dynamics were not related to any metabolites measured in a control region of interest, the motor cortex, in either group (all $R_s < 0.17$, all $p > 0.25$).

The specificity of this effect to GABA and not glutamate argues against the possibility that these findings are driven by differences in the spectral quality between the two groups. Further, spectral fit errors (an indication of data quality) and frequency drift (an indication of subject motion) were within the expected ranges and matched between groups (all $p > 0.46$; Figure S1; Table S2). Repeatability control experiments indicated that our metabolite measurements were highly reliable (coefficient of variation [CVs] < 8%) with a high reproducibility relative to the effect range of our study (SDs < 13% of the group range for each metabolite) (see Supplemental Experimental Procedures). These results confirm previous reports of high test-retest reliability of in vivo MRS measurements [22] and add to a growing literature of replicated relationships between GABA concentration and psychophysical performance, including sensory sensitivity thresholds [23, 24], motor response inhibition [25], and binocular rivalry dynamics [15, 26].

Atypical sensory perception has been noted since the earliest reports of autism [27], although little is known about the neural underpinnings of these symptoms. Interestingly, recent reports of reduced spatial suppression [28], decreased global motion perception [29, 30], larger population receptive fields [31], and more variable evoked responses [32, 33] in autism mirror the perceptual consequences of reduced GABAergic inhibition in animal studies [34, 35]. Computational accounts suggest that

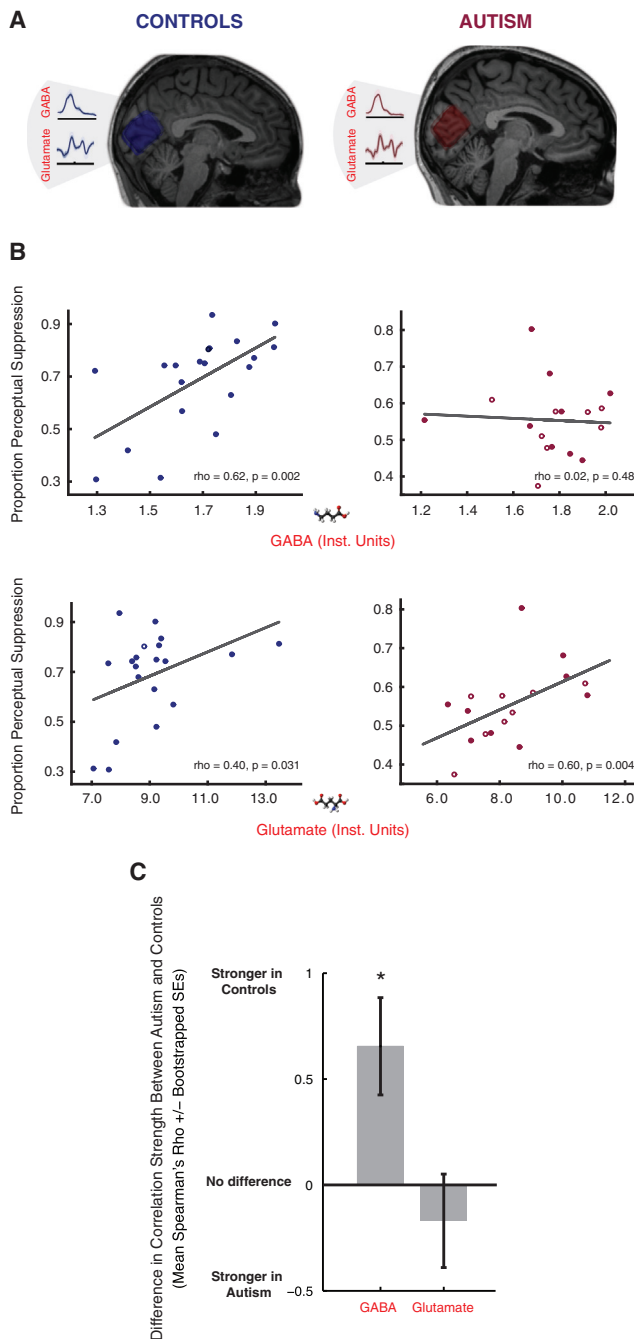


Figure 2. Reduced GABAergic, but Conserved Glutamatergic, Action in the Autistic Visual Cortex

(A) Magnetic resonance spectra were acquired from individuals with and without autism: one example control (left) and autistic (right) participant shown here, with the acquisition region (visual cortex) shown in color (80% probability of VOI placement). Spectra included the neurotransmitters predicted to govern binocular rivalry dynamics from computational models, GABA and glutamate, as well as control metabolites TNAa, TCho, TCr, and Ins.

(B) GABA strongly predicted the strength of perceptual suppression during rivalry in control individuals ($R_s = 0.62$, $p = 0.002$, top left), but this relationship was absent in autism ($R_s = 0.02$, $p = 0.473$, top right). However, glutamate strongly predicted binocular rivalry dynamics in both controls ($\rho = 0.40$, $p = 0.031$, bottom left) and autism ($R_s = 0.60$, $p = 0.004$, bottom right).

reducing inhibition may account for a wide range of autistic perceptual symptoms [36]. One such account predicted reduced perceptual suppression during rivalry in autism but found only a trend toward the finding ([37], but see [38]). Further work is needed to test whether this litany of visual disruptions indeed results from reduced GABAergic signaling in the autistic brain, as we demonstrate here for one robust autistic visual symptom.

Our findings provide evidence for an empirical link between a specific neurotransmitter measured in the brains of individuals with autism and an autistic behavioral symptom. Although perceptual in nature, this symptom strongly predicts higher-order clinical measures of autistic symptomatology and may be well poised to serve as a behavioral marker of a perturbation in GABAergic signaling in the autistic brain. Together with the pivotal roles of GABA in canonical cortical computations [39] and neuro-development [40], these findings point to the GABAergic signaling pathway as a prime suspect in the neurobiology of this pervasive developmental disorder [41]. Future work should replicate these findings and investigate GABAergic signaling in autism at multiple developmental time points to establish whether GABAergic perturbations predict, or arise in response to, the developmental onset of autistic symptomatology.

EXPERIMENTAL PROCEDURES

Participants

We tested 41 adolescents and adults: 21 controls and 20 autism, matched for age and IQ (Table S1). All individuals with autism met DSM-IV diagnostic criteria (15 Asperger's, 1 high-functioning autism, 4 pervasive developmental disorder not otherwise specified [PDD-NOS]), as assessed by an experienced clinician, and met cutoff for the category "Autism Spectrum Disorder" after a research-reliable administration of the ADOS-2 [42]. Twelve participants (1 control, 11 autism) were being treated with psychiatric medications: antidepressants ($n = 8$), antipsychotics ($n = 2$), antiepileptics ($n = 4$), and anxiolytics ($n = 2$). Excluding participants taking medications known to interact with the GABAergic system ($n = 8$) did not qualitatively alter the results. All participants had normal or corrected-to-normal vision and IQ > 70. Written consent was obtained in accordance with a protocol approved by the Massachusetts Institute of Technology Institutional Review Board.

Binocular Rivalry Paradigm and Analysis

Binocular rivalry stimuli and analysis were identical to those used in [11], except that the stimuli were slightly smaller. On each run, two grayscale objects (e.g., a baseball and a piece of broccoli) appeared on the left and right of the screen. Each object was displayed within a tinted square (green or red; width: 2.2°), surrounded by a black circle to support binocular fusion (radius: 3.1°). Stimuli were viewed through a mirror stereoscope, which reflected the left and right sides of the screen into the participants' left and right eyes so that each eye was presented with only one of the two images (red or green).

Testing sessions were composed of two 30-s practice runs and six 45-s experimental runs. Participants were asked to continuously report whether they perceived a fully dominant percept—the red image (right key) or the green image (left key)—or a mixture of the two images (up key).

Since the key press was continuous (sampling rate: 4 ms), a sequence of perceptual transitions was computed as events in which one continuous key press was terminated and another began. For each participant and trial, the frequency of perceptual switches as well as the duration of any percept (red,

(C) GABA was the only molecule to show a significantly stronger effect on rivalry dynamics in controls, as compared to autism, demonstrating a selective disruption in GABAergic action in the autistic brain ($\eta_p^2 = 0.167$, $p = 0.013$). In all plots, medicated individuals are labeled with unfilled circles. Error bars represent 1 SEM. See also Figures S1 and S2 and Table S2.

green, or mixed) were calculated. The primary measure of interest, the proportion of perceptual suppression, was calculated as the proportion of each trial spent viewing a fully dominant percept: (dominant percept durations)/(dominant + mixed percept durations).

See [Supplemental Experimental Procedures](#) for information regarding test-retest reliability of rivalry dynamics.

MRI and MRS Acquisition

Magnetic resonance (MR) data were collected using a Siemens Trio 3T MRI scanner at MIT, equipped with a 32-channel head coil. At the beginning of each scan session, we acquired a high-resolution, whole-brain anatomical volume using a T1-weighted MPRAGE sequence (124 slices; voxels = $1 \times 1 \times 1$ mm; repetition time = 2,530 ms; echo time = 2.94 ms; matrix = $256 \times 256 \times 176$ mm; field of view = $256 \times 256 \times 176$ mm).

Subsequently, single voxel ^1H MR spectra (MRS) were acquired from early visual and motor volumes of interest (VOIs). Prior to MRS acquisition, whole-volume first and second-order shims were adjusted using the automatic GRE shim sequence, and manual shimming was additionally performed within each VOI to optimize magnetic field homogeneity (mean full width at half maximum of water signal: 15.9 Hz [controls], 15.2 Hz [autism]; mean T_2^* : 39.7 ms [controls], 40.7 ms [autism]).

A Mescher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) scan [43] (320 spectral averages; repetition time = 1,500 ms; echo time = 68 ms; eight-step phase cycle) and a localized unsuppressed water scan (four averages; repetition time = 10,000 ms; echo time = 30 ms) were acquired in each VOI. A frequency-selective inversion pulse was applied at 1.9 and 7.5 ppm in alternating spectral lines, and the edit-ON and edit-OFF spectra were subtracted to generate a difference spectrum containing total GABA.

The visual cortex VOI ($2.5 \times 2.5 \times 3.0$ cm) was placed using standard anatomical landmarks [44], angled along the calcarine sulcus and set dorsal to the cerebellum and anterior from the anterior sinus. The motor cortex VOI ($2.5 \times 2.5 \times 3.0$ cm) was centered on the hand knob of the left hemisphere and angled along the primary motor strip [45].

During the MEGA-PRESS scan of visual cortex, participants performed a simple visual task at fixation. During the MEGA-PRESS scan of motor cortex, participants performed a simple finger-tapping task.

MRI Analysis

Individuals' anatomical scans were reconstructed and segmented into gray matter, white matter, and cerebrospinal fluid (CSF) maps using statistical parametric mapping (SPM8, Wellcome Trust Center for Neuroimaging). The tissue composition within each VOI was computed by creating a mask of the portion of the anatomical volume covered by the VOI.

MRS Analysis

GABA concentration was quantified from the MEGA-PRESS difference subspectra using the Gannet GABA analysis toolkit [46]. Gannet models the GABA peak using a simple, five-parameter Gaussian model, fit between 2.19 and 3.55 ppm and the water peak using a Gaussian-Lorentzian function. Gannet applies a correction factor for co-edited macromolecule signal and editing efficiency during water scaling (see below). All other metabolic signals were quantified from the edit-OFF subspectra using TARQUIN [47], which uses a linear combination of simulated basis sets based on quantum calculations to fit spectra in the time domain. Macromolecules are modeled as components of the TARQUIN fit.

In all analyses, metabolite values were scaled to water and expressed in institutional units (IU). Nuclear magnetic resonance (NMR) visible water was estimated at 78%, as each VOI primarily contained gray matter [48], and the following formula was used to calculate the water relaxation factor: $\exp(-TE_{\text{water}}/T_2_{\text{water}})/\exp(-TE_{\text{metabs}}/T_2_{\text{metabs}})$. The resulting metabolite concentrations were corrected for the proportion of gray matter in each VOI. Cramér-Rao lower bound values were reliably < 20% for each metabolite and group (GABA: 6.53 [autism], 5.41 [control]; Glx: 8.88 [autism], 9.12 [control]). Therefore, all fitted spectra were accepted for analysis (Figure S2).

In total, we were able to resolve the metabolic signals of the following molecules: GABA, Glx, TNAA, TCho, TCr, and myo-inositol (Ins). Glutamate and its metabolic precursor glutamine were fitted together (Glx) due to their overlapping resonances at 3T, as were N-Acetylaspartate and N-acetylaspartylgluta-

mate (TNAA), creatine and phosphocreatine (TCr), and glycerophosphocholine and phosphocholine (TCho).

It should be noted that MEGA-PRESS measurements of GABA typically include macromolecule signals with similar resonant frequencies. As a result, they are often referred to as GABA+. All results were qualitatively similar to those reported here when metabolites were quantified using the MRS analysis software LCModel.

See [Supplemental Experimental Procedures](#) for information regarding test-retest reliability of MRS measurements.

Statistical Analyses

Spearman's rank correlations coefficients (R_s) were used to compare the relationship between neurotransmitter concentrations and rivalry dynamics. Two-tailed, uncorrected p values were calculated from permutation tests, and correlation coefficients were calculated from bootstrapped confidence intervals of these correlations, sampled 10,000 times with replacement. The strength of two relationships was computed by performing an F test on the linear regression coefficients of each relationship. All results remained significant using Pearson's coefficients (all $p < 0.02$) and when results were corrected for multiple comparisons between GABA and Glx (all $p < 0.026$).

Participants whose rivalry percept durations were determined to fall outside of 2 SDs of the group mean were excluded from analyses (one control, two autism). One autism participant was unable to complete the MRS scan.

Participants' psychometric data (age and IQ, on which the two groups were matched) did not correlate with any of our experimental variables of interest (all $p > 0.212$). Therefore, statistical analyses were performed without psychometric data included as covariates.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.11.019>.

AUTHOR CONTRIBUTIONS

Conceptualization, C.E.R. and N.K.; Methodology, C.E.R., N.K., and E.-M.R.; Investigation, C.E.R. and E.-M.R.; Formal Analysis, C.E.R.; Visualization, C.E.R.; Writing – Original Draft, C.E.R.; Writing – Reviewing & Editing, C.E.R., N.K., and E.-M.R.

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REFERENCES

1. Rubenstein, J.L.R., and Merzenich, M.M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* 2, 255–267.
2. Canitano, R. (2007). Epilepsy in autism spectrum disorders. *Eur. Child Adolesc. Psychiatry* 16, 61–66.
3. Sanders, S.J., Ercan-Sencicek, A.G., Hus, V., Luo, R., Murtha, M.T., Moreno-De-Luca, D., Chu, S.H., Moreau, M.P., Gupta, A.R., Thomson, S.A., et al. (2011). Multiple recurrent de novo CNVs, including duplications

- of the 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron* 70, 863–885.
4. Shao, Y., Cuccaro, M.L., Hauser, E.R., Raiford, K.L., Menold, M.M., Wolpert, C.M., Ravan, S.A., Elston, L., Decena, K., Donnelly, S.L., et al. (2003). Fine mapping of autistic disorder to chromosome 15q11-q13 by use of phenotypic subtypes. *Am. J. Hum. Genet.* 72, 539–548.
 5. Ma, D.Q., Whitehead, P.L., Menold, M.M., Martin, E.R., Ashley-Koch, A.E., Mei, H., Ritchie, M.D., Delong, G.R., Abramson, R.K., Wright, H.H., et al. (2005). Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *Am. J. Hum. Genet.* 77, 377–388.
 6. Chen, C.-H., Huang, C.-C., Cheng, M.-C., Chiu, Y.-N., Tsai, W.-C., Wu, Y.-Y., Liu, S.-K., and Gau, S.S.-F. (2014). Genetic analysis of GABRB3 as a candidate gene of autism spectrum disorders. *Mol. Autism* 5, 36.
 7. Piton, A., Jouan, L., Rochefort, D., Dobrzaniecka, S., Lachapelle, K., Dion, P.A., Gauthier, J., and Rouleau, G.A. (2013). Analysis of the effects of rare variants on splicing identifies alterations in GABAA receptor genes in autism spectrum disorder individuals. *Eur. J. Hum. Genet.* 21, 749–756.
 8. Fatemi, S.H., Reutiman, T.J., Folsom, T.D., and Thuras, P.D. (2009). GABA(A) receptor downregulation in brains of subjects with autism. *J. Autism Dev. Disord.* 39, 223–230.
 9. Oblak, A.L., Gibbs, T.T., and Blatt, G.J. (2011). Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. *Brain Res.* 1380, 218–228.
 10. Gogolla, N., Leblanc, J.J., Quast, K.B., Südhof, T.C., Fagiolini, M., and Hensch, T.K. (2009). Common circuit defect of excitatory-inhibitory balance in mouse models of autism. *J. Neurodev. Disord.* 1, 172–181.
 11. Robertson, C.E., Kravitz, D.J., Freyberg, J., Baron-Cohen, S., and Baker, C.I. (2013). Slower rate of binocular rivalry in autism. *J. Neurosci.* 33, 16983–16991.
 12. Laing, C.R., and Chow, C.C. (2002). A spiking neuron model for binocular rivalry. *J. Comput. Neurosci.* 12, 39–53.
 13. Seely, J., and Chow, C.C. (2011). Role of mutual inhibition in binocular rivalry. *J. Neurophysiol.* 106, 2136–2150.
 14. Said, C.P., and Heeger, D.J. (2013). A model of binocular rivalry and cross-orientation suppression. *PLoS Comput. Biol.* 9, e1002991.
 15. van Loon, A.M., Knapen, T., Scholte, H.S., St John-Saaltink, E., Donner, T.H., and Lamme, V.A.F. (2013). GABA shapes the dynamics of bistable perception. *Curr. Biol.* 23, 823–827.
 16. Tong, F., and Engel, S.A. (2001). Interocular rivalry revealed in the human cortical blind-spot representation. *Nature* 411, 195–199.
 17. Leopold, D.A., and Logothetis, N.K. (1996). Activity changes in early visual cortex reflect monkeys' percepts during binocular rivalry. *Nature* 379, 549–553.
 18. Miller, S.M., Hansell, N.K., Ngo, T.T., Liu, G.B., Pettigrew, J.D., Martin, N.G., and Wright, M.J. (2010). Genetic contribution to individual variation in binocular rivalry rate. *Proc. Natl. Acad. Sci. USA* 107, 2664–2668.
 19. Wilson, H.R., Blake, R., and Lee, S.H. (2001). Dynamics of travelling waves in visual perception. *Nature* 412, 907–910.
 20. Kang, M.-S., Lee, S.-H., Kim, J., Heeger, D., and Blake, R. (2010). Modulation of spatiotemporal dynamics of binocular rivalry by collinear facilitation and pattern-dependent adaptation. *J. Vis.* 10, 3.
 21. Gaetz, W., Bloy, L., Wang, D.J., Port, R.G., Blaskey, L., Levy, S.E., and Roberts, T.P.L. (2014). GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. *Neuroimage* 86, 1–9.
 22. O'Gorman, R.L., Michels, L., Edden, R.A., Murdoch, J.B., and Martin, E. (2011). In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J. Magn. Reson. Imaging* 33, 1262–1267.
 23. Puts, N., Edden, R., Evans, C.J., McGlone, F., and McGonigle, D. (2011). Regionally specific human GABA concentration correlates with tactile discrimination thresholds. *J. Neurosci.* 31, 16556–16560.
 24. Puts, N.A., Harris, A.D., Crocetti, D., Nettles, C., Singer, H.S., Tommerdahl, M., Edden, R.A., and Mostofsky, S.H. (2015). Reduced GABAergic inhibition and abnormal sensory processing in children with Tourette Syndrome. *J. Neurophysiol.* Published online June 3, 2015. <http://dx.doi.org/10.1152/jn.00060.2015>.
 25. Boy, F., Evans, C.J., Edden, R.A.E., Singh, K.D., Husain, M., and Sumner, P. (2010). Individual differences in subconscious motor control predicted by GABA concentration in SMA. *Curr. Biol.* 20, 1779–1785.
 26. Lunghi, C., Emir, U.E., Morrone, M.C., and Bridge, H. (2015). Short-term monocular deprivation alters GABA in the adult human visual cortex. *Curr. Biol.* 25, 1496–1501.
 27. Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child* 2, 217–250.
 28. Foss-Feig, J.H., Tadin, D., Schauder, K.B., and Cascio, C.J. (2013). A substantial and unexpected enhancement of motion perception in autism. *J. Neurosci.* 33, 8243–8249.
 29. Robertson, C.E., Martin, A., Baker, C.I., and Baron-Cohen, S. (2012). Atypical integration of motion signals in Autism Spectrum Conditions. *PLoS ONE* 7, e48173.
 30. Robertson, C.E., Thomas, C., Kravitz, D.J., Wallace, G.L., Baron-Cohen, S., Martin, A., and Baker, C.I. (2014). Global motion perception deficits in autism are reflected as early as primary visual cortex. *Brain* 137, 2588–2599.
 31. Schwarzkopf, D.S., Anderson, E.J., de Haas, B., White, S.J., and Rees, G. (2014). Larger extrastriate population receptive fields in autism spectrum disorders. *J. Neurosci.* 34, 2713–2724.
 32. Haigh, S.M., Heeger, D.J., Dinstein, I., Minshew, N., and Behrmann, M. (2015). Cortical variability in the sensory-evoked response in autism. *J. Autism Dev. Disord.* 45, 1176–1190.
 33. Dinstein, I., Heeger, D.J., Lorenzi, L., Minshew, N.J., Malach, R., and Behrmann, M. (2012). Unreliable evoked responses in autism. *Neuron* 75, 981–991.
 34. Thiele, A., Herrero, J.L., Distler, C., and Hoffmann, K.-P. (2012). Contribution of cholinergic and GABAergic mechanisms to direction tuning, discriminability, response reliability, and neuronal rate correlations in macaque middle temporal area. *J. Neurosci.* 32, 16602–16615.
 35. Shen, W., McKeown, C.R., Demas, J.A., and Cline, H.T. (2011). Inhibition to excitation ratio regulates visual system responses and behavior in vivo. *J. Neurophysiol.* 106, 2285–2302.
 36. Rosenberg, A., Patterson, J.S., and Angelaki, D.E. (2015). A computational perspective on autism. *Proc. Natl. Acad. Sci. USA* 112, 9158–9165.
 37. Said, C.P., Egan, R.D., Minshew, N.J., Behrmann, M., and Heeger, D.J. (2013). Normal binocular rivalry in autism: implications for the excitation/inhibition imbalance hypothesis. *Vision Res.* 77, 59–66.
 38. Freyberg, J., Robertson, C.E., and Baron-Cohen, S. (2015). Reduced perceptual exclusivity during object and grating rivalry in autism. *J. Vis.* 15, 11.
 39. Turkheimer, F.E., Leech, R., Expert, P., Lord, L.-D., and Vernon, A.C. (2015). The brain's code and its canonical computational motifs. From sensory cortex to the default mode network: A multi-scale model of brain function in health and disease. *Neurosci. Biobehav. Rev.* 55, 211–222.
 40. Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. *Nat. Rev. Neurosci.* 6, 877–888.
 41. American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)* (American Psychiatric Association).
 42. Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Jr., Leventhal, B.L., DiLavore, P.C., Pickles, A., and Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J. Autism Dev. Disord.* 30, 205–223.
 43. Mescher, M., Merkle, H., Kirsch, J., Garwood, M., and Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR Biomed.* 11, 266–272.

44. Muthukumaraswamy, S.D., Evans, C.J., Edden, R.A.E., Wise, R.G., and Singh, K.D. (2012). Individual variability in the shape and amplitude of the BOLD-HRF correlates with endogenous GABAergic inhibition. *Hum. Brain Mapp.* *33*, 455–465.
45. Yousry, T.A., Schmid, U.D., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., and Winkler, P. (1997). Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. *Brain* *120*, 141–157.
46. Edden, R.A.E., Puts, N.A.J., Harris, A.D., Barker, P.B., and Evans, C.J. (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *J. Magn. Reson. Imaging* *40*, 1445–1452.
47. Wilson, M., Reynolds, G., Kauppinen, R.A., Arvanitis, T.N., and Peet, A.C. (2011). A constrained least-squares approach to the automated quantitation of in vivo ¹H magnetic resonance spectroscopy data. *Magn. Reson. Med.* *65*, 1–12.
48. Gasparovic, C., Neeb, H., Feis, D.L., Damaraju, E., Chen, H., Doty, M.J., South, D.M., Mullins, P.G., Bockholt, H.J., and Shah, N.J. (2009). Quantitative spectroscopic imaging with in situ measurements of tissue water T1, T2, and density. *Magn. Reson. Med.* *62*, 583–590.

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Supplemental Information

Reduced GABAergic Action in the Autistic Brain

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Supplemental Figures and Tables:

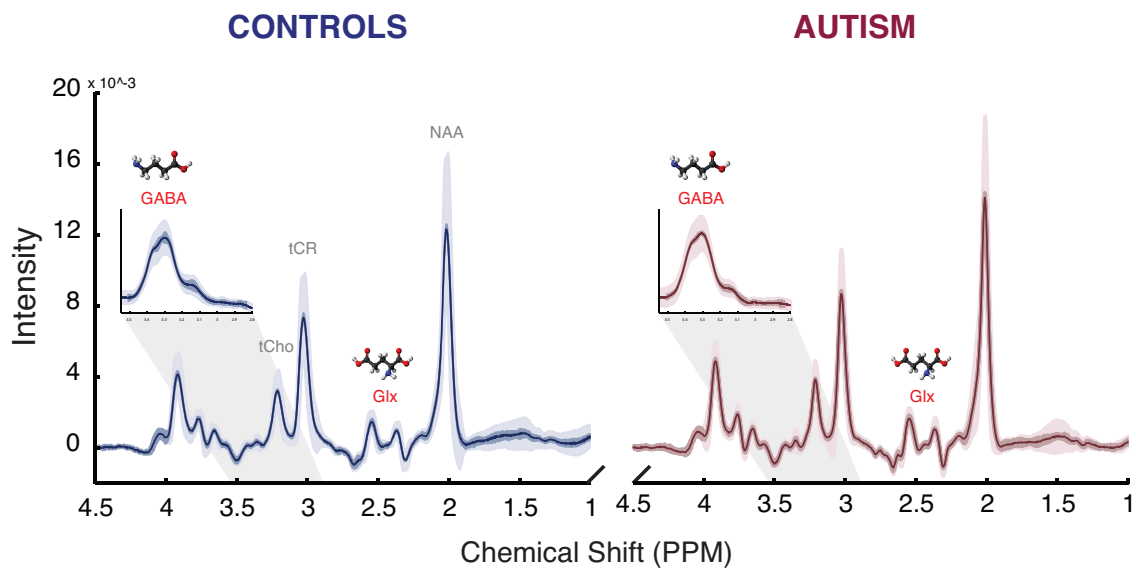


Figure S1. Related to Figure 2. Magnetic Resonance Spectra were highly comparable between the two groups. No differences were observed in the mean concentrations (dark line) or intrasubject SDs (light shading) of the two groups for any metabolites of interest. Further, no differences were observed in the spectral data quality: SDs of spectral fit (dark shading) were comparable between the two groups (Supplementary Table 2).

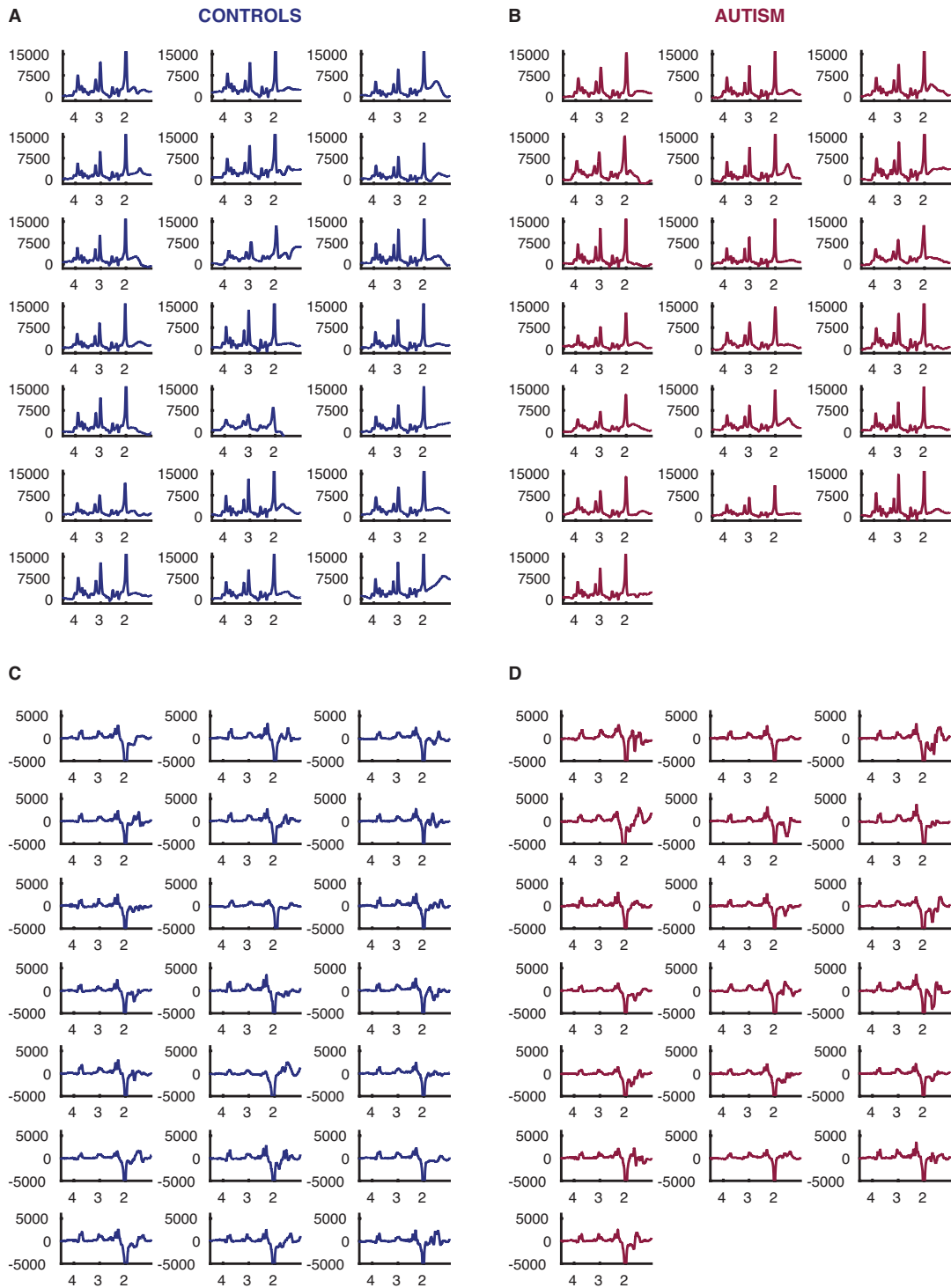


Figure S2. Related to Figure 2. Magnetic Resonance Spectra for each participant in the Control (**blue**) and Autism (**red**) group (**A, C:** Controls edit-OFF and difference spectra; **B, D:** ASC edit-OFF and difference spectra). The quality of MRS spectra was comparable between groups, and CLRBS were <20% for each metabolite of interest for all participants. See Table S2 for quantitative comparisons of the two groups' spectral data.

		Age	IQ	Gender	Autism-Spectrum Quotient (AQ)	Autism Diagnostic Observation Schedule (ADOS)
Controls						
	Mean	29.10	113.95	-	17.2	-
	SEM	8.14	13.7	-	5.95	-
	Min	18	83	-	7	-
	Max	47	132	-	29	-
	N	21	21	21 (19 M)	21	-
Autism						
	Mean	29.61	112.15	-	31.75	10.75
	SEM	9.17	16.8	-	8.07	3.44
	Min	16	77	-	18	7
	Max	49	132	-	47	18
	N	20	20	20 (14 M)	20	20
p-value		0.85	0.66	0.11	0.01	N.A.

Table S1 Psychometric Data. Related to Figures 1 and 2. Groups are matched for age, IQ, and Gender (Female = 1; Male = 2). Psychometric data did not correlate with any of the dependent (rivalry dynamics) or independent (neurotransmitter concentrations) variables of the experiment (all $p > 0.212$). Scores on the Autism Spectrum Quotient (AQ), a self-report questionnaire that quantifies autistic traits across both autistic and control populations, were significantly higher for autistic, as compared with control individuals ($p < 0.01$), although they did not predict rivalry dynamics in either group (both $p > 0.212$). Scores on the Autism Diagnostic Observation Schedule (ADOS-2), an hour-long diagnostic protocol administered to all participants in the autism group, predicted rivalry dynamics in ASD, where individuals with higher symptom severity demonstrated reduced perceptual suppression ($p = 0.027$). Means \pm 1 SEM for each group. *P*-value calculated using two-tailed t-tests.

	GABA Conc. (Inst. Units)	Glx Conc. (Inst. Units)	GABA/Glx (Inst. Units)	Spectral line- width FWHM (NAA, Hz)	Spectral Quality (SD fit / RMS noise)	Water FWHM (Hz)	Frequency Drift STD (Hz)	% Gray Matter	% White Matter	% CSF
Controls	1.8 (0.03)	8.82 (0.25)	0.20 (0.01)	6.68 (0.40)	2.77 (0.10)	8.31 (0.38)	1.07 (0.17)	0.62 (0.01)	0.24 (0.01)	0.13 (0.01)
Autism	1.8 (0.04)	8.35 (0.35)	0.21 (0.01)	7.03 (0.37)	2.72 (0.12)	8.75 (0.33)	0.90 (0.13)	0.63 (0.01)	0.24 (0.01)	0.13 (0.01)
p-value	0.82	0.27	0.12	0.52	0.75	0.38	0.46	0.26	0.62	0.48

Table S2 Magnetic Resonance Data Quality. Related to Figure 2. MR data quality, tissue composition, and the concentration of observed metabolites are statistically indistinguishable between the two groups. Means +/- 1 SEM for each spectral data value for each group. *P*-value calculated through two-tailed t-tests.

Supplemental Experimental Procedures

Binocular Rivalry: Test-Retest Reliability Experiment

To assess the test-retest reliability of our behavioral measure, binocular rivalry dynamics, we recruited 10 additional control participants to participate in our binocular rivalry experiment on two separate days, and measured the test-retest reliability of both perceptual switch rate and the strength of perceptual suppression (identical methods to those reported in *Methods: Binocular Rivalry Paradigm and Analysis*). Binocular rivalry dynamics were highly reliable across behavioral sessions. Both Perceptual Switches and Perceptual Suppression demonstrated high intra-subject consistency across behavioral sessions (Switches: $R = 0.87$, $p < 0.001$; Perceptual Suppression: $R = 0.94$, $p < 0.001$). Additional evidence for high reliability of binocular rivalry dynamics can be found in a recent large-scale longitudinal study of binocular rivalry, which also found high heritability of rivalry dynamics [S1].

Magnetic Resonance Spectroscopy: Measurement Reliability and Sensitivity

To test the reliability of our GABA and Glx measurements, as well as the sensitivity of these measurements relative to the effect range in our main experiment, we ran a repeatability study with five control participants. In each session, we acquired 5 anatomical scans (1 MPRAGE, 4 lower-resolution scans), 5 separate water scans, and 5 separate MEGA-PRESS scans (same acquisition parameters as specified in *Methods: MRI and MRS Acquisition*).

To quantify the reliability of metabolite measurements, we calculated the Coefficient of

Variation (CV; SD of metabolite concentration / mean metabolite concentration) for each participant, which approaches 1 if measurement reliability is low. Confirming previous reports using MEGAPRESS [S2], we found high measurement reliability for both metabolites of interest: measurement error accounted for 7.4% of the signal (CV GABA = 7.0%, CV Glx = 7.8%). To quantify the sensitivity of MEGAPRESS metabolite measurements, we compared the measurement variance determined from our repeatability study to the range of intrasubject variance in our main experiment (SD of metabolite concentration / range metabolite concentration). These results indicated high sensitivity of our measurements: measurement variance spanned about 12.3% of the effect range of our main experiment (GABA = 15.0%, Glx = 9.6%). The impact of measurement error on these results is therefore likely to be low.

Supplemental References

- S1. Miller, S. M., Hansell, N. K., Ngo, T. T., Liu, G. B., Pettigrew, J. D., Martin, N. G., and Wright, M. J. (2010). Genetic contribution to individual variation in binocular rivalry rate. *107*, 9–13.
- S2. O’Gorman, R. L., Michels, L., Edden, R. A., Murdoch, J. B., and Martin, E. (2011). In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J. Magn. Reson. Imaging* *33*, 1262–7.