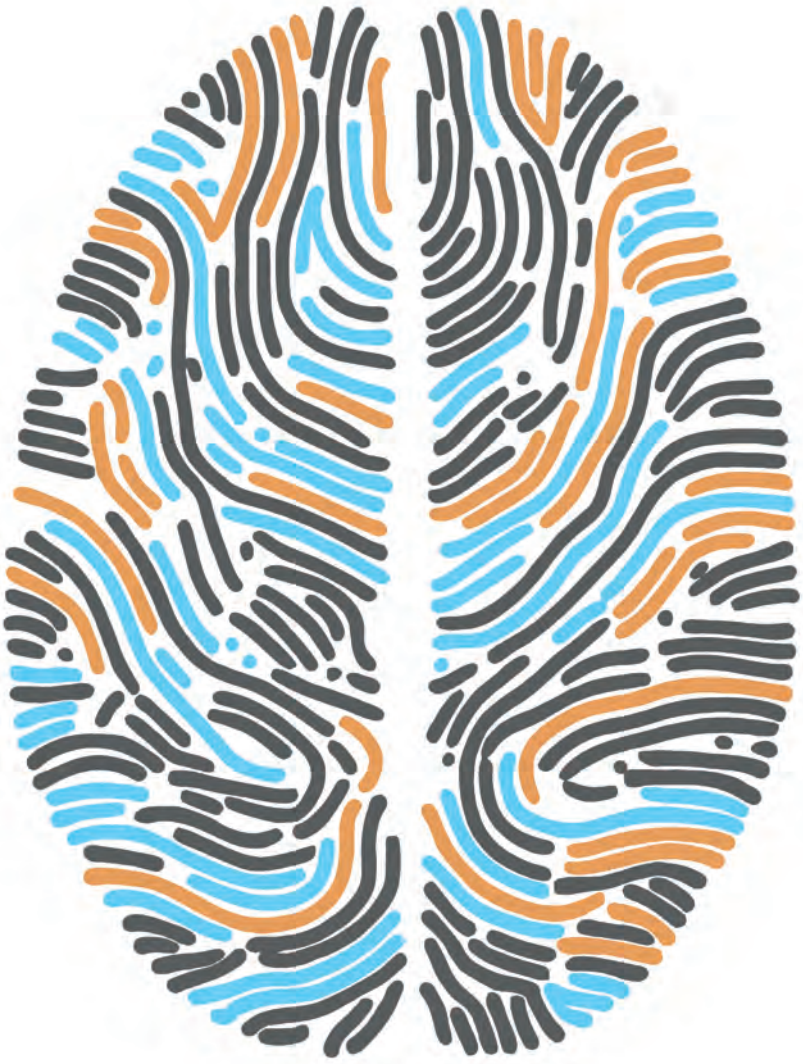
Explorations of the E/I imbalance framework of autism

Neural mechanisms and genetic factors



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according to the decision of the Doctorate Board
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Wednesday, June 11, 2025
at 12.30 pm

by

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So everybody, everywhere
Don't be afraid, don't have no fear
Gonna tell the world
Make it understand

Backstreet boys

Preface

Autism (Autism Spectrum Disorder) is one of the most common neurodevelopmental conditions. Yet, there is much that remains unknown about its underlying mechanisms in the brain, how it develops, and the various expressions of autism across individuals. This thesis presents a series of studies on the excitation/inhibition (E/I) imbalance theory of autism, aiming to disentangle some of the heterogeneities by linking genetic underpinnings of glutamatergic (excitation) and GABAergic (inhibition) functions, neuroimaging measures and behavioral autism characteristics.

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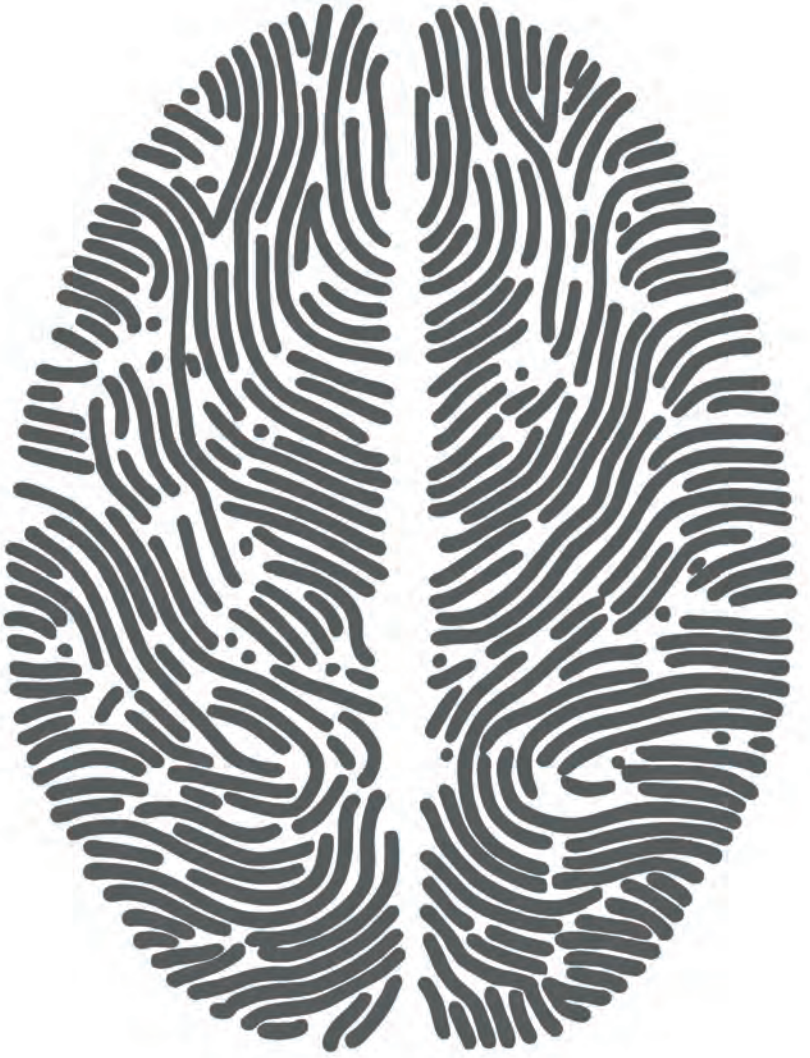


Chapter 1

Introduction

The definition of what we today call Autism Spectrum Disorder, or autism, has changed a lot since it was first described 80 years ago (1). Today, it is defined as a collection of clinical characteristics that include differences in social-communicative behaviors and interactions, repetitive behaviors and differences in sensory processing (2,3). The experiences and lives of autistic people vary widely; as is indicated in its name, it is a spectrum where autism characteristics present in different ways across individuals. For example, repetitive behaviors refer to many kinds of behaviors, including making repeated body movements or sounds (often referred to as stimming), preoccupations with certain topics and having special or circumscribed interests, or repeating words and phrases. Some autistic people are nonverbal, while some are incredibly talkative. The estimated prevalence of autism varies widely, especially across countries and continents, but typically ranges between 0.5 – 2% (4,5). While autism is one of the most prevalent neurodevelopmental conditions, we know little about its causes, development, and varying phenotypic expressions.

We do know that autism is highly heritable, and that genes associated with autism consistently include, among others, genes involved in excitatory and inhibitory functions in the brain (6–8). We also know that both rare genes with strong effects and the combined effects of multiple common genetic variations, each of small effect size, can give rise to autism (7,9).

Many efforts have been made to disentangle the neurobiology of autism; various neuroimaging methods have been used to link differences in brain structure and function to behavioral phenotypes of autism, albeit often with contradictory findings and across several brain regions (10,11). These varying results likely reflect etiological and biological heterogeneities of autism, and approaches that have been used so far have often failed to consider these heterogeneities (i.e. focusing on case control approaches (12)), which this thesis aim to disentangle. In this chapter, I will first introduce the clinical heterogeneity of autism, introduce the excitatory and inhibitory (E/I) imbalance theory and how it relates to autism, followed by ways to investigate this theory. Finally, I will summarize the aims and outline of this thesis.

Clinical heterogeneity

One primary reason for the lack of understanding of the etiology of autism stems from the broad differences in experiences and behaviors between autistic people. Previously, in the fourth edition of the Diagnostic and Statistical Manual of mental

disorders (DSM-IV-TR) published in 1994 (13), what is now known as autism was then separated into several labels with overlapping characteristics including Autistic disorder, Asperger's disorder and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS). In the current fifth edition from 2013 these were all grouped together as Autism Spectrum Disorder (2). To be diagnosed with autism, one needs to express a combination of the symptoms associated with it, within the domains of social communication, stereotyped and repetitive behaviors and sensory processing differences, reaching over a threshold score (what this threshold is depends on the diagnostic tool used). Due to this categorical diagnostic system, individuals who receive an autism diagnosis may express very different symptom combinations within these dimensions.

Dimensionality

The categorical diagnostic classification creates an arbitrary boundary between autistic and neurotypical individuals, which does not reflect clinical, biological nor etiological heterogeneities of autism. Research aiming to understand the etiology of autism has mainly followed this categorical approach, dividing people into autistic vs neurotypical groups and comparing them, effectively clumping all autistic individuals together and only looking at group-level differences (12). This omits differences *between* autistic individuals and has proven to be a rather fruitless approach. A more informative way to unravel the different causes of heterogeneous autism experiences and behaviors is to use a dimensional approach, looking at distinct behavioral traits and how they are mediated in the brain. A dimensional approach considers the quantitative differences both between autistic people, and between autistic and neurotypical people, along dimensions of the characteristics of autism. Understanding these differences is particularly important as there are currently limited support- and treatment options available and most of the care given to autistic people is mainly for things autistic individuals may struggle with that co-occur with their autism, such as anxiety, depression, attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD) and/or intellectual disability (ID), rather than the experiences and needs that arise from autism itself. It is therefore crucial to understand biological underpinnings of the heterogeneous expressions of autism, to be able to identify objective measures that can define subgroups of autistic people, potentially with distinct alterations of E/I. Taking a dimensional approach to understand the underlying mechanisms of autism is therefore a more suitable approach to unravel the complex etiologies of autism. Multiple causal pathways may lead to the same clinical behavioral

trait, where individuals may have various E/I imbalances that lead to the same or similar behaviors, which I discuss further in the following section. Understanding these differences will allow for predicting who may benefit the most from certain treatment- and support options.

Neural heterogeneity

We have yet to answer *how* the behavioral manifestations of autism arise, and unraveling the heterogeneities within autism is particularly challenging due to the sheer complexity of the E/I system in the brain. However, despite not having a complete answer, there is research pointing us in promising directions.

A highly influential conceptual framework to explain the underlying biology of autism is the excitatory/inhibitory (E/I) imbalance theory that poses that autism emerges due to an imbalance between excitation and inhibition in the brain (14,15). Excitation and inhibition, and the balance between them, are fundamental properties of how the brain functions. There is a large body of work supporting the E/I imbalance theory, although findings have been inconsistent in terms of how the scale of this imbalance may be tipped. Initially, the E/I imbalance framework was presented as increased excitation (15), which would explain the higher prevalence of epilepsy in autism, as well as the reduced GABA signaling (γ-aminobutyric acid, the most abundant inhibitory neurotransmitter) that has been observed (14,15). In more recent work, studies have supported this notion by showing evidence of increased glutamate signaling (the main excitatory neurotransmitter) (10,11), and decreased GABA signaling (11,16). In contrast, there is also evidence for increased inhibition, indicated by both decreased glutamate concentrations and increased GABA concentrations, even in the same brain regions where other studies found increased glutamate concentrations (10,11,17–26). Both excitatory and inhibitory metabolite concentrations have been linked to different behavioral characteristics of autism (21,27,28), and both similar and contradictory findings have been made in animal and genetic studies (28–36). These inconsistent findings highlight that the E/I imbalance framework, while influential, has had limited utility in explaining what in the brain is underlying the different representations of autism. This is probably not due to its invalidity, given the large amount of research supporting the theory, but rather the lack of dimensional approaches investigating these relationships, ultimately failing to consider autism heterogeneity (see Figure 1).

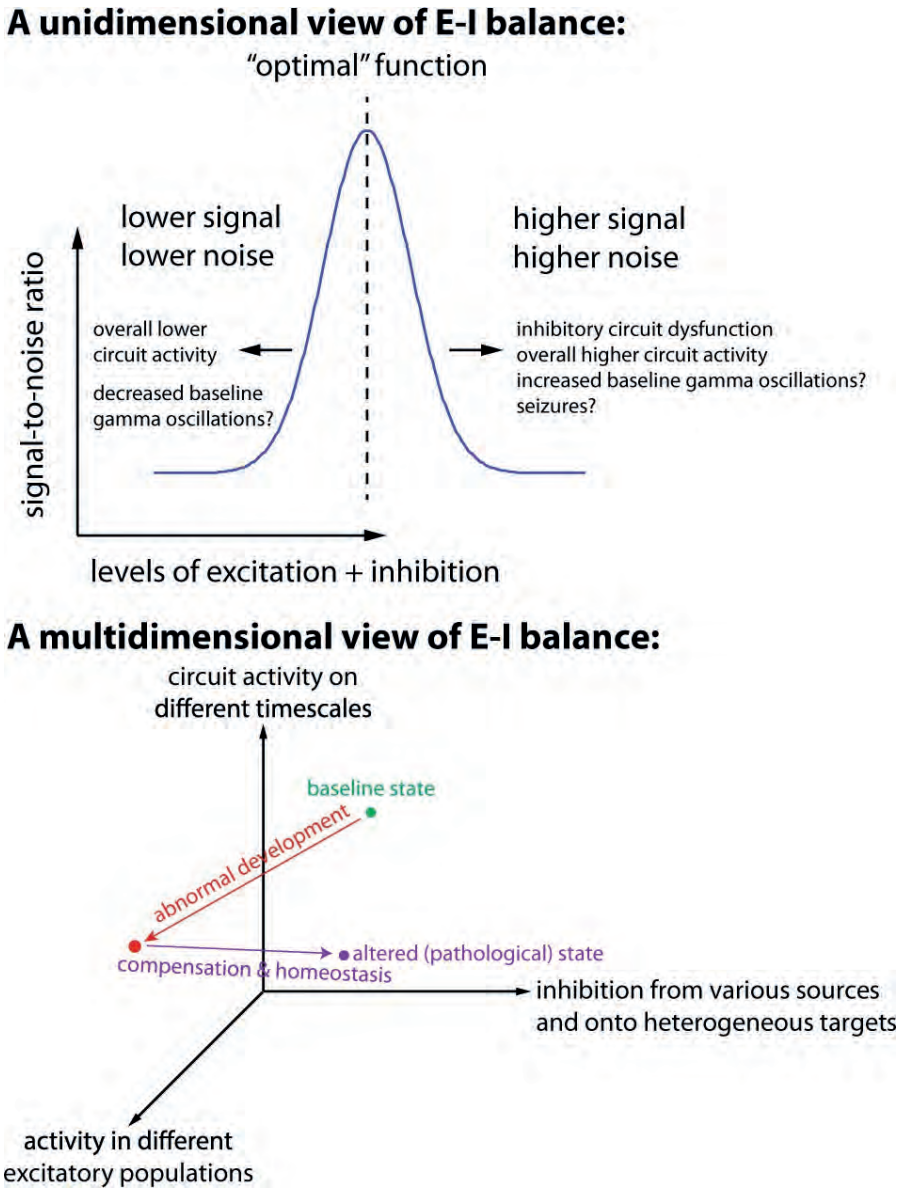


Figure 1: Unidimensional and multidimensional views of E/I imbalance

The top graph shows a unidimensional view of E/I imbalance in the brain, where alterations in the balance would be expressed at a higher overall circuit level. The bottom graph shows a visualization of a multidimensional view of several factors within the E/I system being affected, e.g. genetic variants, gene expression, or compensatory homeostatic mechanisms affecting excitatory and inhibitory circuits in the brain, ultimately underlying various autism traits. Image from (14).

E/I balance is a useful umbrella concept, but there are many mechanisms involved in excitatory and inhibitory function that make up complex, and adaptable, communication pathways across the brain (15,37–40). Many mechanisms are involved in excitatory and inhibitory functions, for example the amount of neurotransmitters within neurons, encapsulation and release of neurotransmitters, receptors on receiving neurons, and re-uptake by transporters to the transmitting neuron (10) to name a few. These are all excitatory and inhibitory functions, depending on the properties of different types of neural mechanisms. All of these are affected by genetics, where some genetic alterations may have strong individual effects on one of these functions, but many common genetic factors could also affect some or several in a combined fashion, resulting in an overall change in the E/I balance (37). A visual representation of some of these functions can be seen in Figure 2 below.

These mechanisms are not independent but make up a homeostatic system that regulates and fluctuates during cortical activity (38,39). This becomes particularly apparent when there are disruptions to the system, which is followed by compensatory mechanisms adjusting the balance between excitation and inhibition back to regular functioning (38). Compensatory mechanisms include neurons adapting their gain; integration of incoming excitatory and inhibitory signals, ultimately altering excitability. Neurons may also adjust receptor densities, and the number of synapses can be increased or decreased. These kinds of mechanisms make up a homeostatic system, where several functions both within neurons and across communication pathways adjust to maintain balance between excitation and inhibition. However, these homeostatic corrective mechanisms could themselves be affected in autism, further leading to maladaptive responses (37). Consequently, there are numerous potential changes leading to imbalances and maladaptive responses, generating various alterations across communication pathways in autism that may even be specific to certain brain regions (37). This can potentially explain and integrate the opposing results that we have seen in autism research so far, as the system could be adapting in various ways across autistic individuals (14,37).

Ways to assess excitation and inhibition

Essentially all aspects of the brain and its functioning pertains to excitatory and inhibitory mechanisms in some way – the structure of the brain reflects communication pathways and their functions, brain activity is the output of

excitatory and inhibitory mechanisms, and metabolite concentrations largely consist of neurotransmitters and functions surrounding them. The methods available for investigating excitation and inhibition capture distinct aspects of brain functioning that together provide different but informative insights into what may be altered in autism.

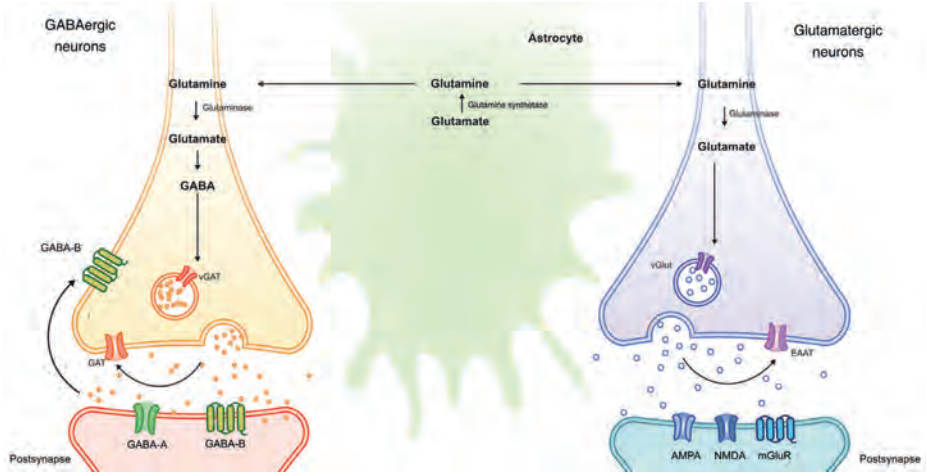


Figure 2: Neuronal communication mechanisms

The process of neuronal communication consists of many complex mechanisms both in a transmitting neuron, a receiving neuron, in the extracellular space between neurons and the supporting cells that surround them. Communication also varies between different types of neurons. For example, neurons require the production of neurotransmitters (e.g. GABA on the left or glutamate on the right), encapsulation into vesicles which are then taken to the membrane of the neuron at the synapse and releasing the neurotransmitters into the synaptic cleft. The membrane of receiving neurons contains receptors that capture these neurotransmitters. Inhibitory neurons tend to suppress postsynaptic neurons, whereas excitatory neurons increase the chances of an action potential. Transporters allow for reuptake of some of the neurotransmitters, to be released for recycling or breakdown. The image illustrates several of these mechanisms, and how they differ between excitatory and inhibitory neurons. All these steps are part of the excitatory and inhibitory mechanisms in the brain, which may be altered in autism (10). These functions are all intrinsically linked and part of a complex system of homeostatic mechanisms that allows for adaptation and habituation, maintaining the balance between excitation and inhibition, and are also affected by genetics (37,38). These compensatory mechanisms may also be affected in autism, leading to maladaptive alterations of the E/I systems. vGlut and vGAT in the presynaptic neurons are vesicular transporters; GAT and EAAT are transporters of neurotransmitters; GABA-A, GABA-B, AMPA, NMDA, mGluR are receptors of their respective neurotransmitters. Image is an adapted version from original image created by Duanghathai Pasanta.

We know that excitatory and inhibitory mechanisms are greatly determined by genetic factors. The heterogeneity of autism could be explained, at least partially, by variations in genetic underpinnings affecting E/I processes (6,37). Investigating selections of genes involved in functions we are interested in can provide a better understanding of how the functions of those genes link to other brain and behavior differences in autism. To understand excitatory and inhibitory mechanisms, a good place to start is to look at the most common excitatory neurotransmitter, glutamate, and the most common inhibitory neurotransmitter, γ-aminobutyric acid (GABA) (38,40,41).

Many factors may influence the path between our genes and our behaviors, and so an intermediate step is to look at the brain and its different functions to examine how genetic differences affect brain functioning, ultimately leading to behavioral autism characteristics. Understanding these relationships is further complicated as they are not static but change throughout development, which are captured by changes e.g. in brain structure and function and the effects of genes, while behavioral characteristics of autism often change within individuals throughout development as well.

Neuroimaging methods are incredibly useful to disentangle the differences in autism manifestations and changes throughout development, and here I focus on magnetic resonance imaging (MRI). Other methods such as EEG, pharmacological approaches and animal models also provide useful insights, but are beyond the scope of this thesis (for more details on EEG measures of E/I balance see (10,42), for pharmacological studies (10,11) and (29) for animal models). With MRI we can capture brain structure, functional brain activity, as well as estimate metabolite concentrations of glutamate and GABA. Box 1 contains more details on how these MR methods work.

Structural MRI captures tissue types involved in several functions of the brain, particularly excitation and inhibition. Neurons are present and reach across the whole brain in pathways, although, communication between neurons mainly happen in gray matter which is estimated with cortical thickness measures from structural MRI. Differences in cortical thickness likely indicate differences in how neurons connect and communicate. Alterations in cortical thickness have consistently been found in autism, both in childhood and through development into adulthood (43,44) reflecting alterations in neuronal pathways ultimately affected by differences in excitation and inhibition.

Hydrogen-Magnetic Resonance Spectroscopy (^{1}H-MRS) quantifies *in vivo* concentrations of metabolites in the brain based on hydrogen protons. Metabolites include glutamate and GABA, whose signals are predominantly from their presence in neurons (40). This is a unique and incredibly useful method as it is the only way we can capture estimated concentrations of metabolites, *in vivo*, in living humans in selected regions of interest.

We can estimate brain activity using functional MRI, reflecting output of temporary alterations between excitation and inhibition which is particularly relevant when aiming to understand how brain functioning may differ in autism. For instance, we can capture brain activity while people perform tasks that require behaviors and abilities often affected in autism, such as inhibitory control, related to the repetitive behavior domain of autism traits (45–47).

These measures are all pieces of the puzzle we need to disentangle how differences in the brain ultimately leads to the various behaviors and experiences of autistic people (10). Fortunately, there are now large datasets available that allow us to look at these things together, such as genetic markers of excitation and inhibition, neuroimaging methods capturing excitation and inhibition and factors affected by it, with behaviors typical to autism. Combining several of these measures within the same individuals, in large datasets, give us a unique opportunity to start to unravel what underlies the heterogeneous spectrum of autism.

Thesis aims and outline

In this thesis, each chapter aims to unravel the complex associations between brain and behavior relating to autism using multimodal measures and analysis methods. We take advantage of large multicenter datasets with both autistic and neurotypical individuals ranging from childhood into adulthood that have been deeply phenotyped by a plethora of behavioral and cognitive measures, combined with multimodal neuroimaging measures as well as genetics. Chapters 2 and 4 report on data from the COMPULS study (48), part of the TACTICS consortium, comprising of longitudinal data from participants between 8-16 years old. This cohort includes autistic participants and participants with OCD, aiming to disentangle overlapping phenotypes between the two conditions. Chapters 3, 4 and 5 report on data from the LEAP study (12), part of the AIMS-2-TRIALS consortium, the largest autism dataset of its kind that includes deeply genotyped and phenotyped autistic and neurotypical participants, combined with neuroimaging data. This too is a longitudinal cohort, including participants between 6-30 years old. These cohorts,

together with openly available datasets of neuroimaging and/or genetics in autistic individuals that are used for replication where possible (the Autism Brain Imaging Data Exchange (ABIDE (49)), the Allen Human Brain Atlas (AHBA, (50)), and the Simon Simplex Collection (SSC (51)), constitute a massive amount of data from both autistic and neurotypical participants. This allows us to finally investigate brain, behavior and genetic links of autism simultaneously, and doing so covering a broad age range from childhood well into adulthood, all of which has currently rarely been looked at together. An overview of all measures that will be used throughout this thesis is outlined in Figure 3 below.

Chapter 2 looks at longitudinal changes in glutamate concentrations in the brain, functional activity during inhibitory control and behavioral differences particularly in the repetitive behavior domain of autism, also looking at these associations in OCD. We focus on repetitive behaviors as they are amongst the most common and impactful autistic traits, and for its presence across the autistic and OCD participants. Using linear mixed effects models we investigate whether changes in glutamate concentrations are associated with repetitive behaviors, and whether such associations differ between autistic adolescents, those with OCD, or those with typical development.

Chapter 3 introduces genetic markers of glutamate and GABA, reflecting excitation and inhibition respectively, combined with structural MRI and behavioral measures of autism characteristics. Here, we start to bridge the links between genetics, brain and behavior domains using competitive gene-set analysis and gene expression analysis.

Chapter 4 builds on the findings of previous chapters using Bayesian Constraint-based Causal Discovery algorithms (BCCD), combining genetic markers of glutamate and GABA, functional activity during inhibitory control, and behavioral measures of autism characteristics. This method estimates causal relationships between these measures across the datasets from Chapters 2 and 3.

Chapter 5 combines both genetic and *in vivo* ^{1}H-MRS measures of glutamate and GABA. Using competitive gene-set analysis to link genetic variation and ^{1}H-MRS measures of glutamate and GABA, as well as using linear models, we investigate the relationships between these markers and their combined associations to behavioral traits of autism.

In the final chapter, **Chapter 6**, we discuss the overarching aim of this thesis and how the findings fit in a broader perspective of future challenges and opportunities to understanding autism.

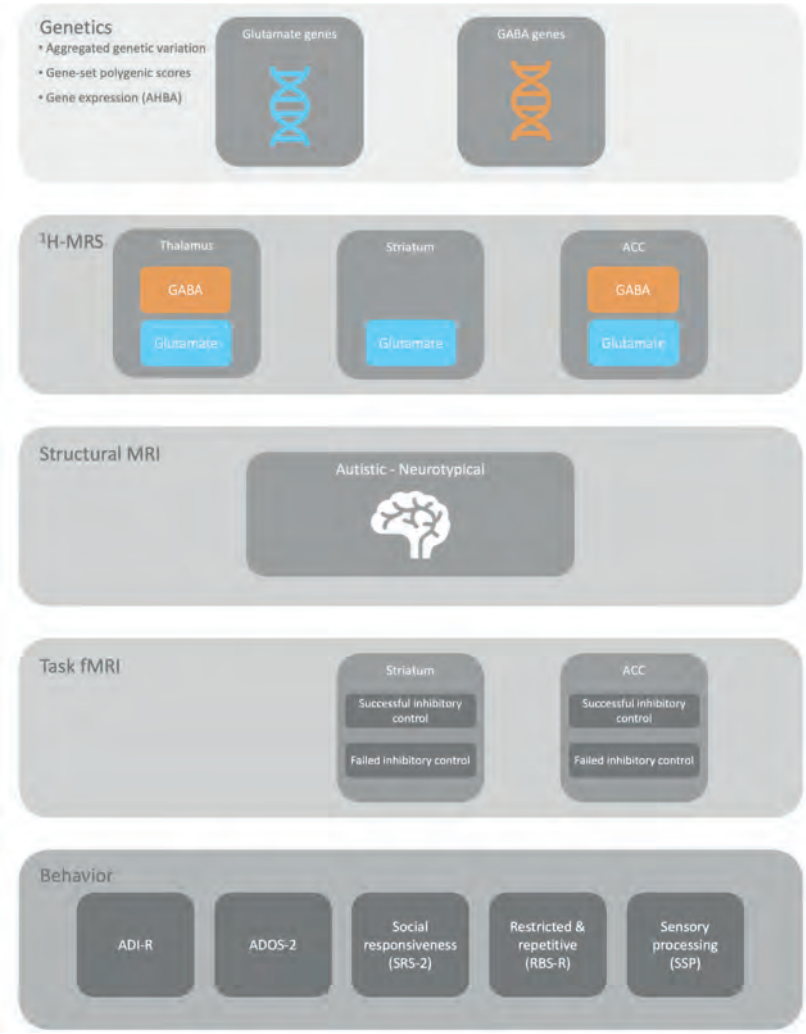


Figure 3. Levels of domains and their measurements included across all chapters

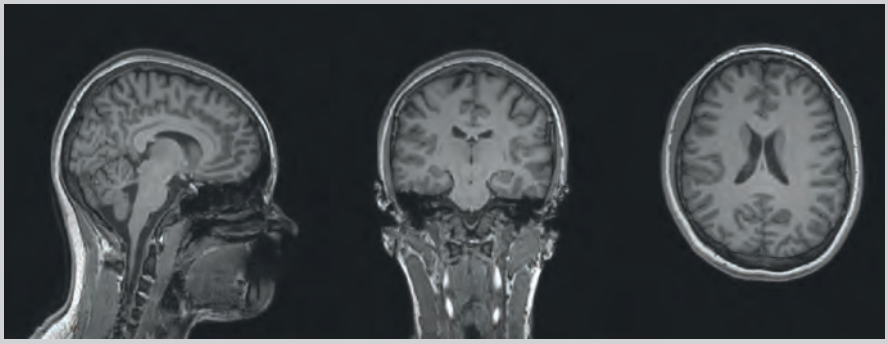
From the top, genetic measures included of selected gene-sets of glutamate and GABA communication pathways genes. AHBA, Allen Human Brain Atlas. ^{1}H-MRS, Proton Magnetic Resonance Spectroscopy, including measures of glutamate and GABA metabolite concentrations in thalamus, striatum and ACC. Structural MRI including T1-weighted data of cortical thickness differences between autistic and neurotypical participants. Task fMRI, fMRI contrasts of successful and failed inhibitory control. Behavioral measures were, from the left; ADI-R, Autism Diagnostic Interview-Revised; ADOS-2, Autism Diagnostic Observation Schedule-2; SRS-2, Social Responsiveness Scale Second Edition; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile.

BOX 1: Magnetic Resonance Imaging and Spectroscopy

Magnetic Resonance Imaging (MRI) is based on nuclear magnetic resonance (NMR), utilizing the magnetic behavior of hydrogen present in chemicals and tissues in the brain. Hydrogen protons act like little magnets, and outside of an MR scanner environment these spin along random directions. The MR scanner is a very strong magnet, making the magnetic spin of the hydrogen protons align along the direction of the magnetic field of the scanner. By applying various kinds of radio frequency (RF) pulses, flipping around and altering this magnetic spin within the brain in different ways, we can measure how these spins then relax back towards the main magnetic field of the scanner. This relaxation of spins vary across different tissue types, metabolites, and change due to blood flow, allowing us to capture these using different sequence types designed with different combinations of RF pulses. This is how we capture the anatomy, metabolism, and brain activity using MR sequences.

Structural MRI

In structural brain imaging we utilize the different signatures of hydrogen protons in water and fat to the magnetic field, giving different signal intensities. This means that once certain RF pulses are applied, the magnetization spins in different types of tissues relax back at different speeds to the main magnetic field of the scanner, giving us distinct signal intensities. In T1-weighted structural imaging this is disentangled into three tissue types: white matter, gray matter, and cerebrospinal fluid (CSF). These are easy to visually distinguish in a T1 structural image of the brain, where tissues with more fat appear brighter (white matter), and tissues with more water appear darker (gray matter) and CSF which contains even more water appear black. In this thesis we are mainly interested in gray matter, extracted in what we call estimations of cortical thickness, as this is where neurons connect and communicate.



Example of T1-weighted structural MRI image.

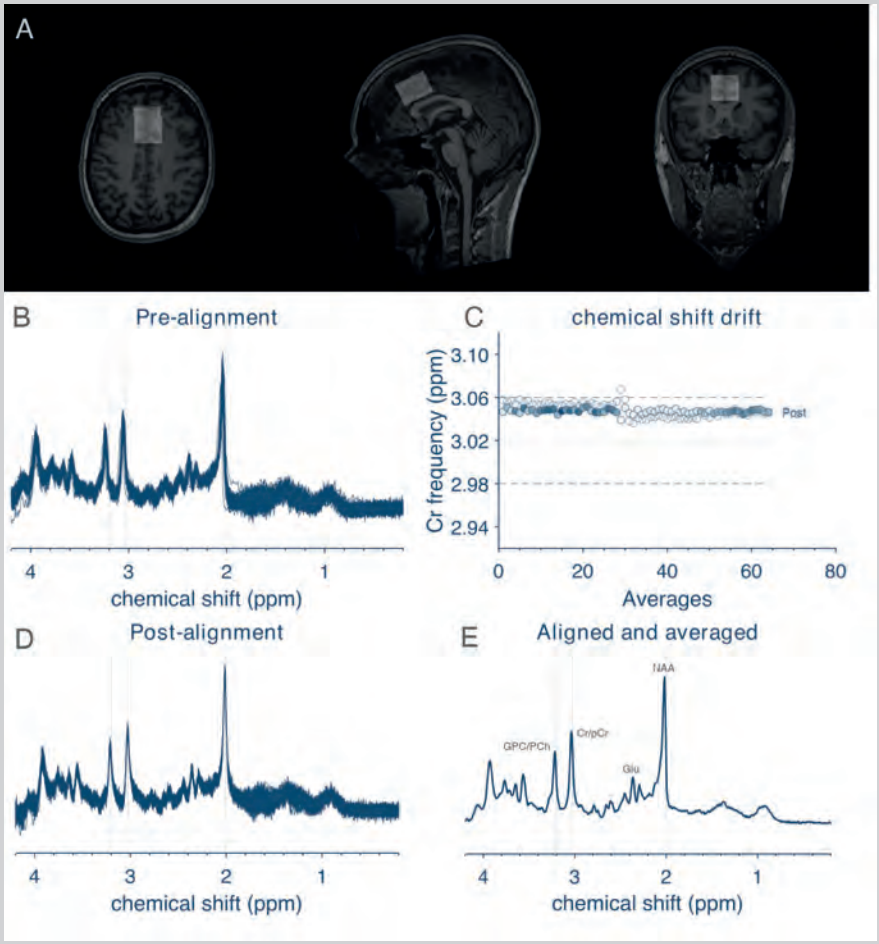
^{1}H-MR Spectroscopy (or ^{1}H-MRS) somewhat differs from other MR modalities. While structural and functional imaging rely on hydrogen protons in water, ^{1}H-MRS allows us to investigate other hydrogen containing molecules. ^{1}H-MRS captures the chemical shift of hydrogen protons, whose reaction to the magnetization of RF pulses varies between chemicals that contains them depending on their chemical environment. ^{1}H-MRS utilizes the differences in resonance frequency between chemicals, capturing metabolites present within cells in the brain. Due to their chemical structure, metabolites differentially affect the relaxation of the spins back to the magnetic field of the scanner, creating different resonance frequencies. Thus, different metabolites will have peaks at distinct resonant frequencies on a 'chemical shift' axis, where the area under the peak for each metabolite is proportional to its concentration. The chemical shift is measured in parts per million and metabolite concentrations are expressed relative to a reference compound, typically water or creatine. This makes ^{1}H-MRS a quantitative measure (52).

Water and fat have an about 10 000 times stronger signal than the metabolites we are interested in ^{1}H-MRS. Compared to T1 imaging, which utilizes the strong differences in signal from water and fat, we here need to remove those signals to reliably capture the metabolites in. Water is removed by suppressing the water signal. To avoid lipid contamination in the signal due to fat tissue, we avoid placing the voxel of measurement close to tissues outside the brain.

The ^{1}H-MRS signal is averaged across the whole area where acquisition was made, meaning that we do not get regional specificity across the brain using many small voxels, as in structural or functional MRI, but instead use one larger voxel, typically around 8-30 ml in total volume, which is placed over a region of interest (52). In ^{1}H-MRS, an acquisition is typically repeated many times and then averaged to increase signal-to-noise.

In this thesis we use two types of ^{1}H-MRS sequences: edited and unedited. While ^{1}H-MRS allows for detecting different metabolites, there is significant overlap between metabolites (e.g. two metabolites that both contain the same amino group will have overlapping signals). This complicates measurement of low concentration metabolites which are often masked by larger concentration molecules. Edited sequences are designed to capture signals from these low concentration metabolites by selectively affecting the metabolite of interest and cancelling frequencies that are not of interest. An example where this is very useful is when we want to measure GABA concentrations (53) where we

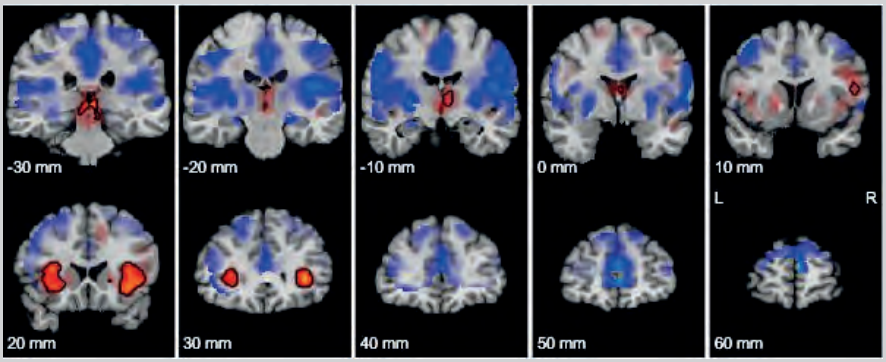
'selectively edit' for GABA (54). Unedited spectra on the other hand, capture the whole frequency range between water and fat, allowing us to capture a broader range of spectra of metabolites with higher concentrations and have higher signal to noise ratio (SNR). Glutamate can be measured well with unedited sequences.



Example of ^{1}H-MRS spectra. (A) shows the voxel placement in the anterior cingulate cortex (ACC). B-E shows an unedited ^{1}H-MRS spectrum, where E contains the sum spectrum of the average of the repeated measures of acquisition. Some peaks are labelled for their respective metabolite: GPC/PCh, glycerophosphocholine/phosphocholine; Cr/pCR, Creatine/Phosphocreatine; Glu, Glutamate; NAA, N-acetylaspartate.

Functional MRI

Functional MRI (fMRI) captures changes in blood flow in the brain, which reflects brain activity. When regions in the brain are more active the cells in that region requires more oxygen, which is delivered via the blood stream. Oxygenated blood (meaning blood coming *from* your heart) contains oxyhemoglobin, which is slightly more magnetic than deoxygenated blood (blood going *towards* your heart), making the relaxation times of oxygenated blood slightly longer. This means that increased brain activity increases the signal captured in fMRI, which is how we can measure brain activity. After processing the fMRI data, which is often done comparing for example brain activity while performing a task compared to when a person is not performing a task, we get contrast images showing where there is increased or decreased brain activity while performing the task, compared to when not performing the task.



Example of task-based fMRI contrast of brain activity during an inhibitory control task. Red and blue colors indicate regions with different levels of brain activity during failed inhibition of a button press compared to a successful button press during the task.

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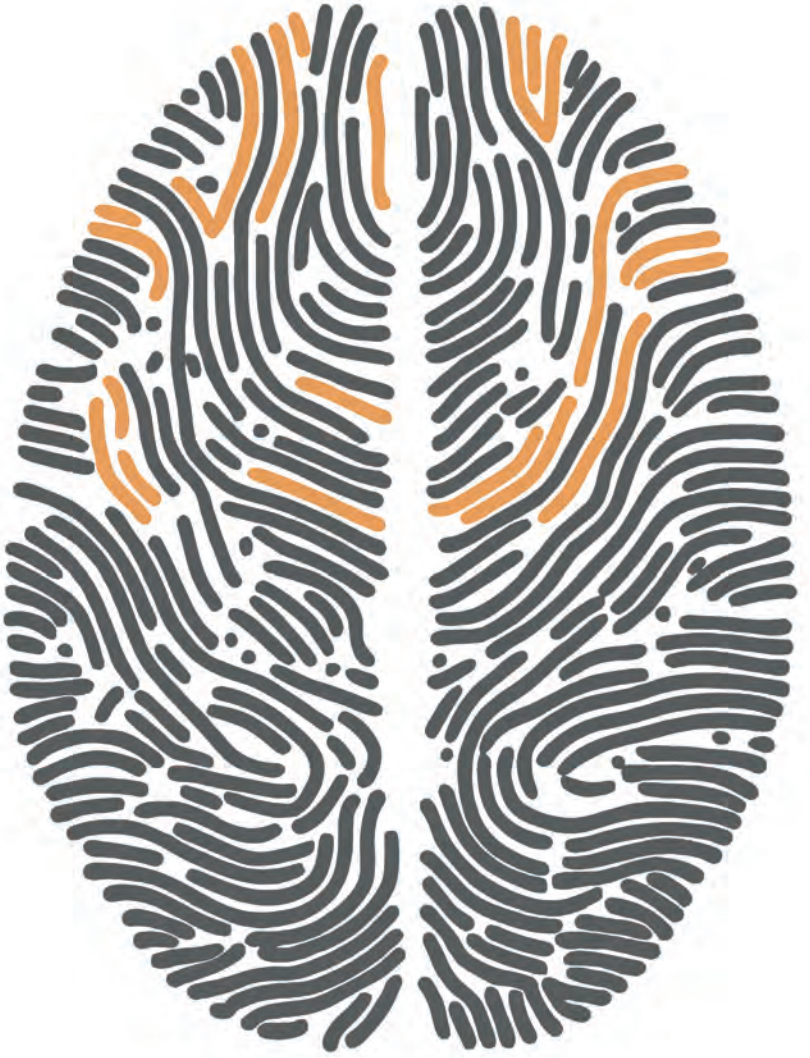

Chapter 2

Developmental changes in fronto-striatal glutamate and their association with functioning during inhibitory control in autism spectrum disorder and obsessive compulsive disorder

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This chapter is based on:
Hollestein, V., Buitelaar, J. K., Brandeis, D., Banaschewski, T., Kaiser, A., Hohmann, S., Oranje, B., Gooskens, B., Durston, S., Williams, S. C. R., Lythgoe, D. J., & Naaijen, J. (2021). **Developmental changes in fronto-striatal glutamate and their association with functioning during inhibitory control in autism spectrum disorder and obsessive compulsive disorder.** NeuroImage: Clinical, 102622. https://doi.org/10.1016/j.nicl.2021.102622

Language has been slightly adjusted from the published manuscript for consistency across this thesis.

Abstract

Autism spectrum disorder (autism) and obsessive compulsive disorder (OCD) show overlapping symptomatology and difficulties in inhibitory control, which are associated with altered functioning and glutamatergic signaling in fronto-striatal circuitry. These parameters have never been examined together. The purpose of this study was to investigate functioning during inhibitory control and its association with fronto-striatal glutamate concentrations across these conditions using a multicenter, longitudinal approach. Adolescents were either autistic (n=24), had OCD (n=15) or neurotypical (n=35) and underwent two magnetic resonance imaging (MRI) sessions with a one-year interval. This included proton magnetic resonance spectroscopy (^{1}H-MRS; n=74) and functional MRI during an inhibitory control task (n=53). We investigated ^{1}H-MRS data and fMRI data separately as well as integrated in a multimodal analysis using linear models focusing on diagnosis and continuous measures of overlapping compulsivity symptoms. ACC glutamate was reduced over time in the autism group compared with the neurotypical group, while striatal glutamate decreased over time independent of diagnosis. Increased compulsive behavior seemed to be associated with increased striatal activity during failed inhibitory control. The integrated analyses showed differential involvement of increased striatal glutamate during failed but decreased striatal glutamate during successful inhibitory control in the OCD group compared to the neurotypical and autism groups, suggesting different underlying mechanisms for OCD compared to autism.

Introduction

Although autism spectrum disorder (autism) and obsessive compulsive disorder (OCD) are two separate neurodevelopmental conditions with distinct diagnostic characteristics (1), they are highly co-occurring and a comparison of symptoms has suggested more similarities than differences between the two (2–4). However, not much is known about underlying mechanisms of the behaviors common among those with these conditions; restricted and repetitive patterns of behavior and/or compulsivity. The latter is defined as a repetitive, irresistible urge to perform certain behaviors or thoughts, and diminished control over this urge (5). Repetitive and compulsive behaviors are associated with difficulties in inhibitory control in tasks such as the stop-signal task (3,6). Fronto-striatal areas are known to be involved in inhibitory control and are regulated by the excitatory neurotransmitter glutamate (7–9). Within fronto-striatal circuity, the striatum is thought to be involved in driving the repetitive and compulsive behaviors, while frontal regions, such as the anterior cingulate cortex (ACC) is responsible for exerting inhibitory control (7,10–15). Imaging studies focusing on autism and/or OCD have shown altered fronto-striatal structure and function as well as altered glutamate conentrations, suggesting a possible shared underlying mechanism affecting repetitive and compulsive behaviors (10,11,16). Here, we investigated this by using a multicenter, longitudinal approach looking at associations of fronto-striatal glutamate and repetitive and compulsive behaviors on neural activity during inhibitory control in a childhood/adolescent cross-condition population.

In studies using the stop-signal task in autism and OCD, there have been inconsistent results. Some studies found no behavioral differences in autism and OCD compared with neurotypical participants (17–19), while others have found worse performance in participants with OCD (5,6,20–23), demonstrating difficulties in inhibitory control. However, these differences are more commonly found in adults with OCD than children and adolescents (24). Altered activity in fronto-striatal areas during inhibitory control has been found in both conditions as well (18,23,25–27), showing reduced activity during inhibition in ACC. Additionally, in autism increased activation has been found in left striatum compared to neurotypical participants, while this was decreased in OCD (27). Contrarily, some studies found altered functional activity despite not finding behavioral differences in response inhibition compared to neurotypical participants (28,29). In a previous study using a partly overlapping sample of the current study, no behavioral or neural alterations were found during inhibitory control in autistic and OCD participants (19).

The excitatory neurotransmitter glutamate is highly relevant for fronto-striatal functioning and inhibitory control. Altered concentrations of glutamate, investigated using Proton Magnetic Resonance Spectroscopy (^{1}H-MRS), have been linked to repetitive behaviors and compulsivity (7,30), which seem to differ in individuals with autism and OCD compared to neurotypical participants across development. A meta-analysis of ^{1}H-MRS studies investigating fronto-striatal glutamate in autism, OCD and attention-deficit/hyperactivity disorder (ADHD) reported that increased glutamate concentrations in striatum seems to be present across these conditions (7). In the ACC, on the other hand, glutamate concentrations were often higher in children and adolescents with these conditions while in adults the opposite pattern was found, with lower concentrations compared to neurotypical participants, suggesting a developmental shift (7). In a study investigating glutamate concentrations and neural functioning during inhibitory control, increased ACC glutamate was associated with decreased activity in striatum, but this was unrelated to any clinical diagnosis (9).

Evidence from these previous studies strongly suggests that investigating the interplay between glutamate and functional activity during inhibitory control is an important step for understanding the mechanistic underpinnings of behaviors across neurodevelopmental conditions. In a study including the first time of measure of the participants in this study, increased ACC glutamate was found in both autism and OCD groups, and a positive association between ACC glutamate and compulsive behaviors was found, while there were no group differences in striatal glutamate nor any association with behavior (8). In the current study we followed up this sample with a second timepoint of measurements using a multimodal, multicenter study design. With this developmental data we aimed to investigate whether changes in fronto-striatal glutamatergic alterations and functioning during inhibitory control differed across (atypical) neurodevelopment and whether there were any changes over time. Based on previous findings, we expected increased glutamate concentrations in fronto-striatal brain regions in the autism and OCD groups, especially in the ACC. As repetitive and compulsive behaviors likely decrease over time, we expected inhibitory control to be associated with these behaviors differently over time. In addition, we expected a differential role for glutamate here, which may affect functioning differently in autism and OCD as compared to the control group. These were exploratory analyses as the link between fronto-striatal functioning and neurochemistry has not been investigated in these groups before.

Methods and Materials

Participants

We included 74 participants (autistic = 24, OCD = 15, neurotypical = 35) for the ^{1}H-MRS analysis, between 8 and 16 years old at time-point 1 (T1), and between 9 and 17 years at timepoint 2 (T2). A previous manuscript describing the spectroscopy results of T1 included a total amount of n=133 participants (8). Reasons for drop-out for this longitudinal study were loss of interest by the participants and quality restrictions regarding the spectra. For the combined ^{1}H-MRS and fMRI analysis we included 53 participants. The participants were recruited at three different locations across Europe (Radboud University Medical Center and the Donders Institute for Brain, Cognition and Behavior, Nijmegen, The Netherlands (N = 38), Kings College London, London, United Kingdom (N = 17), Central Institute of Mental Health, Mannheim, Germany (N = 19)) in the multicenter study COMPULS, part of the TACTICS consortium (https://cordis.europa.eu/project/id/278948/reporting). Another site was excluded due to too few participants surviving quality control (N=3). The inclusion criteria were IQ > 70, ability to speak and comprehend the native language of the location of recruitment and being of Caucasian descent (for further details, see (8)). To confirm DSM-IV-TR (31) diagnoses of autism and OCD, we used the Autism Diagnostic Interview-Revised (ADI-R) (32) and Children´s Yale Brown Obsessive Compulsive Scale (CYBOCS) (33) for autism and OCD respectively. Participants in the autism and OCD groups were not allowed to have a diagnosis of the other condition of interest. Neurotypical participants were confirmed to not score in the clinical range for any DSM IV axis I diagnoses using the Child Behavior Checklist (CBCL) and the Teacher Report Form (TRF) (34), assessment of ADHD symptoms were measured using the Conners Parent Rating Scale (CPRS-R, (35). Repetitive and compulsive behaviors were measured using the Repetitive Behavior Scale – Revised (RBS-R) (36). Information on medication use was collected on the measurement days via parental report. Participants were asked to abstain from stimulant medication 48 hours before scanning. None of the participants received non-pharmacological treatment during the study. Ethical approval for the study was obtained for all centers separately and participants and their parents gave written informed consent for participation.

Stop-Signal Task

To measure inhibitory control, participants completed a modified visual version of the stop-signal task (SST) (37) during an fMRI session. For details of the design of the task see Figure S1 in the supplement. To ensure consistency across sites, task instructions were given according to a standard operating procedure (SOP).

Neuroimaging

Imaging Acquisition

Participants were familiarized with the MRI settings and practiced the SST using a dummy scanner at T1. At T2, the task was practiced again if needed. The data were acquired from the three study locations, all using 3 Tesla scanners (Siemens Trio and Siemens Prisma, Siemens, Erlangen, Germany; Philips Achieva, Philips Medical Systems, Best, The Netherlands; General Electric MR750, GE Medical Systems, Milwaukee, Wi, USA). Structural T1-weighted scans were acquired based on the ADNI GO protocols (38,39), which were used for registration of the functional scans and voxel placement for the ^{1}H-MRS.

Spectra were acquired using a point resolved spectroscopy sequence (PRESS) with a chemically selective water suppression (CHESS) (40) from the midline pregenual ACC and the left dorsal striatum covering caudate and putamen with an 8 cm^3 voxel size (2x2x2). Voxel locations were adjusted to maximize the amount of gray matter (GM) and minimize the cerebrospinal fluid (CSF) content to keep the quality of the data as high as possible. The locations of all voxel placements are shown in the supplement (Figure S2 and S3). Details on the structural, functional and ^{1}H-MRS scan parameters can be found in Table S1 in the supplement.

Imaging Analysis

fMRI. From the 74 participants included in analysis based on available ^{1}H-MRS data, 53 had available fMRI data included in analysis (ACC: autistic= 15, OCD=11, neurotypical= 27; Striatum: autistic=13, OCD=9, neurotypical= 24). Preprocessing of the fMRI data was performed using FSL (https://fsl.fmrib.ox.ac.uk/fsl/docs/#/). The first five volumes from each scan were removed to account for equilibration effects. Head movement correction was performed by realigning to the middle volume (MCFLIRT; (41)). A Gaussian kernel with full width at half maximum (FWHM) of 6 mm was used for grand mean scaling and spatial smoothing. ICA-AROMA (42,43) was then used to remove signal components related to secondary-head motion artefacts, subsequently followed by nuisance regression to remove signal from CSF and white matter (WM), and high-pass filtering (100 sec). These images were co-registered to each participants' anatomical scan using boundary-based registration within FSL-FLIRT (44). The anatomical scans were spatially normalized using a 12-parameter affine registration to MNI152 standard space, which was refined by non-linear registration FSL-FNIRT (45). The images were then brought into standard space by applying the resulting warp fields to the concatenated functional image. Neural activation during inhibitory control was analyzed using

SPM12 (Statistical Parametric Mapping release 12, https://www.fil.ion.ucl.ac.uk/spm/). For the whole-brain analysis during the stop-task, the first level models included two contrasts of interest; (1) failed stop – successful go, to isolate failed inhibitory control and (2) successful stop – failed stop, to isolate successful inhibitory control. For the second level analyses regarding differences across groups and times of measure, we used a full-factorial design where t-contrasts were applied to the first level contrast maps. To investigate the association between our spectral data and the fMRI data we extracted the mean beta weights during both failed and successful inhibitory control from the ACC and dorsal striatum regions of interest as extracted from the ^{1}H-MRS voxels. This was done using the MarsBar toolbox (46).

^{1}*H-MRS*. Glutamate concentrations were estimated using Linear Combination Model (LCModel), using water as reference (47,48). Example fitted spectra for ACC and striatum can be seen in Figure 1. As different tissues contain different amounts of water, correction for tissue percentage and partial volume effects was calculated using the formula:

$$Metabolite_{corrected} = Metabolite_{Raw} \times \left(\frac{(43\,300 \times f_{GM} + 35\,880 \times f_{WM} + 55\,556 \times f_{CSF})}{35\,880}\right) \times \left(\frac{1}{(1 - f_{CSF})}\right)$$

where 43 300 is the water concentration in millimolar for GM, 35 880 for WM and 55 556 for CSF, as described in the LCModel manual (47).

Criteria for quality control were the signal-to-noise ratio being ≥ 15, Cramér-Rao lower bounds ≤ 20%, and FWHM ≤ 0.1 parts per million. This resulted in 74 participants included in the analysis of ACC glutamate (autistic = 24, OCD = 15, neurotypical = 35), and 55 participants included for striatal glutamate (autistic = 18, OCD = 11, neurotypical = 26). To check for possible influences of glutamine we performed quality controls of glutamine concentrations, which only survived quality control measures for one participant for the ACC voxel and ten participants for the striatum voxel. We therefore do not report Glx (glutamate + glutamine) measures and report only glutamate. The raw glutamate levels can be found in Table S3 in the supplement.

Statistical analyses

Statistical analyses were performed using the R-software package (49), unless otherwise stated.

We first investigated changes in fronto-striatal glutamate concentrations, neural activation and behavioral responses during inhibitory control over time separately.

Changes in these scores over time were calculated by subtracting glutamate levels, or neural responses, in the spectral regions of interest and measures of compulsivity and inhibitory control at T1 from T2. These are reported as change-scores (Δ). Diagnosis, ΔRBS-R total and ΔRBS-R compulsivity scores were used as predictors in separate models. Age, sex and scan-site were included as covariates of non-interest in all analyses. Because age and sex did not affect the results nor influenced the model(s), they were removed from further analyses. To test general effects of time we used linear mixed effects models, where participant was added as a random factor to account for within subject variability across time (lme4 package (50)). Additionally, we investigated whether ADHD characteristics was associated with glutamate concentrations by including the CPRS-R scores in separate models. As there were no associations of ADHD characteristics, CPRS-R scores were not included in subsequent models in analyses.

Secondly, we combined spectral and functional analyses into a multimodal model investigating whether changes over time in one modality were associated with changes over time in the other modality using the ^{1}H-MRS voxels as regions of interest. Specifically, we investigated whether changes in glutamate concentrations in either region (ΔGluACC/Str) were associated with changes in neural activation (ΔbetaACC/Str) in the same region and whether this was different across groups and continuous measures of repetitive behavior. This resulted in twenty-four models listed in Table S2 in the supplement.

All reported *p*-values in all statistical tests are corrected for multiple comparisons by the false discovery rate (FDR, q <0.05), unless otherwise stated. Effect sizes are indicated as *r*.

Results

Demographics

No differences were found between groups in age, IQ or sex. Table 1 shows an overview of the demographics and clinical variables of the largest subsample used. For the repetitive and compulsive behaviors we used the RBS-R total scores and the compulsivity subscale scores at T1, T2 as well as the change over time (Δ). Although there was no general effect of time on these measures, there were significant differences between autistic, OCD, and neurotypical participants at all time-points. See Figure 2 for a summary of these results.

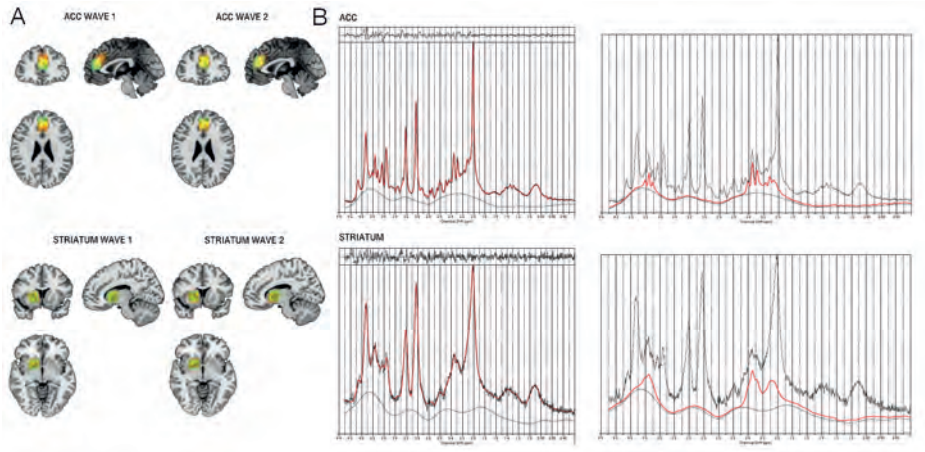

Figure 1: ^{1}H-MRS voxel placement

A: Superposition on the MNI152 template of all individual voxel placements in ACC and striatum, for autism (red), OCD (blue) and neurotypical (yellow) groups. The placements were consistent across diagnoses, as seen by the large overlap of voxels. For voxel placements across sites, see supplement. **B**: Example spectra of a 3T proton magnetic resonance spectroscopy (^{1}H-MRS) Linear Combination (LC) Model spectral fit in ACC and striatum from one of the control participants. The top of the images represents the residuals. The black line represents frequency-domain data, the red line is the LCModel fit. The right images show the fits for glutamate only. For examples of LCModel spectral fits and glutamate fits for each site, see Figure S5 in the supplement.

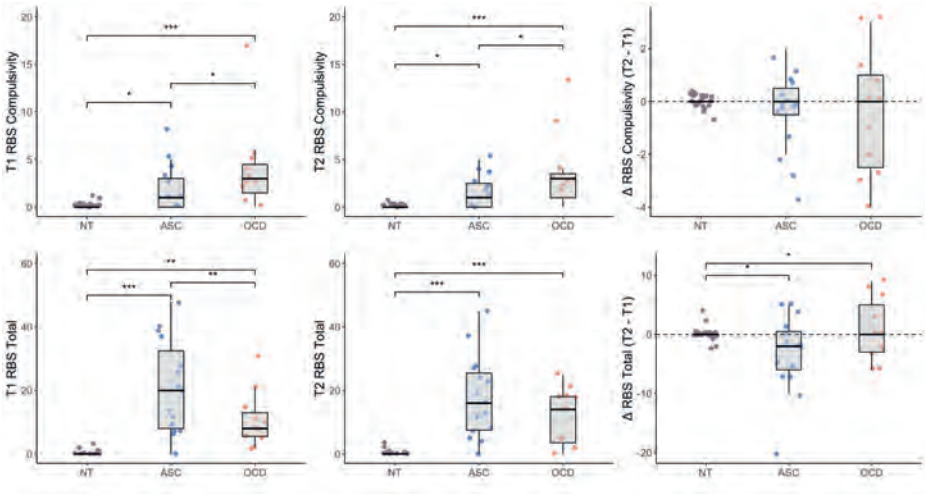

Figure 2: Repetitive and compulsive behaviors

Group differences in RBS-R compulsivity (upper panel) and RBS-R total scores (lower panel) at T1, T2 and over time. The OCD (N=15) group showed higher compulsivity than both autism (ASC) (N=24) and neurotypical (NT)(N=35) groups at both time-points without any differences in changes. Total RBS-R scores were highest in the autism group at both time-points while this group simultaneously showed the largest decrease in these symptoms between T2 and T1. * p <0.05, ** p <0.01, *** p <0.001.

Table 1: Demographic characteristics (based on the largest subsample group in analysis)

	Autism		**OCD**		**Neurotypical**	
Sex, m/f	17/7		9/6		21/14	
	Mean	SD	Mean	SD	Mean	SD
Age 1	11.38	1.64	11.95	2.49	10.70	1.38
Age 2	12.92	1.62	13.38	2.51	12.20	1.46
IQ[a]	109.38	15.07	109.89	15.56	111.84	11.05
RBS 1						
Total	24.86	24.46	15.67	19.09	0.80	2.14
Stereotype	2.79	3.27	2.00	2.80	0.06	0.24
Self-harm	1.38	2.06	1.40	2.77	0.06	0.34
Compulsivity	3.46	5.74	4.73	5.00	0.20	0.63
Ritualistic	5.17	6.03	3.73	4.30	0.08	0.28
Insist on sameness	9.71	8.61	2.87	5.89	0.26	0.92
Limited interests	2.46	2.78	0.93	1.16	0.14	0.36
RBS 2						
Total	20.61	19.48	11.86	9.71	0.46	1.06
Stereotype	2.26	2.25	1.71	1.69	0.03	0.17
Self-harm	1.96	3.62	0.71	1.69	0.00	0.00
Compulsivity	2.43	3.46	3.71	3.73	0.06	0.24
Ritualistic	3.83	4.31	2.29	2.91	0.12	0.33
Insist on sameness	7.63	6.34	2.07	2.62	0.18	0.53
Limited interests	2.30	2.75	1.36	1.45	0.06	0.24
MEDICATION[b]						
Stimulant	2		0		0	
Antipsychotic	0		1		0	
Antidepressant	1		5		0	

Autism, Autism Spectrum Disorder; OCD, Obsessive Compulsive Disorder; NT, neurotypical; SD, standard deviation; RBS, Repetitive Behavior Scale (36). KWχ2, Kruskal-Wallis Chi-Square. Post hoc tests were Bonferroni corrected. The number of participants per group is the largest subsample available across analyses. A: IQ was collected during the first time of measure. B: Medication use is indicated from first time of measure, changes in the second measure can be found in the supplement. 1 and 2 in the left column indicate first (T1) and second (T2) point of measure.

	Test statistic	p-value	Post-hoc
	KWχ2=0.81	0.667	
	KWχ2=5.61	0.061	
	KWχ2=5.27	0.072	
	KWχ2=0.50	0.781	
	KWχ2=49.75	<0.001	OCD & ASD > NT
	KWχ2=31.44	<0.001	OCD & ASD > NT
	KWχ2=20.18	<0.001	OCD & ASD > NT
	KWχ2=29.12	<0.001	OCD & ASD > NT
	KWχ2=40.04	<0.001	OCD & ASD > NT
	KWχ2=42.22	<0.001	ASD > OCD & NT
	KWχ2=20.42	<0.001	ASD & OCD > NT
	KWχ2=44.26	<0.001	OCD & ASD > NT
	KWχ2=31.99	<0.001	OCD & ASD > NT
	KWχ2=16.68	<0.001	ASD > OCD & NT
	KWχ2=30.08	<0.001	OCD & ASD > NT
	KWχ2=20.17	<0.001	OCD & ASD > NT
	KWχ2=37.76	<0.001	OCD > ASD > NT
	KWχ2=24.63	<0.001	OCD & ASD > NT

Spectral quality

Groups did not differ in mean voxel percentage GM, WM or CSF in both voxels (all *p*-values > 0.05). Percentage GM in striatum, however, was lower the second time of measure compared to the first one ((b=-0.07, $t_{(52)}$=- 2.97, *p*= 0.004), independent of diagnosis. Voxel placement during T1 and T2 and across scan-sites can be seen in the supplement in Figures S2 and S3. No differences were found between diagnostic groups or time-points for any of the measures. The autistic group showed, compared to the neurotypical group, an increase in glutamate Cramér-Rao lower bound (CRLB) over time (b=0.009, $t_{(71)}$=2.49, *p*=0.015), although with the highest CRLB of 14%, guaranteeing sufficient quality of these spectra at both timepoints (51).

Fronto-striatal glutamate

There was a negative association between diagnosis and ΔGluACC (b=-1.55, t=$_{(0.68)}$=-2.28, *p*= 0.026, *r*= 1.00), which indicated a larger decrease in ACC glutamate in the autistic group over time compared with the neurotypical group, but not OCD (*p* > 0.05; Figure 3A). In addition, the RBS-R total score was associated with ACC glutamate as well, where an increase over time in repetitive behaviors was related to a decrease over time in ACC glutamate (b=-0.12, $t_{(0.05)=-}$2.330, *p*=0.026, *r*=1.00; Figure 3B).

There was no effect of diagnostic status or any of the continuous measures on ΔgluStr (all *p*-values > 0.05). However, striatal glutamate decreased significantly over time, independent of group (b= -0.65, $t_{(52)}$ = -2.77, *p*= 0.023, *r*= 0.36).

Stop-Signal Task

All groups showed common patterns of brain activation during failed as well as successful inhibitory control, where there was activation in areas typically associated with inhibitory control, such as ACC and striatum (Figure S4). No significant differences in neural activation between groups were found at any time-point in any of our contrasts (all *p*-values > 0.05). However, using continuous measures of compulsivity and our fronto-striatal regions of interest, we found an effect of Δcompulsivity on Δstriatal activity (b=1.88, $t_{(0.51)}$=3.70, *p*=0.002, *r*= 0.98) during failed inhibitory control, where an increase in compulsivity over time was associated with an increased striatal activation, reflecting higher activity at T2, compared to T1. Behavioral results regarding the SST are described further in the supplement.

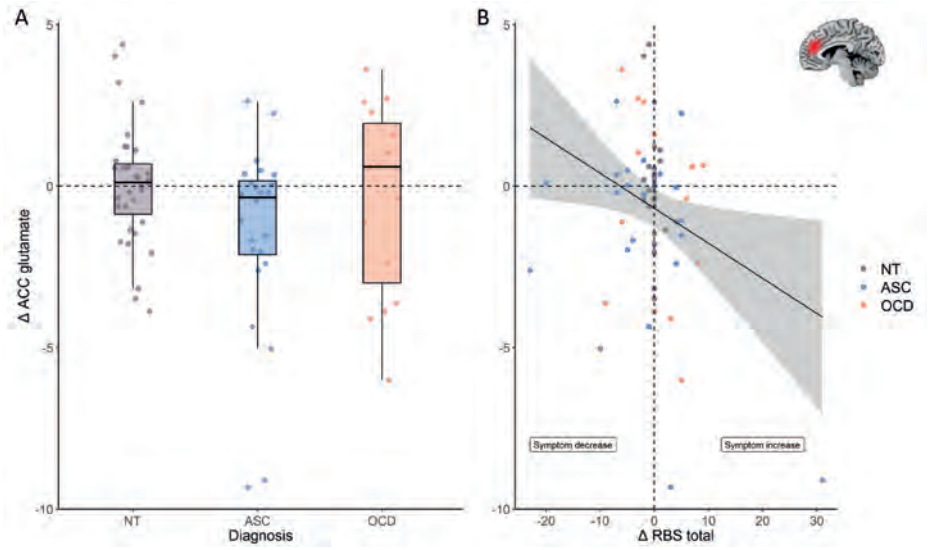

Figure 3: ACC glutamate

A: Glutamate concentrations, shown in institutional units (i.u.), decreased over time in the autism (ASC) (N=24) group (blue) compared with the neurotypical group (NT) (N=35) (gray). Plot was created using ggplot2 (52) and in-house adapted violin plots (53). * p <0.05. **B**: Effects of changes of changes in RBS-R total score on changes in ACC glutamate (in i.u.). The linear regression line shows a negative association of Δ RBS-R total score with changes Δ ACC glutamate, independent of group. The shaded area represents the 95% confidence interval. Dots on the vertical dashed line represent participants that did not change in RBS-R total scores. *Note*: this figure shows raw data-points, not model estimates.

Association between fronto-striatal glutamate and functioning

Failed inhibitory control

During failed inhibitory control there was a negative interaction between diagnosis and ΔgluStr on ΔbetaStr. This interaction showed that in the OCD group, an increase in striatal glutamate over time was associated with a decrease over time in activity in the same region compared to the neurotypical (b=-7.46, $t_{(2.19)}$=-3.412, p= 0.003, r= 0.92), and autism groups (b=7.73, $t_{(2.30)}$=3.36, p= 0.003, r= 0.91); see Figure 4A. There was no significant difference between the autism and neurotypical groups (all p-values > 0.05). No associations were found regarding the ACC or any interactions between glutamatergic changes and continuous measures of compulsivity (all p-values > 0.05).

Successful inhibitory control

During successful inhibitory control, there was a positive interaction between diagnosis and gluStr on ΔbetaStr. This time, again in OCD, an increase in striatal

glutamate over time was associated with an increase in striatal activity control compared to the neurotypical (b=0.96, $t_{(0.41)}$=2.33, p= 0.025, r= 0.96), and the autism (b=1.04, $t_{(0.43)}$=2.40, p= 0.025, r= 0.96) groups, see Figure 4B. There was again no significant difference between autism and neurotypical groups (all p-values > 0.05) nor any other significant associations for the ACC or continuous measures of compulsivity (all p-values > 0.05).

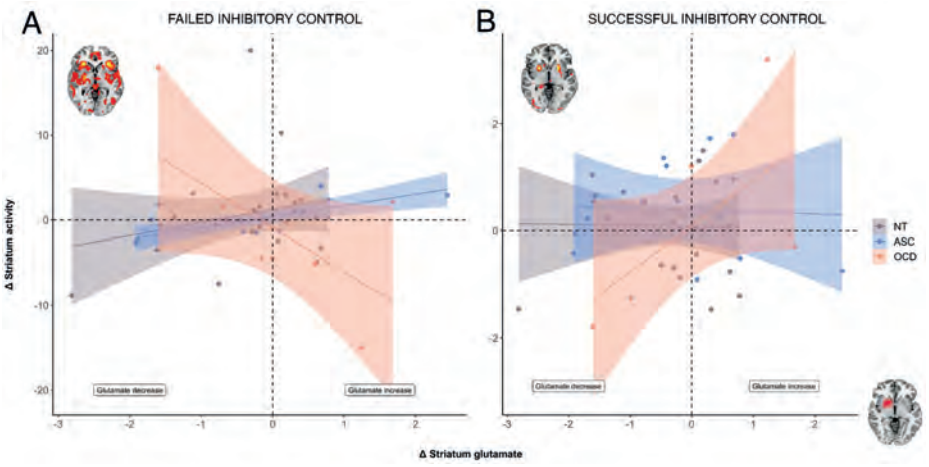

Figure 4: Failed and Successful inhibitory control

A: During failed inhibitory control, an increase in striatal glutamate (i.u.) was associated with a decrease in striatal BOLD signal in the OCD (N=9) group (salmon) compared to the neurotypical group (NT) (N=24) (gray) and autism (ASC) (N=13) (blue) groups. **B**: During successful inhibitory control, an increase in striatal glutamate (i.u.) was associated with an increase in striatal BOLD in the OCD group compared to neurotypical and autism groups. Brain activity is shown on the axial slice for both failed and successful inhibitory control outlining the striatal voxel as an overlay. Activity is presented at p <0.01 (uncorrected) for visualization purposes. The shaded areas represent the 95% confidence intervals. *Note:* This figure shows raw data-points, not model estimates

Discussion

This is the first study that used a multicenter, longitudinal, transdiagnostic approach to investigate the associations of repetitive behaviors and compulsivity with fronto-striatal glutamate concentrations and functioning during inhibitory control in a childhood/adolescent cross-condition population.

Our ^{1}H-MRS only results showed that over time there was a reduction in ACC glutamate in autistic compared with neurotypical participants, while an increase in repetitive behaviors over time was associated with decreased glutamate in the

same region. Previous studies investigating autistic children have shown higher glutamate concentrations in ACC (54–56), while studies looking at autistic adults have found both lower and higher glutamate concentrations in ACC compared to neurotypical participants (7,57). Our finding may therefore reflect changes in development in autism being different from neurotypical development. We found no such differences in the OCD group, although they did not significantly differ from the autism group either, and previous studies with OCD have shown inconsistent results (7). This may be due to a larger heterogeneity in the disorder, and future studies considering possible subtypes of OCD may successfully disentangle such differing results. However, the previous study investigating an overlapping sample (however, larger) at T1 found increased ACC glutamate in both autism and OCD (8). In the striatum we found that glutamate decreased over time independent of diagnosis. This is in line with the study that found no group differences in striatal glutamate during the first time of measure (8), which is also reflected at T2. Alterations in metabolite concentrations are also known to occur in neurotypical development (58), and our finding may reflect such development in striatum, independent of a clinical diagnosis.

Regarding neural activation, we did not find any group differences, time effects nor effects of our continuous measures in our whole brain analyses for neither failed nor successful inhibitory control. This was in line with the findings of T1 by Gooskens et al. (19). However, other studies with similar behavioral results still found altered brain activation during inhibitory control (17,23,26,27,29). Although we were not able to find any whole-brain differences, looking at our region of interest we found that an increase in compulsivity over time was associated with increased striatal activation over time, but only during failed inhibitory control. Increased compulsivity may thus be associated with more difficulties with inhibition, resulting in more striatal activity, reflecting an increased cognitive demand. Our longitudinal TACTICS study on inhibitory control in autism and OCD found improvements in SSRT over time, regardless of group (19). In our partly overlapping subsample in this study, as shown in the supplement, we do not replicate this finding but show that males performed better than females. That we did not find a general improvement may, however, be due to a lack of power and/or a larger proportion of males in this subsample.

Integrating all these analyses for the first time in a multimodal fashion, investigating the association between developmental changes in glutamate concentrations as well as fronto-striatal functioning, resulted in differential findings across failed and successful inhibitory control. While during failed inhibitory control, OCD participants

showed decreased striatal activity with an increase in striatal glutamate over time, the reverse was found for successful inhibitory control; increased concentrations were associated with increased activity, again in the OCD group. Both these findings were significant compared with neurotypical and autistic participants. These results suggest differential involvement of striatal glutamate in neural activation patterns in OCD compared with neurotypical and autistic participants during different aspects of inhibitory control. To successfully inhibit responses, more glutamate resulted in more activity, suggesting a compensatory mechanism to fulfill the cognitive demands of the task, even though behaviorally there were no differences in performance. As these results show significant changes over time in our ~1 year time window between measurements, our results also suggest there may be critical differences in neural measurements in childhood/adolescent neurodevelopmental populations. This needs further investigation but may explain inconsistent results in neuroimaging results with child/adolescent populations in these conditions.

Considering that the OCD group showed higher compulsivity scores compared to the neurotypical and autism groups without any changes over time (Figure 2), associations of both changes of glutamate in OCD and compulsivity on striatal activity during failed inhibitory control may point towards the same mechanistic differences for achieving the same neural activation. A recent study using a network analysis has suggested that compulsivity as seen in OCD and repetitive behaviors as seen in autism represent distinct features of these conditions (59), rather than symptom overlap between the two, which has also been suggested (60,61). Our OCD and autism results do not overlap, but were found within the different regions of the fronto-striatal circuit (OCD findings in the striatum, autism findings in the ACC). This indeed suggests that compulsivity in OCD and repetitive behaviors in autism have distinct mechanistic underpinnings that are regionally specific and differently regulated by glutamate, despite the seemingly similar behavioral phenotypes. Considering the very limited research on these measures during adolescence, even more so in OCD than in autism, these results are an important step towards increasing understanding of underlying mechanisms of development in compulsivity-related disorders. Further studies should confirm this initial finding, but this may contribute to targeted glutamate altering interventions in OCD.

Strengths of the current study were combining categorical and dimensional analyses, with a longitudinal approach to investigate the relationship between repetitive and compulsive behaviors, fronto-striatal glutamate as well as functioning. There were also some limitations. Firstly, the OCD group was smaller than the autism group, which may have led to less power and the possibility of false negatives. However,

we still found significant associations with changes in glutamate concentrations affecting changes in functional activity in OCD. Furthermore, the percentage GM in striatum decreased over time, suggesting worse voxel placement. However, these changes were not different across diagnostic groups and therefore probably did not affect our main findings. As ability to speak their native language and IQ > 70 were inclusion criteria in this study, this may have resulted in excluding autistic participants with higher support needs. Therefore, our autism specific results may not be generalizable to the entire population of autistic individuals. There are also difficulties performing multicenter studies, where data quality may differ across sites. However, we did manage to control for these effects in our models and our results were likely not affected by left-over site effects. Future studies should use a true longitudinal model with a longer time-period in between and preferably a larger sample size to increase the understanding of these integrated mechanisms underlying autism and OCD. To further investigate similarities and differences between these conditions regarding compulsivity and repetitive behaviors we also suggest using a larger battery of measures of compulsivity and repetitive behaviors, to disentangle what variations of these features differ between these diagnostic groups, and what their underlying mechanisms are.

In conclusion we found, over time, significant associations in OCD of increased glutamate concentrations in striatum with decreased functional activity in striatum during failed inhibitory control, and an opposite effect of increased striatal glutamate concentrations with increased striatal activity during successful inhibitory control. Increased compulsivity was also associated with increased striatal activity during failed inhibitory control. While glutamatergic alterations were differently involved during neural activation in OCD, there were no general changes in glutamate in the OCD group over time compared with neurotypical participants. In the autism group on the other hand, we found ACC glutamate to decrease more over time compared with neurotypical participants. These results should be replicated in an independent sample, but this study has given new insights into the alterations of glutamate in autism and OCD during development in adolescence, and its role in functional activity.

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Supplement

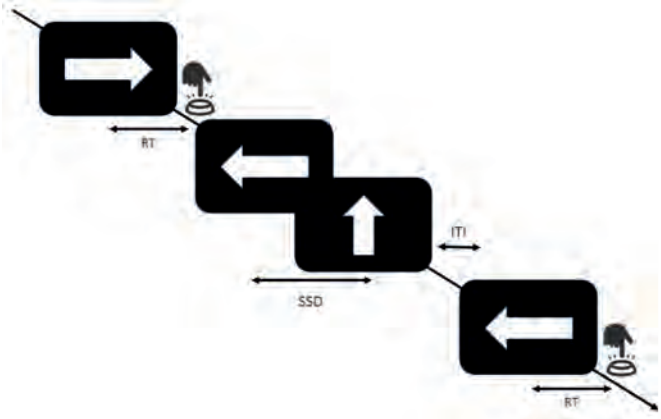

Figure S1: Stop-signal task

Arrows were presented on a screen; the task was to press a button indicating the direction the arrow was pointing at. In 20% of trials the arrow was followed by a stop cue of an arrow pointing upwards, instructing to withhold a response. The stop-signal delay (SSD) between stimulus onset and stop-signal was adaptive, where the SSD after successful inhibition increased with 50 ms while after failed inhibition it decreased with 50 ms. This ensured participants success to inhibit in approximately 50% of the stop-trials. The inter-trial interval (ITI), the time between the trials, was randomly jittered between 1.6 and 2.0 seconds.

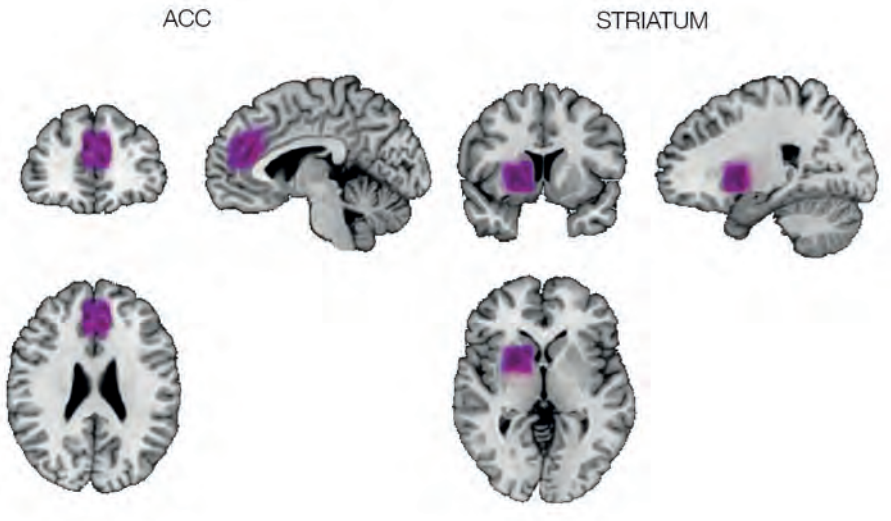

Figure S2: Voxel overlays across timepoints

Superposition on the MNI152 template of all individual voxel placements in ACC (left) and striatum (right), across times (First time of measure, blue; Second time of measure, red). The placements are consistent across times of measures.

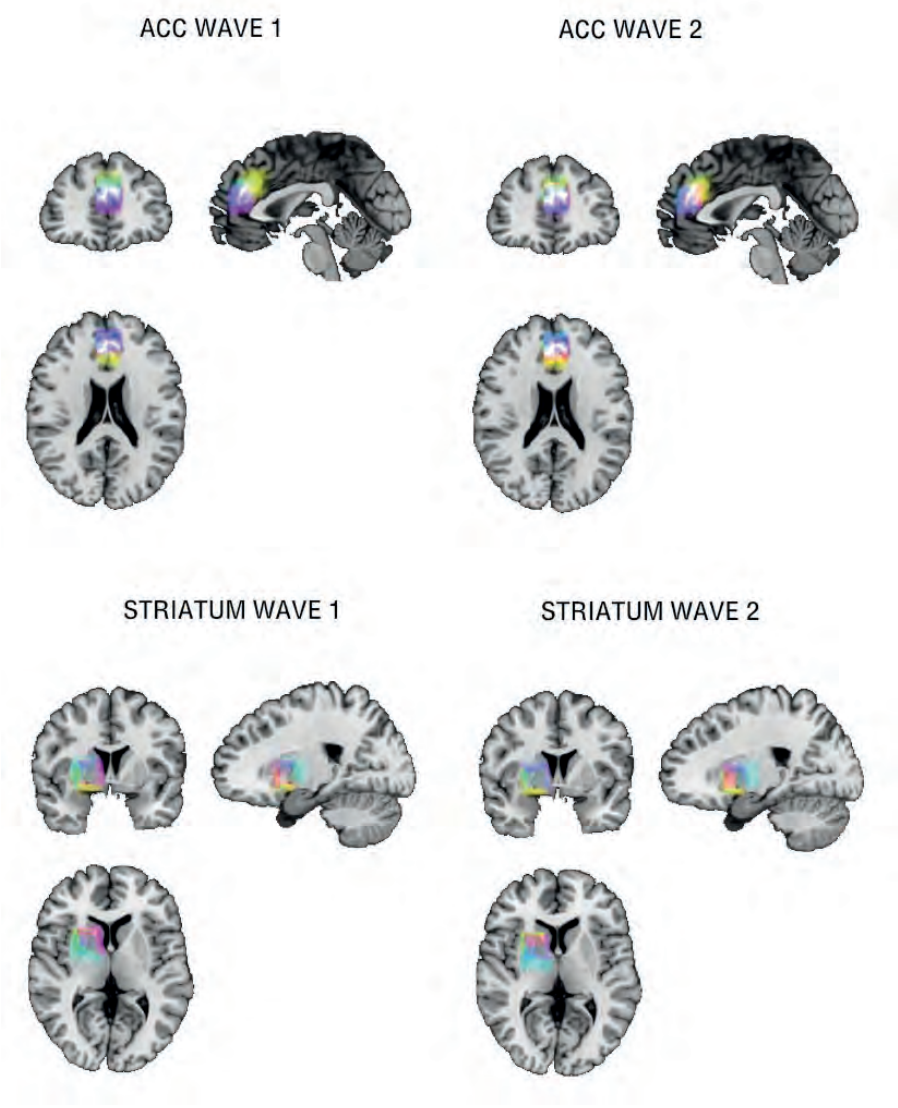

Figure S3: Voxel overlays across sites

Superposition on the MNI152 template of all individual voxel placements in ACC and striatum, for all sites (London, blue; Mannheim, yellow; Nijmegen, pink). The placements are consistent across and within sites. For more detail across-site acquisition, see (1) and (2).

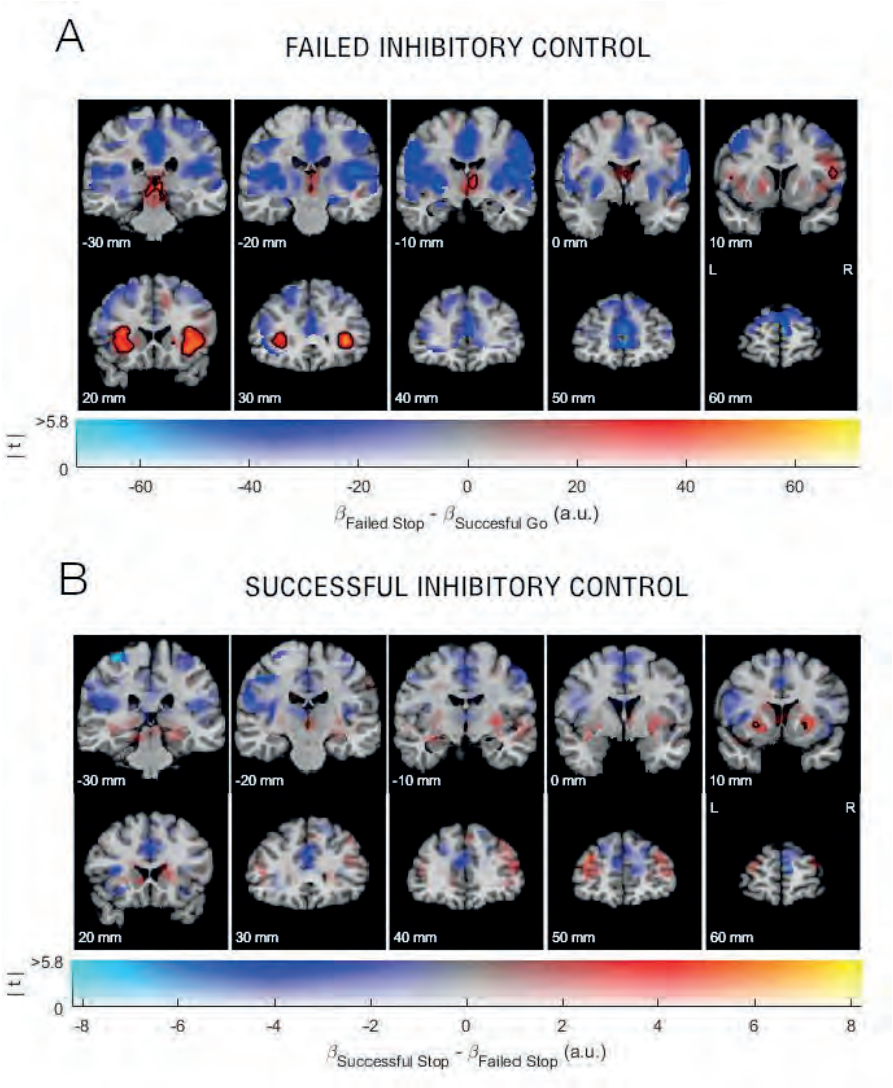

Figure S4: Task activation contrasts

Task activation across all groups during (**A**) failed inhibitory control (failed stop – successful go) and (**B**) successful inhibitory control (successful stop – failed stop), which showed common patterns of activation. The colors reflect uncorrected activation, voxels with a black line around the color reflect survived correction at p_{FWE} = 0.05 showing fronto-striatal activation during cognitive control. The numbers below the color bars reflect beta-values. Neuroimaging data are plotted using a procedure introduced by (3) and implemented by (4).

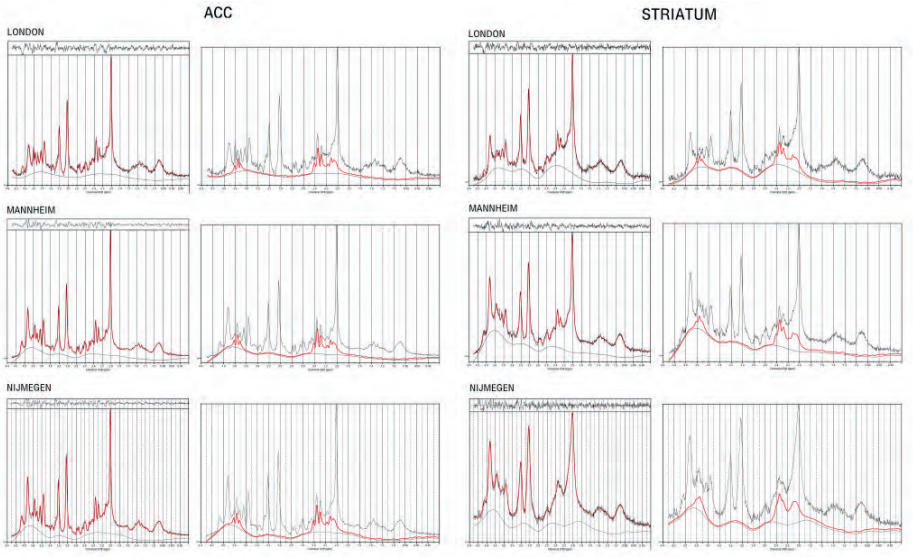

Figure S5: Example spectra

Example spectra of a 3T from proton magnetic resonance spectroscopy (^{1}H-MRS) Linear Combination (LC) Model spectral fit in ACC and striatum across all sites from separate participants. The top of the images represents the residuals. The black line represents frequency-domain data, the red line is the LCModel fit. The right images show the fits for glutamate only.

Table S1: Scan sequences

Sequence	Site	TR/TE/TI (ms)	Flip angle	Field of view (mm)	Matrix RL/ AP/slices	Voxel – size (mm)	Gap (%)	Parallel Imaging	Averages Water suppressed/ unsuppressed
T1	Nijmegen (Siemens)	2300*/2.98/900	9	256	212/256/176	1.0´1.0´1.2	NA	2	NA
	Mannheim (Siemens)	2300*/2.98/900	9	270	212/254/176	1.1´1.1´1.2	NA	2	NA
	London (GE)	7.31*/3.02/400	11	270	256/256/196	1.1´1.1´1.2	NA	1.75	NA
^{1}H-MRS PRESS	All	3000/30/-	NA	NA	NA	20´20´20	NA	NA	96/16
Functional MRI	All	2070/35/-	74	192	192/192/36	3.0´3.0´3.0	13	2	NA

*As provided by the manufacturer. GE defines a TR as the time between excitation pulses, while Siemens defines TR as the time between inversion recovery pulses.

Details multimodal analysis

Linear models were used for our multimodal statistical analyses using the lm function available in the base package in R (RStudio Team, 2016). Our integrated analyses of the ^{1}H-MRS and fMRI data resulted in twenty-four models: Δbeta in ACC or striatum during failed or successful inhibitory control were dependent variables (4), and our (continuous) predictors of interest were ΔGluACC or ΔGluStr (2), together with diagnostic status, ΔRBS compulsivity or ΔRBS total (3); 4 x 2 x 3 = 24 models. Site was added as a predictor of non-interest to all models, to account for site-effects on our measures. All models are listed in Table S2. These models test associations of the predictors (right side of Table S2) on neural activity in our regions of interest during inhibitory control (left side of Table S2).

For analyses of glutamate concentrations in ACC and striatum associated with time and diagnosis independently, linear mixed effects models were used using the lme4 package (Bates et al., 2014). The lmer function was used to fit linear mixed-effects models:

GluACC ~ Diagnosis * Time + Site + (1|Participant)
GluStr ~ Diagnosis * Time + Site + (1|Participant)

For analysis of SSRT group comparison over time the following model was used:

SSRT ~ Diagnosis * Time + Site + (1|Participant)

The linear mixed effects models account for within subject variability over time by adding participant as a random factor.

Table S2: Linear regression models of multimodal analyses

Failed inhibitory control		
	ΔbetaACC	~ ΔGluACC * Diagnosis + Site
	ΔbetaACC	~ ΔGluACC * Δ RBS Total score + Site
	ΔbetaACC	~ ΔGluACC * Δ RBS Compulsivity + Site
	ΔbetaACC	~ ΔGluStr * Diagnosis + Site
	ΔbetaACC	~ ΔGluStr * Δ RBS Total score + Site
	ΔbetaACC	~ ΔGluStr * Δ RBS Compulsivity + Site
	ΔbetaStr	~ ΔGluACC * Diagnosis + Site
	ΔbetaStr	~ ΔGluACC * Δ RBS Total score + Site
	ΔbetaStr	~ ΔGluACC * Δ RBS Compulsivity + Site
	ΔbetaStr	~ ΔGluStr * Diagnosis + Site
	ΔbetaStr	~ ΔGluStr * Δ RBS Total score + Site
	ΔbetaStr	~ ΔGluStr * Δ RBS Compulsivity + Site
Successful inhibitory control		
	ΔbetaACC	~ ΔGluACC * Diagnosis + Site
	ΔbetaACC	~ ΔGluACC * Δ RBS Total score + Site
	ΔbetaACC	~ ΔGluACC * Δ RBS Compulsivity + Site
	ΔbetaACC	~ ΔGluStr * Diagnosis + Site
	ΔbetaACC	~ ΔGluStr * Δ RBS Total score + Site
	ΔbetaACC	~ ΔGluStr * Δ RBS Compulsivity + Site
	ΔbetaStr	~ ΔGluACC * Diagnosis + Site
	ΔbetaStr	~ ΔGluACC * Δ RBS Total score + Site
	ΔbetaStr	~ ΔGluACC * Δ RBS Compulsivity + Site
	ΔbetaStr	~ ΔGluStr * Diagnosis + Site
	ΔbetaStr	~ ΔGluStr * Δ RBS Total score + Site
	ΔbetaStr	~ ΔGluStr * Δ RBS Compulsivity + Site

ΔbetaACC/ ΔbetaStr: Changes in neural activation in ACC/striatum between time-point 1 (T1) and time-point 2 (T2), during failed or successful inhibitory control. ΔGluACC/ ΔGluAStr: Changes in glutamate concentration in ACC/striatum between T1 and T2. "~" indicates that the variables on the right side are associated with the dependent variable on the left hand side. The "*" between the variables of interest indicates that the model assesses these variables both independently and their interaction effects.

Table S3: Raw glutamate levels at T1 and T2

Diagnosis	ACC T1	ACC T2	STR T1	STR T2
Autism	10.46646461	8.496578355	NA	NA
Autism	9.502124709	9.887580258	8.247075833	6.352032402
Autism	10.45382499	8.428490578	NA	NA
Autism	9.808432659	9.344632423	6.673729796	6.263571408
Autism	9.03451682	9.839876294	6.941402246	6.487048865
Autism	8.459706588	8.431155876	6.536547776	4.619588719
Autism	10.12216162	10.4795116	4.877940024	7.320578073
Autism	12.28622171	7.932510748	4.56654398	5.242980373
Autism	9.528864663	9.623110444	5.694679044	6.126748945
Autism	8.762677237	7.701688908	7.093335165	6.294975144
Autism	8.678187241	9.172212197	NA	NA
Autism	15.74052401	15.49530981	9.466125109	9.237021295
Autism	22.9679727	13.85815441	9.251698141	11.4537308
Autism	16.79127614	14.39287469	8.051919207	9.004611253
Autism	14.73175341	16.9883202	9.28496324	9.112406219
Autism	13.63378153	16.26286471	9.060665591	7.549782644
Autism	14.17480125	12.66238483	9.056145735	7.362930309
Autism	12.12576328	11.93199961	NA	NA
Autism	9.161339697	9.192108149	NA	NA
Autism	10.09289446	8.424581493	NA	NA
Autism	10.61616663	10.41175931	6.46558221	7.254520547
Autism	10.9101927	8.294028346	6.704347808	5.705254053
Autism	20.07059274	15.04236284	6.313574257	6.402479415
Autism	17.61304837	8.295779564	6.927463488	6.594419822
Neurotypical	9.099615118	8.483204361	7.037234954	5.448861084
Neurotypical	11.90266353	10.1138775	8.379938904	7.000655466
Neurotypical	9.703567829	9.071750281	7.80662892	6.190655013
Neurotypical	9.390472411	9.032679628	6.445081954	6.760872956
Neurotypical	9.688986736	8.583364547	NA	NA
Neurotypical	11.67860923	14.28663776	NA	NA
Neurotypical	12.75080489	9.575479647	6.614020154	6.303383585
Neurotypical	7.664403144	7.655782073	8.646897829	5.834470139
Neurotypical	9.424915728	9.03764206	6.412028842	7.0184551
Neurotypical	11.35464345	9.890793357	7.099357725	7.158730934
Neurotypical	14.18055305	10.30268859	6.500372186	6.313729084
Neurotypical	8.3599096	9.949890644	6.344679076	6.160679051
Neurotypical	9.675412031	9.555373079	NA	NA
Neurotypical	9.532707858	8.17309906	5.971965993	6.647455325
Neurotypical	9.554553965	10.11461868	6.188594005	5.432526183
Neurotypical	8.349128716	9.130057346	NA	NA

Table S3: Continued

Diagnosis	ACC T1	ACC T2	STR T1	STR T2
Neurotypical	8.845944014	9.417837617	6.252027577	5.607146096
Neurotypical	9.468649279	9.049949317	4.859555094	5.479611342
Neurotypical	14.53116037	11.055685	9.642807214	9.761815574
Neurotypical	11.92673935	15.96033783	11.03101319	7.788142954
Neurotypical	15.70143114	18.91421894	7.214572233	6.967431191
Neurotypical	15.02416225	13.30618856	8.520991737	8.595460476
Neurotypical	13.61447189	14.73748597	9.814362703	5.817394356
Neurotypical	10.8603263	10.68386152	NA	NA
Neurotypical	10.38257723	10.58082587	NA	NA
Neurotypical	11.79813299	13.02120024	NA	NA
Neurotypical	14.25759296	18.65058519	7.763861404	8.162968401
Neurotypical	9.722633896	10.94151912	7.176305053	7.359308648
Neurotypical	10.54027896	11.11133059	7.462122855	6.350588479
Neurotypical	9.969426778	10.08564778	7.538577217	5.966455811
Neurotypical	9.988677383	10.25062889	7.257006015	8.034547418
Neurotypical	10.55478596	10.94177261	NA	NA
Neurotypical	9.578996296	9.882392411	NA	NA
Neurotypical	13.57801178	11.51251984	7.014844338	6.712492963
Neurotypical	10.50094717	11.10952951	6.496388591	5.760879882
OCD	13.53502686	9.430361242	5.592711434	4.605072501
OCD	9.947264788	10.54916246	7.556859004	5.955580429
OCD	10.08391323	10.7309773	6.900305508	7.096163611
OCD	10.67196045	13.28778394	7.404535854	6.701631818
OCD	14.57241252	10.94868352	6.65173351	6.705290913
OCD	9.625646412	9.238435885	5.329838352	5.325569179
OCD	9.316942712	10.35378667	5.431188384	6.662102284
OCD	10.74705388	8.375389987	NA	NA
OCD	8.194909742	11.80746102	6.14599681	6.142299143
OCD	17.78596703	13.89954418	10.13001278	11.80747576
OCD	23.88093187	17.86656965	NA	NA
OCD	12.65959818	14.95887336	NA	NA
OCD	14.9685669	17.69010235	7.334637431	7.923531508
OCD	13.88118108	15.47390753	NA	NA
OCD	10.6982443	9.595557239	6.996366196	6.843144398

Medication use over time

During the first time of measure, in the autism group two people used stimulants, and one anti-depressants. In the OCD group five people used antidepressants and one anti-psychotics. In the second time of measure, one of the participants using stimulants and the participant using anti-depressants now also used antipsychotics. An additional participant used antidepressants, and one antipsychotics and stimulants. In the OCD group two were no longer on antidepressants, the one using antipsychotics in the first time of measure now also used antidepressants, and one participant had started using stimulants. None of the neurotypical participants used medication at any time of measure.

Stop-Signal task (behavioral)

Analysis

The behavioral measure of interest on the SST was the stop-signal reaction time (SSRT), which was calculated using the integration method (5,6), where the reaction time (RT) of correct go trials was rank ordered, then the *n*th go-RT was selected, where *n* was derived by multiplying the number of correct go-trials by the probability that the participant respond to a stop signal. The SSRT was then estimated by subtracting the mean SSD from the *n*th go-RT (7). Participants were excluded from analysis for excessive motion or when they showed an SSRT < 50 ms as it is indicative of not performing the task properly, for example by constantly pressing buttons without paying attention to cues which results in atypically short response times on correct go-trials. This resulted in 41 participants included for stop-task analysis (autistic = 12, OCD = 8, neurotypical = 21). Data from T1 and T2 were initially analyzed separately allowing investigation of group differences without the possible influence of time. Shapiro-Wilk normality tests showed that there was no normal distribution in either time of measure, and therefore Fligner-Killeen tests of homogeneity of variance were used, which showed that there was equal variance between diagnosis groups in both T1 and T2. Consequently, Kruskal-Wallis tests were used to analyze differences in SSRT between groups (autistic, OCD or neurotypical) for T1 and T2 independently. To investigate behavioral differences between groups on the stop-task measured by the SSRT, Kruskal-Wallis tests were used to compare groups, and a mixed effects model was used to analyze changes between groups over time of measure, including the same covariates as described before.

Results

There were no group differences in SSRT at T1 ($c^2_{(2)}$= 2.84, $p > 0.1$) or T2($c^2_{(2)}$=2.64, $p > 0.1$), showing similar performance across groups. Across T1 and T2 a significant effect of sex was found (b = -101.34, $t_{(34.3)}$=-2.21 p = 0.03, r = 0.35), indicating an improvement in stop-task performance in males, but not in females. There were also no significant group differences in task performance across T1 and T2.

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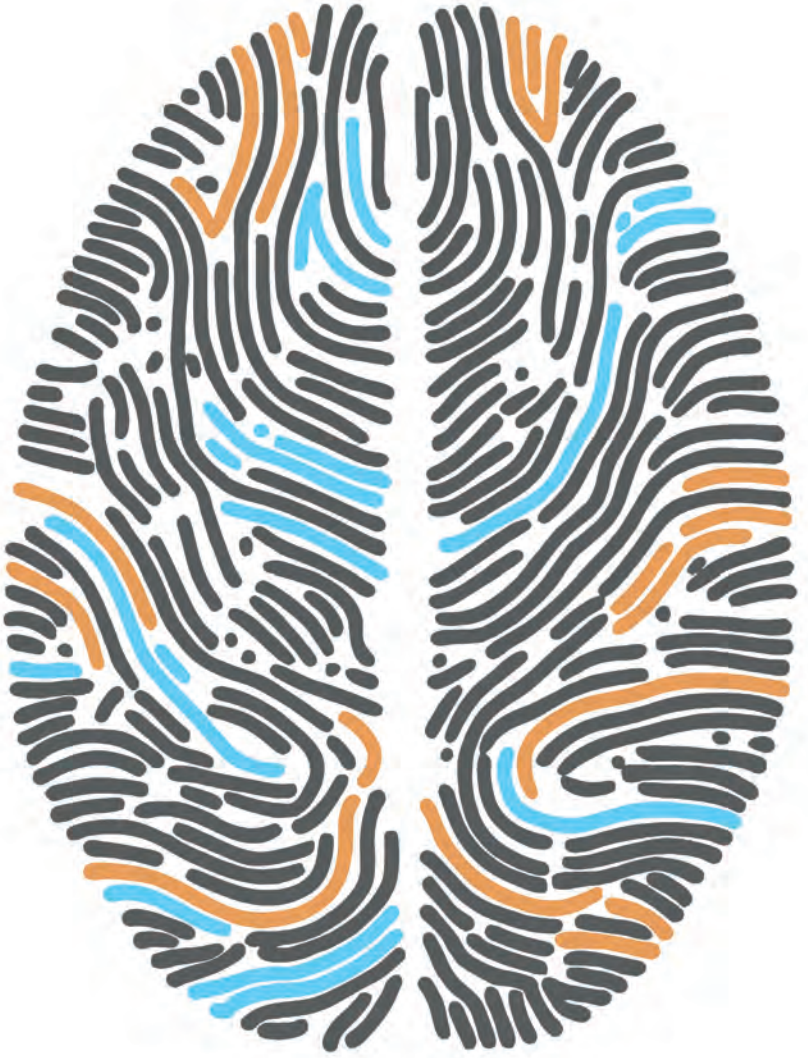

Chapter 3

Excitatory/inhibitory imbalance in autism: the role of glutamate and GABA gene-sets in symptoms and cortical brain structure

This chapter is based on:
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Language has been slightly adjusted from the published manuscript for consistency across this thesis.

Abstract

The excitatory/inhibitory (E/I) imbalance hypothesis posits that imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) mechanisms underlies the behavioral characteristics of autism. However, how E/I imbalance arises and how it may differ across autism symptomatology and brain regions is not well understood. We combined competitive gene-set analysis and gene-expression profiles in relation to cortical thickness (CT) to investigate relationships between genetic variance, brain structure and autism symptomatology of participants from the AIMS-2-TRIALS LEAP cohort (autistic = 359, male/female = 258/101; neurotypical = 279, male/female = 178/101) aged 6 to 30 years. Using competitive gene-set analyses we investigated whether aggregated genetic variation in glutamate and GABA gene-sets could be associated with behavioral measures of autism symptoms and brain structural variation. Further, using the same gene-sets, we corelated expression profiles throughout the cortex with differences in CT between autistic and neurotypical participants, as well as in separate sensory subgroups. The glutamate gene-set was associated with all autism symptom severity scores on the Autism Diagnostic Observation Schedule-2 (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R) within the autistic group. In adolescents and adults, brain regions with greater gene-expression of glutamate and GABA genes showed greater differences in CT between autistic and neurotypical control participants although in opposing directions. Additionally, the gene expression profiles were associated with CT profiles in separate sensory subgroups. Our results suggest complex relationships between E/I related genetics and autism symptom profiles as well as brain structure alterations, where there may be differential roles for glutamate and GABA.

Introduction

Autism spectrum disorder (autism) is a neurodevelopmental condition characterized by challenges in social interaction and communication, restricted and repetitive patterns of behavior and/or atypical sensory processing (1). One influential hypothesis regarding its underlying mechanisms is the excitatory/inhibitory (E/I) imbalance hypothesis, which suggests that an imbalance between excitatory (predominantly glutamatergic) and inhibitory (predominantly GABAergic (γ-aminobutyric acid)) mechanisms in the brain underlies symptomatology (2). Causal links have been suggested, but so far with suggestions for both overexcitation and overinhibition (2–6). However, understanding the mechanisms of *how* E/I imbalance is underlying autism symptomatology is complex. The heterogeneity and polygenic nature of autism, and previous opposing findings of E/I imbalance, may be evidence of differential involvement across autism characteristics or brain regions.

Mechanisms of E/I imbalance may have genetic underpinnings. Autism is a polygenic condition where several genetic variants together give rise to the expression of the phenotype. Progress in identifying common genetic variants associated with autism have included genes encoding proteins involved in glutamate and GABA receptors and transporters (7–10). De novo mutations are also known to underlie a significant portion of the prevalence of autism, where additional links between genes involved in excitatory and inhibitory signaling have been found (11). Several studies have suggested glutamatergic and GABAergic genetic links to behavioral autism phenotypes (3,4,12–14). These phenotypes have been linked to changes in glutamate and GABA concentrations in the brain as well (4,15).

Genetic and behavioral changes in autistic individuals can additionally be linked to brain structure, where a role for E/I imbalance seems plausible. Differences in cortical thickness (CT) have consistently been found in autism and have been shown to differ throughout development as well (16,17). More specifically, both increased and decreased cortical thickness has been found in autism mainly in fronto-temporal, fronto-parietal, limbic areas and fronto-striatal circuits (16–22). However, we do not yet have a clear understanding of what is causing these differences, although there is strong evidence that genetic factors play a role (21). Cell-type specific gene-expression has been shown to be associated with differences in cortical thickness in several neurodevelopmental disorders, among which autism (23,24). Some genes within these cell-type specific gene-sets relate to cellular E/I function, but a similar relation has not yet been investigated focusing specifically on genetic pathways involved in excitatory and inhibitory signaling. Although not yet investigated

directly, it is plausible that alterations in glutamate and GABA functioning relate to morphological differences such as CT. For instance, glutamate and GABA receptors play a role in dendritic growth, a process with genetic underpinnings found to be altered in autism (25–27). Dendrite growth is also linked to cortical thickness (23,28). Altered dendritic growth, and associated genes, have been linked to autism symptomatology, especially repetitive behaviors (25,29,30). To understand mechanistic underpinnings of morphological differences in autism it is important to get a better understanding of these links between E/I imbalance and how it may relate to structural differences. This has the potential to increase understanding of the links between molecular and genetic mechanisms of autism and macroscopic measures such as cortical thickness, aiding in the development of markers for subtyping and targeted treatment options in autism (19).

In the current study we wanted to integrate parts of the E/I puzzle by taking a multimodal approach focusing on aggregated (common) genetic variation, different autism phenotypes and their association with brain structure. One relatively understudied part of the autism phenotype comprises sensory symptoms. These are especially interesting as they have been suggested promising to unscramble the autism heterogeneity (31) as well as for their shown link with E/I imbalance using Proton-Magnetic Resonance Spectroscopy (^{1}H-MRS) (32). Additionally, previous investigations within this dataset have shown differences in CT between those with severe and low sensory processing difficulties in brain regions enriched for genes that are expressed in excitatory neurons in the developing cortex (19).

Here we used a competitive gene-set approach (33–35), investigating the role of aggregated genetic variation in glutamate and GABA gene-sets in behavioral autism phenotypes and cortical thickness. By considering several (common) genetic variants in the same analysis, the power of the study in explaining phenotypic variance is increased. In short, this tests whether genes in the gene-set are more strongly correlated with the phenotype of interest than other genes (33). This method has shown utility in other neurodevelopmental disorders showing aggregated genetic effects rather than using single candidate-gene associations (29,36,37). Additionally, using the same gene-sets, we investigated whether their expression profiles across the cortex could be associated with differences in cortical thickness between autistic and neurotypical participants. Building on the previous findings focusing on sensory symptoms (18,31), we further extended these analyses linking this E/I related gene-expression to cortical thickness profiles in separate sensory subgroups.

By integrating these approaches, we can deepen our understanding of the links between aggregated genetic variation in glutamate and GABA pathway signaling sets and different behavioral autism phenotypes as well as brain structure. Based on previous findings regarding excitatory or inhibitory alterations in autism, we expected to find differential involvement of the glutamate and GABA genes across the autism phenotypes, reflected in the competitive gene-set analysis. We also attempted to further confirm such differences with the exploratory analyses using gene-expression in association with structural brain differences in both the autism versus neurotypical groups as well as in the sensory symptom subgroups.

Methods

Participants

We included participants from the Longitudinal European Autism Project (LEAP), part of the AIMS-2-TRIALS clinical research programme (https://www.aims-2-trials.eu/) (38–40). Our sample consisted of 638 participants (autistic = 359, neurotypical = 279) for whom structural MRI data was available that passed quality control (19). Phenotypic, genetic and brain imaging data were collected at six study centers across Europe: Institute of Psychiatry, Psychology and Neuroscience, King's College London (IoPPN/KCL, UK), Autism Research Centre, University of Cambridge (UCAM, UK), University Medical Centre Utrecht (UMCU, Netherlands), Radboud University Medical Centre (RUMC, Netherlands), Central Institute of Mental Health (CIMH, Germany), and the University Campus Bio-Medico (UCBM) in Rome, Italy.

Inclusion criteria for the autism group were an existing diagnosis of autism and an age-range between 6 and 30 years. Symptoms were additionally assessed using the Autism Diagnostic Observation Schedule Second Edition (ADOS-2; (41)) and the Autism Diagnostic Interview-Revised (ADI-R; (42)). For the neurotypical participants, exclusion criterion comprised of parent- or self-report of any psychiatric disorder. Individuals who had a normative T-score of 70 or higher on the Social Responsiveness Scale Second Edition (SRS-2) were excluded. Some individuals in the autism and neurotypical groups had intellectual disability (ID) (autistic=53, neurotypical=25), defined as an IQ score between 40 and 74. Ethical approval was obtained through ethics committees at each study site. All participants or legal guardian (where applicable) provided written informed consent. For further details of the recruitment of participants in this study see (19,38,39).

Phenotypic measures

The phenotypic measures used were part of a larger test battery (see (39)). Here we included three questionnaires focusing on the core autism symptoms: the Social Responsiveness Scale-Revised (SRS-2) (43), the Repetitive Behavior Scale-Revised (RBS-R) (44), and the Short Sensory Profile (SSP) (45). For these questionnaires, we used self- or parent-report ratings, depending on age and diagnostic group. We additionally made use of sensory symptom subgroups used in this sample previously, created based on the SSP scores and factor mixture modelling (19,31).

Genotyping

Genotyping was performed at the Centre National de Recherche en Génomique Humaine (CNRGH) using the Infinium OmniExpress-24v1 BeadChip Illumina. Sample quality controls such as sex check (based on the X chromosome homozygosity rate or the median of the Log R ratio of the X and Y chromosomes), Mendelian errors (transmission errors within full trios) and Identity By State were performed using PLINK 1.90. Imputation of 17 million SNPs was performed using the 700k genotyped SNPs on the Michigan Imputation Server (46). The HRC r1.1 2016 reference panel for a European population was used, as most individuals in the LEAP cohort were from European ancestry. Only autosomes were imputed. Linkage disequilibrium-based SNP pruning was done for SNPs with a MAF>1% and SNPs with an $R2 < 0.1$ in windows of 500kb were selected. This resulted in 546 participants with genotypic data (autistic= 304, neurotypical= 242).

Selection of the glutamate (n=72 genes) and GABA (n=124 genes) gene-sets was based on Ingenuity Pathway Analysis software (http://www.ingenuity.com), a frequently updated database for genetic pathway analysis. Supplement Tables S1 and S2 show an overview of the included genes. In case of any significant associations between the aggregated genetic variation in the gene-sets and the phenotypes of interest, we explored smaller gene-sets containing genes encoding glutamate/GABA receptors and transporters specifically because of their more direct role in neurotransmitter signaling (47). Those are referred to as glu-RT (n=32) and GABA-RT (n=26) and are defined in the supplement Tables S1 and S2.

Neuroimaging

Structural brain images were acquired on 3T MRI scanners at all sites, with T1-weighted MPRAGE sequence (TR=2300ms, TE=2.93ms, T1=900ms, voxels size=1.1x1.1x1.2mm, flip angle=9°, matrix size=256x256, FOV=270mm, 176 slices). For a summary of scanner details and acquisition parameters at each site, see Table S3 in the supplement. The processing of all neuroimaging data was conducted at

one site for all available data. For each image a model of the cortical surface was computed using FreeSurfer v6.0 (https://surfer.nmr.mgh.harvard.edu/), using a fully automated and validated procedure (48–51). Subsequently, each reconstructed surface went through strict quality assessments, described in detail in (19). This quality assessment included visual inspection of reconstruction errors by independent raters, manual editing where needed, and examination of the Euler number of each FreeSurfer surface reconstruction resulting in the conclusion that there were no differences in the complexity of the reconstructed cortical surface between participant groups. This resulted in parcellated regional CT measures for all the 638 participants included in our study, with 34 regions in each hemisphere using the Desikan-Killiany atlas (52). A more detailed description of the processing of the cortical thickness data can be found in a previous publication (see (19)).

Gene-expression data

Gene-expression data were acquired from post-mortem human brains from the Allen Human Brain Atlas (AHBA) (53), using data from six donors (aged 24-57 years, one female) of the left hemisphere only. These whole-brain gene-expression data are open source and can be downloaded from the Allen Institute for Brain Science; http://www.brain-map.org. For more details on how these data were obtained, see (53).

Using previously described procedures (23,24,54), these gene-expression data were mapped onto the 34 cortical regions defined by FreeSurfer's Desikan-Killiany Atlas (52). These gene-expression profiles were then used in the two-step procedure described by (55) to select the most consistent profiles for inclusion in our analyses. First, the correlations of gene-expressions to the median expression values across donors were calculated, and the genes showing consistent correlation profiles were selected (donor-to-median correlation rho >0.446). Secondly, we used data from the BrainSpan Atlas, where gene-expression data in a wide age-range of donors are available (www.brainspan.org). Donors were selected within the age range of our LEAP dataset (6-30 years), which gave us 9 donors (male/female = 5/4). We calculated correlations to the median expression values in the 11 cortical regions in the AHBA-to-FreeSurfer data that were also included in the BrainSpan Atlas, using methods as described by (24). We then selected genes that correlated between the profiles of the two atlases higher than r=0.52 (one-sided *test* $p < 0.05$), which resulted in 2293 genes available in total. The overlap with the gene-sets left 29 genes in our glutamate pathway gene-set, and 42 genes in our GABA pathway gene-set. The median expression profiles across regions for these genes constitute the interregional gene-expression profiles used in our analyses.

Analyses

All analyses included the linear effects of age, sex, IQ and site as covariates. All tests were corrected using the false discovery rate (FDR; $q < 0.05$ was considered significant) unless otherwise described.

Gene-set analysis

To investigate associations between aggregated genetic variation within the glutamate and GABA gene-sets and the autism phenotypes of interest (SRS-2 total score, RBS-R total score, SSP total score, and ADOS-2 and ADI-R (the last two for the autism group only)) and cortical thickness, we performed competitive gene-set analysis using MAGMA (Multi-marker Analysis of GenoMic Annotation) software (version 1.10, (33)). This analysis is performed in two steps. First, gene-based *p*-values are calculated for each gene (excluding genes located on the X-chromosome, see supplement Tables S1 and S2) on our phenotypes of interest, using a multiple linear principal components regression using F-tests. Secondly, the association of the set is tested, aggregating the gene-based *p*-values using competitive analysis. This gene-set analysis is done with an intercept-only linear regression model for the gene-set, which tests whether the aggregated genetic variation of the genes in a gene-set is more strongly associated with the phenotype of interest than all other genes in the genome (33).

Cortical thickness and clinical phenotypes

To test associations between cortical thickness (CT) and our phenotypes of interest (SRS-2 total score, RBS-R total score, SSP total score, ADOS-2 and ADI-R), we used linear regression models in the R-software package (56). This was done in the left hemisphere only, due to the expression profile analysis being performed only in the left hemisphere. In addition to age, sex, IQ and site we added quadratic age effects and total mean cortical thickness as covariates as well, as described previously in (19).

Expression profiles

To investigate associations between expression profiles of the glutamate and GABA gene-sets and brain structure we used correlation across interregional profiles of CT with interregional profiles of gene-expression (23,24,57). Profiles of CT were created by subtracting the average CT per region in the neurotypical group from the average CT in the autism group, as has been done previously (54). In order to rule out effects being caused by heterogeneity in the sample, the groups were matched for age, sex and IQ using MatchIt (58) in R-software (56) performing nearest neighbor matching, resulting in n=279 participants in each group. As

cortical thickness is strongly associated with age (59), we additionally decided to do these profile correlation analyses for children, adolescents and adults separately. To verify any of these associations analyses were replicated using structural imaging data of CT from the multi-site open-source ABIDE database (60). We included participants in the same age range as our own sample (6-30 years), which resulted in data from 874 participants matched for age, sex and IQ (n =437 in both groups). Details on these analyses and results can be found in the supplement and Figure S1. Building upon previous results from our group (19,31) and to parse some of the autism heterogeneity, we additionally performed these gene-expression analyses with interregional CT profiles in separate sensory subgroups (low, n=375; moderate, n=37; severe, n=37). These subgroups were defined previously (31).

The interregional expression profiles of the genes in our glutamate and GABA pathway gene-sets were then correlated with the CT-difference interregional profiles (autism minus neurotypical across different age-groups and CT-average interregional profiles in sensory subgroups), which provided a distribution of correlation coefficients per gene-set. The distributions of correlation coefficients between the gene-expression and CT-difference interregional profiles were then tested for significance using a resampling approach of 10,000 random samples, as described in (23,24). In this approach, a random set of genes of the same size as the set being tested is selected (from the 2293 available) 10,000 times with the average correlation each time being used to create a null distribution. A two-tailed significance test was used to test the gene-set of interest against the null distribution.

Results

Demographics

Demographic and clinical characteristics are shown in Table 1. No differences were found between the autism and neurotypical groups in age. The autism group had a higher female-to-male ratio compared to the neurotypical group and the neurotypical group had a higher IQ than the autism group. As expected, the autism group had significantly higher scores on the SRS-2 and RBS-R and scored lower in the SSP (where lower scores indicate higher sensory sensitivity). Information on medication use can be found in Table S4 in the supplement.

Table 1: Demographic and clinical characteristics

		NT (N=279)		Autism (N=359)		Test statistic		p-value
Sex, m/f		178/101		258/101		KWχ2=4.71		0.03
	N	**Mean**	**SD**	**Mean**	**SD**		**df**	
Age		17.33	5.91	17.50	5.52	t = 0.38	576.91	0.70
IQ		104.79	19.72	98.88	18.25	t = -3.92	617.31	< 0.001
SRS-2	555	28.88	23.36	88.99	30.79	t = 26.15	551.34	< 0.001
RBS-R	436	2.59	8.39	16.34	13.94	t = 12.83	423.49	< 0.001
SSP	325	176.66	15.74	139.43	27.27	t = -15.60	322.22	< 0.001
ADI-R								
Social	345	-	-	16.70	6.68	-	-	-
Communication	345	-	-	13.24	5.63	-	-	-
Restricted repetitive	345	-	-	4.30	2.66	-	-	-
ADOS-2								
Calibrated severity	353	-	-	5.40	2.76	-	-	-
Social affect	351	-	-	6.02	2.63	-	-	-
Restrictive repetitive	351	-	-	4.62	2.71	-	-	-

NT, Neurotypical; autism, Autism Spectrum Disorder; SD, standard deviation; df, degrees of freedom; SRS-2, Social Responsiveness Scale 2nd edition; RBS-R, Repetitive Behavior Scale - Revised; SSP, Short Sensory Profile; ADI-R, Autism Diagnostic Interview-Revised; Restricted repetitive, Restrictive Repetitive Behaviors domain; Communication, ADI Communication domain; Social, ADI Social domain; ADOS-2, Autism Diagnostic Observation Schedule 2nd edition; Calibrated severity, ADOS-2 Calibrated Severity Score; Social affect, ADOS-2 Social Affect. KWχ2, Kruskal-Wallis Chi-Square. Post hoc tests were Bonferroni corrected (alpha lower than 0.05).

Gene-set analysis

Aggregated genetic variation within the glutamate gene-set (n=72 genes) was associated with autism symptoms as defined by significant associations with all the ADI-R and ADOS-2 subscales (all $q < 0.05$, see Table 2). Repeating these analyses in the smaller glu-RT gene-set did not give the same significant results. No associations were found for any of the questionnaire scores. Genetic variation within the GABA gene-set (n=124 genes) was nominally significantly associated with sensory processing (SSP total scores; $q = 0.07$) after FDR correction. Repeating these analyses in the smaller, more specific GABA-RT set gave a similar result ($q = 0.06$), see Table 2. To investigate these trend associations further, we performed similar post-hoc association analyses with all the SSP subscales. None of these were significantly associated with the genetic variation within the GABA gene-set (all q-values > 0.05). For more details see Table S5 in the supplement. Repeating the gene-set analyses with the questionnaires in the autism group separately did not result in any significant associations.

We additionally investigated gene-set associations with CT in the FreeSurfer cortical regions in the left hemisphere. There were some nominally significant (uncorrected *p*-values <0.05) associations, although none survived FDR-correction. The details of these results can be seen in supplement Tables S6 and S7.

Table 2: Glutamate and GABA and phenotypes competitive gene-set analysis results

Glutamate: Pathway gene-set (N=72)	**BETA**	**P**	**P_{FDR}**	**SE**
SRS	0.075	0.247	0.247	0.109
RBS-R	0.111	0.144	0.162	0.104
SSP	0.108	0.143	0.162	0.101
Diagnosis	0.171	0.048	-	0.103
ADI communication domain	**0.197**	**0.028**	**0.042**	**0.103**
ADIrestricted and repetitive behaviors domain	**0.197**	**0.028**	**0.042**	**0.103**
ADI social domain	**0.197**	**0.028**	**0.042**	**0.103**
ADOS restricted and repetitive behaviors	**0.225**	**0.014**	**0.042**	**0.103**
ADOS social affect	**0.225**	**0.014**	**0.042**	**0.103**
ADOS total score	**0.225**	**0.015**	**0.042**	**0.103**
Glutamate: Receptors/transporters gene-set (N=31)				
SRS	-0.016	0.538	0.559	0.170
RBS-R	-0.024	0.559	0.559	0.163
SSP	0.188	0.116	0.278	0.157
Diagnosis	0.075	0.319	-	0.161
ADI communication domain	0.077	0.315	0.406	0.160
ADI restricted and repetitive behaviors domain	0.077	0.315	0.406	0.160
ADI social domain	0.077	0.315	0.406	0.160
ADOS restricted and repetitive behaviors	0.186	0.124	0.278	0.160
ADOS social affect	0.186	0.123	0.278	0.160
ADOS total score	0.186	0.124	0.278	0.160
GABA: Pathway gene-set (N=124)	**BETA**	**P**	**P_{FDR}**	**SE**
SRS	-0.050	0.721	0.728	0.085
RBS-R	-0.013	0.562	0.632	0.081
SSP	**0.151**	**0.028**	0.248	0.079
Diagnosis	0.040	0.311	-	0.081
ADI communication domain	0.048	0.274	0.413	0.080
ADI restricted and repetitive behaviors domain	0.048	0.275	0.413	0.080
ADI social domain	0.048	0.274	0.413	0.080
ADOS restricted and repetitive behaviors	0.037	0.321	0.413	0.080
ADOS social affect	0.037	0.321	0.413	0.080
ADOS total score	0.037	0.321	0.413	0.080

Table 2: Continued

GABA: Receptors/transporters gene-set (N=23)				
SRS	0.102	0.318	0.358	0.215
RBS-R	-0.329	0.946	0.946	0.205
SSP	**0.340**	**0.022**	0.198	0.198
Diagnosis	0.133	0.256	-	0.202
ADI communication domain	0.117	0.281	0.358	0.202
ADI restricted and repetitive behaviors domain	0.117	0.281	0.358	0.202
ADI social domain	0.117	0.281	0.358	0.202
ADOS restricted and repetitive behaviors	0.105	0.302	0.358	0.202
ADOS social affect	0.105	0.301	0.358	0.202
ADOS total score	0.105	0.301	0.358	0.202

N, number of genes in analysis. Diagnosis was indicated as a binary variable. SRS-2, Social Responsiveness Scale, Second Edition;; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile; ADI, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule-2nd edition; PFDR p-value corrected using False discovery rate (FDR); SE, standard error of the regression coefficient. Significant results (pFDR<0.05) marked in bold.

Cortical thickness and phenotypes

Our group previously showed vertex-wise group differences in cortical thickness between autism and neurotypical participants in the current sample (19). Here we did not repeat these analyses but instead focused on the continuous measures of autism symptoms using the ADI-R and ADOS-2 in the autism group and the SRS-2, RBS-R and SSP questionnaires in the entire sample.

Cortical thickness in the frontal pole was positively associated with restricted and repetitive behaviors as reflected by the RBS-R total score (b = 0.05, t = 3.33, q = 0.03). No other results survived multiple comparisons corrections, although nominally significant negative associations were found between all ADI-R subscales and precuneus CT (communication q=0.26, social q= 0.19, restricted and repetitive behaviors q=0.05) as well as a nominally significant positive relation between the ADOS-2 total score and the social affect subscale and CT in the insula (q=0.16, q=0.13, respectively).

Gene-expression profiles

While the interregional profiles of group differences in cortical thickness (autism minus neurotypical) were not significantly associated with gene-expression profiles across our glutamate and GABA gene-sets in the full sample (all q-values >0.05), splitting into groups of children, adolescents and adults gave some

opposing results. In adolescents (autistic = 101, neurotypical = 100), the interregional profile of group differences in cortical thickness was positively associated with interregional variation in expression of both glutamate (t=2.25, *q*=0.030, Cohen's d= 0.70) and GABA genes (t=3.28, *q*=0.005, Cohen's d=1.24). In adults, on the other hand (autistic = 124, neurotypical = 115), the group difference profile was negatively associated with expression, again for both gene-sets (glutamate: t=-2.99, *q*=0.005, d=-0.93; GABA: t=-3.17, *q*=0.005, d=-0.93), reflecting differences in CT between autistic participants and NT changing with age. In children, no such associations were found. See Figure 1 for the distributions of the correlation coefficients and Figure 2 for CT differences and example genes for each gene-set.

These results were replicated in the independent ABIDE cohort for adolescents. In adults, however, positive associations between the expression profiles and CT differences were found, as opposed to the negative associations found in our LEAP sample (see supplement and Figure S1).

Investigating the interregional profiles of CT in sensory subgroups separately gave positive associations with the interregional profiles of gene-expression in all groups (LOW: glutamate: t=3.02, *q*=0.004, Cohen's d = 0.94; GABA: t=3.19, *q*=0.004, Cohen's d=1.21; MODERATE: glutamate: t=3.18, *q*=0.003, Cohen's d = 0.99; GABA: t=3.30, *q*=0.003, Cohen's d=1.25; SEVERE: glutamate: t=3.03, *q*=0.004, Cohen's d = 0.95; GABA: t=3.18, *q*=0.004, Cohen's d=1.20), see also Figure S2. To investigate possible differences *between* the sensory subgroups we calculated interregional CT-difference profiles between groups as well, however this gave no significant associations. These results could not be replicated in ABIDE due to unavailability of the SSP questionnaire in that sample.

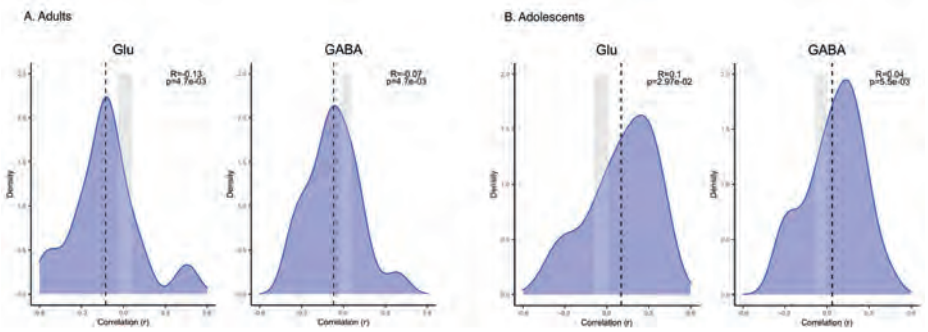

Figure 1: Distributions of correlation coefficients between cortical thickness difference and gene-expression

Distributions of the inter-regional correlation coefficients between differences in cortical thickness (CT) and profiles of gene-expression in adults (A) and adolescents (B). The CT-difference profiles were obtained from our LEAP data, and the expression profiles from the Allen Human Brian Atlas (AHBA), in our glutamate-pathway and GABA-pathway gene-sets. The x-axes show the correlation coefficient between CT-difference and expression profile for all genes in the gene-set; the y-axes show the estimated probability density for the correlation coefficients; the vertical dashed-lines indicates the average expression-CT difference correlation coefficient across all the genes in a gene-set; and the edges of the gray boxes indicates the 2.5% and 97.5%-critical values obtained from the empirical null distribution of the average expression-thickness correlation coefficient. If a vertical line sits outside the gray box, it implies that there is a significant association between gene-set and differences in CT at the unadjusted 5% significance level.

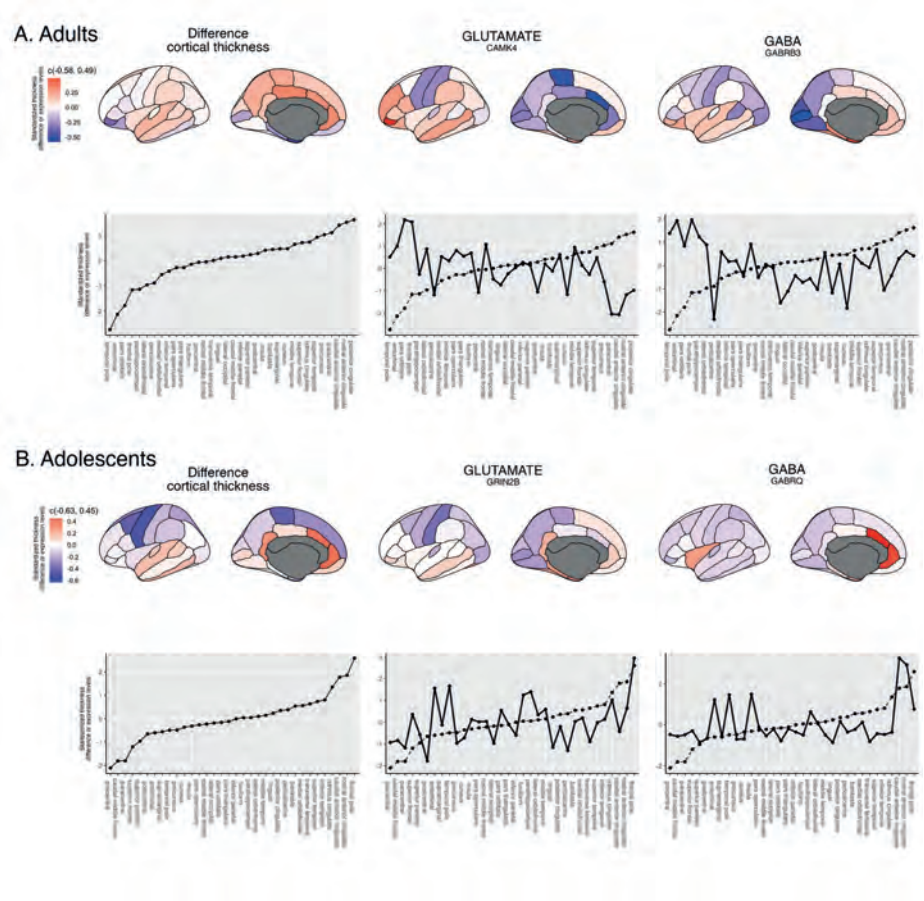

Figure 2: Gene-expression and cortical thickness difference from highest correlating genes

Lateral and medial views of differences in cortical thickness (CT) between autistic and neurotypical control participants in adults (A) and adolescents (B), and gene-expression levels of the genes from the glutamate and GABA (pathway) gene-sets with highest (negative in adults, positive in adolescents) correlation. Plots on the bottom row of each panel show standardized profiles of CT-differences between autistic and neurotypical control participants (dotted lines) in each age group and the gene-expression (solid lines) for the most strongly correlated gene in each respective gene-set. FreeSurfer regions on the x-axes are ordered from low to high thickness. Figures were created using ggplot2 (Ginestet, 2011) and ggseg (Mowinckel & Vidal-Piñeiro, 2020).

Discussion

We took a multimodal approach to investigate the role of E/I imbalance associated gene-sets in relation to behavioral phenotypes and brain structure in autism. The most important takeaway of our results is that the glutamate and GABA gene-sets were differently associated with autism symptoms, and that the expression profiles of these genes throughout the cortex were associated with differences in cortical thickness between autistic and neurotypical participants, depending on age. Aggregated genetic variation in the glutamate gene-set was associated with autism symptom severity on all core symptom subscales of the ADI-R and ADOS-2 (in autistic participants), while variation in the GABA gene-set showed association with sensory symptoms in the entire group, although this did not survive strict multiple comparisons correction. In adolescents and adults, but not in children, regions with greater gene-expression of glutamatergic and GABAergic genes showed greater differences in CT between autism and neurotypical groups, but in opposite directions. In adolescents, this association was positive, suggesting overall higher cortical thickness in the autism group than in the neurotypical group, while in adults this was negative, indicating an overall higher CT in neurotypical as opposed to autistic participants. These results provide a better understanding of the mechanistic underpinnings of the E/I imbalance hypothesis of autism, by supporting the notion that E/I imbalance varies across behavioral autism characteristics and differences in CT between autistic and neurotypical groups.

The findings of associations between genetic variation in the glutamate gene-set and ADI-R and ADOS-2 subscale scores, and the trend associations of these subscale scores with cortical thickness in the precuneus and insula, areas known to be involved in somatosensory and visuospatial processing, interoception and self-reflection (61,62), suggest that glutamate genes linked to broader autism characteristics. Additionally, the trend associations of GABA gene-sets on SSP total score and the association with cortical thickness profiles in the sensory symptom subgroups suggest a particular role for GABAergic genes in sensory processing, which supports previous findings of links between brain GABA concentrations and sensory processing differences (32,63,64).

The lack of significant associations between aggregated variation in the glutamate and GABA gene-sets with repetitive behaviors and social responsiveness (RBS-R and SRS-2) may be considered surprising as previous studies have found links between glutamate and GABA concentrations in several brain regions and/or metabolite altering drugs with these behaviors (65–70). However, studies investigating *in vivo*

measures of alterations of glutamate and GABA in autism have had inconsistent results that could be due to several factors; the heterogeneity of autism, differences in study populations and brain regions investigated, or differences in processing pipelines during analysis. Furthermore, here we focused on behavioral autism characteristics and genetic information, not *in vivo* brain concentrations of glutamate and GABA. We did however find links between repetitive behaviors (RBS-R) and CT in the frontal pole, where increased RBS-R scores were associated with increased CT. Measures from the ADI-R and ADOS-2 diagnostic tools (in the autism group) were differently associated with CT in the precuneus and insula, although this was only at trend-level.

We did not find direct associations of CT with SSP scores, although previous work on this dataset did find associations of differences in CT between sensory subgroups in right premotor cortex and supplementary motor areas, regions enriched for genes expressed in excitatory neurons in developing cortex (19). In support of this, we found that regions with greater expression of genes from both gene-sets also showed greater CT in all sensory subgroups, although there were no significant differences between sensory subgroups. This show that there are likely associations of glutamatergic and GABAergic gene-expression to alterations in sensory processing but that differences may be too subtle between groups to show any differences. These associations, combined with the trend significant associations of aggregated genetic variance of the GABA gene-set are in line with previous work indicating that alterations of GABA are associated with altered sensory processing in autism (32,71,72). It is also possible that we did not see significant associations with CT-difference scores between these groups due to lower number of participants on the moderate and severe groups (n=37, n=18 respectively).

Interregional variation in expression of glutamatergic and GABAergic genes was associated with the group differences in CT in adolescents and adults, but not in children, nor in the overall sample. Regions with greater expression of both glutamate and GABA genes showed greater differences in CT between autistic and neurotypical participants. These results taken together suggests possible genetic underpinnings of excitation/inhibition imbalance affecting autism symptoms. Furthermore, it suggests that there may be important differences in trajectories across development which may be mediated through altered cortical thickness. This is in line with previous work on this cohort finding differences in CT in regions enriched for genes involved in autism, where degree of deviance in CT from the neurotypical range correlated with increased polygenic scores for autism and symptom severity (19). This needs to be investigated further, and future studies

should preferably include measures of metabolite concentrations to draw further conclusions about these relationships.

Our results need to be interpreted with caution, as the presence of glutamate and GABA protein encoding genes does not directly translate to metabolite concentrations, and genetic alterations might not translate to a common phenotype across individuals (12). Additionally, genes differ in coding for loss- or gain-of function, leading to reduced or increased protein function, further complicating any interpretation of *direction* of glutamate and GABA involvement in autism symptoms. However, our results strongly indicate critical roles of glutamate and GABA genes in these specific phenotypes and that the link between these measures needs to be investigated in more detail to increase our understanding of the mechanisms connecting genetics, glutamate and GABA neurotransmitters and autism symptomatology. More direct investigations of the E/I imbalance hypothesis are needed to investigate excitation and inhibition *in vivo* in relation to brain functioning. Promising new techniques combining different imaging methods, causal discovery analysis, and pharmacological interventions and longitudinal studies, will allow us to do this in the future through which we hope to further increase our understanding of how chemical imbalance in the brain is associated with functioning. Ultimately, E/I balance may be manipulated using glutamate- and/or GABA- influencing pharmacological treatments. One study already showed decreased glutamate and GABA concentrations after bumetanide treatment to be positively associated with autism symptom improvement (71).

Strengths of this study were the combination of genetic, structural and phenotypic data from the same cohort, which gave us the opportunity to for the first time analyze these data together. Another strength was the relatively large number of participants available giving us more confidence in our results. There were also some limitations. Firstly, there were fewer females than males included in this study, a common problem in autism research. Furthermore, the gene-expression data were only used in the left hemisphere. However, the gene-expression data used in the expression profile analyses was robust and only included if the interregional profiles were similar across another dataset (BrainSpan), increasing the confidence in the robustness of these profiles. Another limitation is that the AHBA donors were all neurotypical adults, and we do not know whether genes are expressed differently in autism. Additionally, there were differences in ages of participants recruited at different sites, which has been investigated in an initial analysis of the LEAP cohort (38). We also did not fully replicate our gene-expression profile results in the independent ABIDE cohort (see the supplement and Figure S1). However,

the results were largely overlapping showing similar effects, although in opposite direction in the adult group compared to the adults in our LEAP sample. This shows that heterogeneity of the autistic and neurotypical participants have a large influence on the results.

In conclusion, we found that glutamate genes are associated with core behavioral autism characteristics and GABA genes may be associated with sensory processing, and that increased expression of glutamate and GABA genes are associated with larger differences in CT between autistic and neurotypical participants, in adolescents and adults but not in children. This support the hypothesis that the influence of E/I imbalance varies across autism phenotypes and brain regions, suggesting that glutamate and GABA genes play different roles underlying different autism phenotypes and that this may change during development. We also showed the importance of linking structural brain measures, genetic and behavioral phenotype data together to gain a deeper understanding of possible E/I imbalance mechanisms in autism.

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Conflict of interest statements

Prof. Banaschewski served in an advisory or consultancy role for ADHS digital, Infectopharm, Lundbeck, Medice, Neurim Pharmaceuticals, Oberberg GmbH, Roche, and Takeda. He received conference support or speaker's fee by Medice and Takeda. He received royalities from Hogrefe, Kohlhammer, CIP Medien, Oxford University Press; the present work is unrelated to these relationships. Prof. Buitelaar has been in the past 3 years a consultant to / member of advisory board of / and/ or speaker for Takeda/Shire, Roche, Medice, Angelini, Janssen, and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. The remaining authors declare no potential conflict of interest.

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Supplement

Table S1: Summary table of all genes in the glutamate gene-set.

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ALDH5A1	7915	6	24495197	24537435	+	297
CALM1	801	14	90863327	90874619	+	47
CALML5	51806	10	5540658	5541533	-	6
CAMK4*	814	5	110559947	110830584	+	1538
DLG4	1742	17	7093209	7123369	-	102
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GLS	2744	2	191745547	191830278	+	290
GLUD1	2746	10	88809959	88854776	-	186
GLUD2	2747	X	120181462	120183796	+	
GLUL	2752	1	182350839	182361341	-	55
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2*	2783	7	100271363	100276792	+	19
GNB3*	2784	12	6949375	6956564	+	34
GNB5*	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12*	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2*	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4*	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041
GOT1*	2805	10	101156627	101190530	-	146
GOT1L1	137362	8	37791799	37797664	-	17
GOT2	2806	16	58741035	58768246	-	229
GRIA1*	**2890**	**5**	**152870084**	**153193429**	**+**	**1819**
GRIA2	**2891**	**4**	**158141736**	**158287227**	**+**	**425**
GRIA3*	**2892**	**X**	**122317996**	**122624766**	**+**	

Table S1: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GRIA4	**2893**	**11**	**105480800**	**105852819**	**+**	**1505**
GRID1	2894	10	87359312	88126250	-	4622
GRID2*	2895	4	93225453	94695707	+	7119
GRIK1	**2897**	**21**	**30909254**	**31312282**	**-**	**2258**
GRIK2*	**2898**	**6**	**101841584**	**102517958**	**+**	**3720**
GRIK3	**2899**	**1**	**37261128**	**37499844**	**-**	**963**
GRIK4*	**2900**	**11**	**120382465**	**120859514**	**+**	**2775**
GRIK5*	**2901**	**19**	**42502468**	**42574278**	**-**	**138**
GRIN1	**2902**	**9**	**140033609**	**140063214**	**+**	**86**
GRIN2A*	**2903**	**16**	**9847265**	**10276611**	**-**	**3419**
GRIN2B*	**2904**	**12**	**13713684**	**14133022**	**-**	**2569**
GRIN2C	**2905**	**17**	**72838162**	**72856966**	**-**	**93**
GRIN2D	**2906**	**19**	**48898132**	**48948188**	**+**	**222**
GRIN3A*	**116443**	**9**	**104331634**	**104500862**	**-**	**942**
GRIN3B	**116444**	**19**	**1000437**	**1009723**	**+**	**108**
GRINA	2907	8	145064226	145067596	+	9
GRIP1	23426	12	66741178	67463014	-	4124
GRM1*	**2911**	**6**	**146286032**	**146758782**	**+**	**2121**
GRM2*	**2912**	**3**	**51741081**	**51752629**	**+**	**16**
GRM3*	**2913**	**7**	**86273230**	**86494193**	**+**	**1110**
GRM4*	**2914**	**6**	**33989623**	**34123399**	**-**	**1020**
GRM5*	**2915**	**11**	**88237256**	**88796846**	**-**	**3817**
GRM6	**2916**	**5**	**178405328**	**178422124**	**-**	**141**
GRM7*	**2917**	**3**	**6902802**	**7783218**	**+**	**5656**
GRM8*	**2918**	**7**	**126078652**	**126892428**	**-**	**4521**
HOMER1	9456	5	78669647	78809659	-	705
HOMER2	9455	15	83517729	83654905	-	736
HOMER3	9454	19	19040010	19052041	-	42
PICK1*	9463	22	38453262	38471708	+	92
SLC17A1	6568	6	25783125	25832287	-	297
SLC17A2	10246	6	25912982	25930954	-	109
SLC17A6*	**57084**	**11**	**22359667**	**22401049**	**+**	**208**
SLC17A7*	**57030**	**19**	**49932655**	**49945617**	**-**	**39**
SLC17A8*	**246213**	**12**	**100750857**	**100815837**	**+**	**347**
SLC1A1	**6505**	**9**	**4490427**	**4587469**	**+**	**544**

Table S1: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
SLC1A2	**6506**	**11**	**35272752**	**35441610**	**-**	**1155**
SLC1A3	**6507**	**5**	**36606457**	**36688436**	**+**	**420**
SLC1A4*	6509	2	65215579	65250999	+	145
SLC1A6	**6511**	**19**	**15060845**	**15121455**	**-**	**503**
SLC1A7	**6512**	**1**	**53552855**	**53608304**	**-**	**472**
SLC38A1	81539	12	46576838	46663208	-	441
SUCLG2	8801	3	67410884	67705038	-	1963

All genes in table were included in the glutamate pathway gene-set. Genes marked in bold are the genes that were included in the reduced glutamate receptors/transporters gene-set (n=32). NSNPS, number of single nucleotide polymorphisms (SNPs). Two genes were excluded from the gene-set analyses (*GLUD2, GRIA3*) due to the position on the X-chromosome, resulting in n=72 genes. Genes marked with an asterisk (*) were included in the gene-expression analyses (n= 23).

Table S2: Summary table of all genes in the GABA gene-set.

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ADCY1*	107	7	45614125	45762715	+	760
ADCY10	55811	1	167778357	167883608	-	659
ADCY2*	108	5	7396343	7830194	+	2563
ADCY3	109	2	25042038	25142602	-	694
ADCY4	196883	14	24787555	24804277	-	81
ADCY5	111	3	123001143	123167924	-	858
ADCY6	112	12	49159975	49182820	-	81
ADCY7*	113	16	50278830	50352046	+	333
ADCY8*	114	8	131792546	132053012	-	1901
ADCY9*	115	16	4012650	4166186	-	1082
ALDH5A1	7915	6	24495197	24537435	+	297
ALDH9A1*	223	1	165631449	165667900	-	239
AP1B1	162	22	29723669	29784754	-	255
AP1G2	8906	14	24028777	24038754	-	14
AP2A1	160	19	50270180	50310369	+	165
AP2A2	161	11	925809	1012245	+	487
AP2B1*	163	17	33913918	34053436	+	746
AP2M1	1173	3	183892634	183901879	+	53
AP2S1	1175	19	47341423	47354203	-	35

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
CACNA1A	773	19	13317256	13617274	-	1465
CACNA1B	774	9	140772241	141019076	+	880
CACNA1C*	775	12	2079952	2807115	+	3692
CACNA1D*	776	3	53529076	53847179	+	1844
CACNA1E*	777	1	181452447	181775920	+	1671
CACNA1F*	778	X	49061523	49089833	-	
CACNA1G*	8913	17	48638429	48704835	+	310
CACNA1H*	8912	16	1203241	1271772	+	422
CACNA1I	8911	22	39966758	40085740	+	591
CACNA1S	779	1	201008635	201081694	-	505
CACNA2D1	781	7	81575760	82073031	-	3150
CACNA2D2*	9254	3	50400230	50540892	-	656
CACNA2D3	55799	3	54156620	55108584	+	5930
CACNA2D4	93589	12	1901123	2027870	-	775
CACNB1	782	17	37329709	37353956	-	89
CACNB2*	783	10	18429373	18830688	+	2968
CACNB3	784	12	49208215	49222726	+	46
CACNB4*	785	2	152689285	152955593	-	1246
CACNG1	786	17	65040652	65052913	+	56
CACNG2*	10369	22	36956916	37098690	-	720
CACNG3*	10368	16	24266874	24373737	+	675
CACNG4	27092	17	64960980	65029518	+	432
CACNG5	27091	17	64831235	64881941	+	373
CACNG6	59285	19	54494403	54515920	+	115
CACNG7	59284	19	54412704	54447195	+	105
CACNG8	59283	19	54466290	54493469	+	111
CATSPER1	117144	11	65784223	65793988	-	45
CATSPER2	117155	15	43922772	43941039	-	63
CATSPER3	347732	5	134303596	134347397	+	207
CATSPER4	378807	1	26517119	26529033	+	107
DNM1	1759	9	130965634	131017528	+	223
GABARAP	**11337**	**17**	**7143738**	**7145753**	**-**	**5**
GABBR1*	**2550**	**6**	**29570005**	**29600962**	**-**	**219**
GABBR2	**9568**	**9**	**101050364**	**101471479**	**-**	**2637**
GABRA1	**2554**	**5**	**161274197**	**161326965**	**+**	**283**

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GABRA2*	**2555**	**4**	**46246470**	**46392056**	**-**	**727**
GABRA3*	**2556**	**X**	**151334706**	**151619831**	**-**	
GABRA4	**2557**	**4**	**46920917**	**46996424**	**-**	**406**
GABRA5*	**2558**	**15**	**27111866**	**27194357**	**+**	**158**
GABRA6	**2559**	**5**	**161112658**	**161129598**	**+**	**81**
GABRB1*	**2560**	**4**	**47033295**	**47432801**	**+**	**2058**
GABRB2	**2561**	**5**	**160715426**	**160975130**	**-**	**1268**
GABRB3*	**2562**	**15**	**26788693**	**27018935**	**-**	**1332**
GABRD*	**2563**	**1**	**1950768**	**1962192**	**+**	**10**
GABRE*	**2564**	**X**	**151121596**	**151143156**	**-**	
GABRG1*	**2565**	**4**	**46037786**	**46126082**	**-**	**496**
GABRG2	**2566**	**5**	**161494648**	**161582545**	**+**	**435**
GABRG3	**2567**	**15**	**27216429**	**27778373**	**+**	**2556**
GABRP	**2568**	**5**	**170210723**	**170241051**	**+**	**193**
GABRQ*	**55879**	**X**	**151806637**	**151821825**	**+**	
GABRR1	**2569**	**6**	**89887223**	**89941007**	**-**	**344**
GABRR2	**2570**	**6**	**89966840**	**90025018**	**-**	**405**
GABRR3	**200959**	**3**	**97705527**	**97754148**	**-**	**264**
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GNA11	2767	19	3094408	3121468	+	144
GNA12	2768	7	2767739	2883963	-	883
GNA13	10672	17	63005407	63052920	-	84
GNA14*	9630	9	80037995	80263232	-	1496
GNA15	2769	19	3136191	3163766	+	201
GNAI1	2770	7	79764140	79848725	+	383
GNAI2*	2771	3	50264120	50296786	+	114
GNAI3	2773	1	110091186	110138465	+	181
GNAL*	2774	18	11689014	11885684	+	1003
GNAO1*	2775	16	56225251	56391356	+	866
GNAQ	2776	9	80335189	80646219	-	1344
GNAS	2778	20	57414756	57486250	+	323
GNAT1	2779	3	50229043	50235129	+	12
GNAT2	2780	1	110145889	110155705	-	45
GNAZ	2781	22	23412669	23467224	+	256

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2*	2783	7	100271363	100276792	+	19
GNB3*	2784	12	6949375	6956564	+	34
GNB4*	59345	3	179113876	179169371	-	290
GNB5*	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12*	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2*	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4*	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041
GPHN	10243	14	66974125	67648525	+	3011
GPR37	2861	7	124385655	124406079	-	81
KCNH2	3757	7	150642044	150675402	-	179
KCNN1	3780	19	18062111	18110133	+	207
KCNN2*	3781	5	113698016	113832197	+	840
KCNN3*	3782	1	154669938	154842754	-	925
KCNN4	3783	19	44270685	44286269	-	72
KCNQ2	3785	20	62031561	62103993	-	607
KCNQ3	3786	8	133133105	133493004	-	2095
MRAS*	22808	3	138066490	138124377	+	307
NSF	4905	17	44668035	44834830	+	108
OPN1SW	611	7	128412543	128415844	-	20
RPS27A	6233	2	55459039	55462989	+	27
SLC32A1	140679	20	37353105	37358015	+	20
SLC6A1	**6529**	**3**	**11034420**	**11080935**	**+**	**267**
SLC6A11	**6538**	**3**	**10857917**	**10980146**	**+**	**739**
SLC6A12	**6539**	**12**	**299243**	**323740**	**-**	**169**
SLC6A13	**6540**	**12**	**329787**	**372039**	**-**	**322**
UBA52	7311	19	18674576	18688270	+	83
UBB	7314	17	16284367	16286059	+	7

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
UBC	7316	12	125396192	125399587	-	23
UBD	10537	6	29523389	29527702	-	42
UBQLN1	29979	9	86274878	86323168	-	265

All genes in table were included in the GABA pathway gene-set. Genes marked in bold are the genes that were included in the reduced GABA receptors/transporters gene-set (n=26). NSNPS, number of single nucleotide polymorphisms (SNPs). Four genes were excluded from gene-set analyses (*CACNA1F, GABRA3, GABRE, GABRQ)*) due to the position on the X-chromosome, resulting in n=128 genes. Genes marked with an asterisk (*) were included in the gene-expression analyses (n= 39).

Cortical thickness data processing and quality assessments

All data was processed using the default FreeSurfer v6.0.0 software (http://surfer.nmr.mgh.harvard.edu/). The surface reconstructions were then visually inspected for reconstruction errors and rated by three independent raters, blind to group membership. After manual editing the (310) images were (re)preprocessed and visually (re)assessed. To assess the influence of the data quality on subsequent results, a previous study (1) examined the Euler number of each FreeSurfer surface reconstructions following manual editing. As the Euler number is calculated in each hemisphere, the sum of values across hemispheres were computed, creating one value per subject. They found no significant differences in the total Euler number between groups, indicating that the diagnostic groups have matching surface reconstruction quality. Additionally, covarying for the total Euler number in their initial analyses did not significantly affect the results. This shows that results are largely unaffected by the quality of surface reconstruction.

Table S3: Scanner parameters across sites

Site	Manufacturer	Model	Software version	Acquisition sequence	Slices	TR [s]	TE [ms]	FA [°]	Coverage	Thickness [mm]	Resolution [mm³]	FOV
Cambridge	Siemens	Verio	Syngo MR B17	Tfl3d1_ns	176	2.3	2.95	9	256*256	1.2	1.1*1.1*1.2	270
KCL	GE Medical systems	Discovery mr750	LX MR DV23.1_ V02_1317.c	SAG ADNI GO ACC SPGR	196	7.31	3.02	11				
Mannheim	Siemens	TimTrio	Syngo MR B17	MPRAGE ADNI	176	2.3	2.93	9				
Nijmegen	Siemens	Skyra	Syngo MR D13	Tfl3d1_16ns	176	2.3	2.93	9				
Rome	GE Medical systems	Signa HDxt	24/LX/MR HD16.0_ V02_1131.a	SAG ADNI GO ACC SPGR	172	5.96	1.76	11				
Utrecht	Philips Medical Systems	Achieva/ Ingenia CX	3.2.3/3.2.3.1/ 5.1.9/5.1.9.1	ADNI GO 2	170	6.76	3.1	9				

Abbreviations: FA, flip angle; FOV, field of view; TE, echo time; TR, repetition time.

Table S4: Medication information

	Autistic	Neurotypical
N	122	20
Antidepressants	34	6
SSRIs	29	5
Tetracyclic (TeCA)	2	1
Tricyclic (TCA)	3	0
Antiepileptics	11	2
Antimigraine preparations	4	0
Antipsychotics	28	1
Aripiprazole	6	0
Clozapine	1	0
Pipamperone	2	0
Quetiapine	1	0
Risperidone	18	1
Anxiolytics	2	1
Drugs used in Addictive Disorder	0	1
Hypnotics & Sedatives	40	2
Hyoscine butylbromide	1	0
Melatonin	38	2
Niaprazine	1	0
Other Analgesics & Antipyretics	4	4
Opioids	1	0
Others	3	4
Psychostimulants & Other drugs	47	9
used to treat ADHD	3	2
Atomoxetine	1	0
Dexamfetamine	43	7
Methylphenidate hydrochloride		

Note: Participants may have taken up to 3 different types of medication across the listed categories during study participation.

Table S5: Glutamate and GABA and SSP subscale competitive gene-set analysis results

Glutamate: Pathway gene-set (N=72)	**BETA**	**P**	**P_{FDR}**	**SE**
SSP Auditory filtering	0.008	0.469	0.729	0.101
SSP Low energy/weak	0.115	0.129	0.362	0.101
SSP Movement sensitivity	-0.082	0.789	0.850	0.102
SSP Tactile sensitivity	-0.021	0.581	0.772	0.104
SSP Taste/smell sensitivity	-0.112	0.867	0.867	0.100
SSP Underresponsive/seeks attention	0.1585	0.061	0.212	0.102
SSP Visual/auditory sensitivity	-0.028	0.606	0.772	0.102
Glutamate: Receptors/transporters gene-set (N=31)				
SSP Auditory filtering	0.096	0.270	0.541	0.156
SSP Low energy/weak	0.355	0.012	0.152	0.156
SSP Movement sensitivity	0.104	0.254	0.541	0.158
SSP Tactile sensitivity	0.273	0.045	0.208	0.161
SSP Taste/smell sensitivity	0.023	0.442	0.729	0.155
SSP Underresponsive/seeks attention	0.318	0.0218	0.152	0.158
SSP Visual/auditory sensitivity	-0.092	0.719	0.839	0.158
GABA: Pathway gene-set (N=124)				
SSP Auditory filtering	-0.004	0.522	0.905	0.080
SSP Low energy/weak	0.022	0.391	0.905	0.080
SSP Movement sensitivity	-0.029	0.640	0.905	0.081
SSP Tactile sensitivity	-0.076	0.825	0.905	0.082
SSP Taste/smell sensitivity	-0.104	0.905	0.905	0.080
SSP Underresponsive/seeks attention	0.117	0.075	0.526	0.081
SSP Visual/auditory sensitivity	-0.049	0.726	0.905	0.081
GABA: Receptors/transporters gene-set (N=23)				
SSP Auditory filtering	0.061	0.383	0.836	0.206
SSP Low energy/weak	-0.201	0.836	0.836	0.206
SSP Movement sensitivity	0.211	0.153	0.537	0.207
SSP Tactile sensitivity	-0.172	0.793	0.836	0.212
SSP Taste/smell sensitivity	-0.083	0.657	0.836	0.205
SSP Underresponsive/seeks attention	0.228	0.137	0.537	0.208
SSP Visual/auditory sensitivity	-0.176	0.801	0.836	0.208

N, number of genes in analysis. Diagnosis was indicated as a binary variable. SSP, Short Sensory Profile; P_{FDR} p-value corrected using False discovery rate (FDR); SE, standard error of the regression coefficient. Significant results (pFDR<0.05) marked in bold.

Competitive gene-set analysis on cortical thickness

Table S6: Glutamate - left hemisphere competitive gene-set analysis

FreeSurfer region	NGENES	BETA	*P*	P_{FDR}
Banks of superior temporal sulcus	72	-0.028	0.603	0.967
Caudal anterior cingulate cortex	**72**	**0.182**	**0.042**	0.776
Caudal middle frontal gyrus	72	-0.047	0.671	0.967
Cuneus	72	-0.090	0.809	0.967
Entorhinal cortex	72	0.010	0.461	0.967
Frontal pole	72	0.030	0.388	0.967
Fusiform gyrus	72	-0.045	0.669	0.967
Inferior parietal cortex	72	-0.090	0.801	0.967
Inferior temporal gyrus	72	-0.115	0.867	0.967
Insula	72	0.030	0.387	0.967
Isthmus-cingulate cortex	72	-0.125	0.883	0.967
Lateral occipital gyrus	72	-0.107	0.851	0.967
Lateral orbital frontal cortex	72	0.164	0.064	0.776
Lingual gyrus	72	0.105	0.157	0.967
Medial orbital frontal cortex	72	0.075	0.244	0.967
Middle temporal gyrus	72	-0.056	0.698	0.967
Paracentral lobule	72	0.046	0.330	0.967
Parahippocampal gyrus	72	-0.030	0.611	0.967
Pars opercularis	72	-0.077	0.765	0.967
Pars orbitalis	72	0.157	0.068	0.776
Pars triangularis	72	-0.054	0.694	0.967
Pericalcarine cortex	72	-0.001	0.503	0.967
Postcentral gyrus	72	-0.200	0.971	0.971
Posterior cingulate cortex	72	-0.088	0.792	0.967
Precentral gyrus	72	-0.045	0.664	0.967
Precuneus cortex	72	-0.012	0.548	0.967
Rostral anterior cingulate cortex	72	-0.130	0.888	0.967
Rostral middle frontal gyrus	72	-0.038	0.640	0.967
Superior frontal gyrus	72	-0.057	0.702	0.967
Superior parietal cortex	72	-0.051	0.686	0.967
Superior temporal gyrus	72	-0.160	0.936	0.967
Supramarginal gyrus	72	-0.166	0.939	0.967
Temporal pole	72	-0.047	0.667	0.967
Transverse temporal cortex	72	-0.106	0.846	0.967

NGENES, number of genes in analysis. Significant associations are marked in bold.

Table S7: GABA - left hemisphere competitive gene-set analysis

FreeSurfer region	NGENES	BETA	P	P_{FDR}
Caudal anterior cingulate cortex	124	-0.021	0.599	0.956
Caudal middle frontal gyrus	124	0.086	0.150	0.555
Cuneus	124	0.066	0.211	0.652
Entorhinal cortex	124	-0.090	0.867	0.956
Frontal pole	124	0.084	0.154	0.555
Fusiform gyrus	124	0.119	0.074	0.458
Inferior parietal cortex	124	-0.046	0.716	0.956
Inferior temporal gyrus	124	-0.079	0.829	0.956
Insula	124	0.017	0.415	0.940
Isthmus-cingulate cortex	124	-0.101	0.894	0.956
Lateral occipital gyrus	124	0.036	0.331	0.812
Lateral orbital frontal cortex	124	-0.070	0.806	0.956
Lingual gyrus	124	0.082	0.163	0.555
Medial orbital frontal cortex	124	-0.078	0.829	0.956
Middle temporal gyrus	**124**	**0.227**	**0.004**	0.127
Paracentral lobule	124	-0.145	0.956	0.956
Parahippocampal gyrus	124	-0.004	0.519	0.956
Pars opercularis	124	-0.047	0.715	0.956
Pars orbitalis	124	0.094	0.129	0.555
Pars triangularis	124	0.116	0.081	0.458
Pericalcarine cortex	**124**	**0.172**	**0.020**	0.347
Postcentral gyrus	124	-0.073	0.813	0.956
Posterior cingulate cortex	124	-0.057	0.755	0.956
Precentral gyrus	124	0.004	0.482	0.956
Precuneus cortex	124	-0.078	0.825	0.956
Rostral anterior cingulate cortex	124	-0.030	0.643	0.956
Rostral middle frontal gyrus	124	0.118	0.080	0.458
Superior frontal gyrus	**124**	**0.155**	**0.031**	0.352
Superior parietal cortex	124	0.036	0.335	0.812
Superior temporal gyrus	124	-0.054	0.746	0.956
Caudal anterior cingulate cortex	124	-0.114	0.918	0.956
Supramarginal gyrus	124	-0.129	0.937	0.956
Temporal pole	124	0.049	0.280	0.793
Transverse temporal cortex	124	-0.094	0.875	0.956

NGENES, number of genes in analysis. Significant associations are marked in bold.

Replicating gene-expression analysis using ABIDE data

The ABIDE cortical thickness (CT) data was acquired from the open source data base (http://fcon_1000.projects.nitrc.org/indi/abide/) (2), where we selected participants within the same age-range (6-30 years) as our LEAP sample, matched for age, sex and IQ (autistic =437, male/female = 385/52, neurotypical =437, male/female = 349/88). To replicate the analysis performed in our LEAP sample, we calculated CT-difference scores between the autism and neurotypical groups as described in the manuscript.

Gene expression analysis using the ABIDE CT-difference interregional profiles showed no significant associations in the whole sample. However, separating the participants into age groups (children, adolescents and adults, see also main manuscript), showed similar results to our findings in LEAP, especially for the adolescents (autistic = 188, neurotypical = 181), where the same positive association between CT differences and both glutamate and GABA expression profiles were found (glutamate: t=1.94, q=0.059, Cohen's d=0.61; GABA: t=3.56, q=0.003, Cohen's d=1.34), although only nominally significant for glutamate. As opposed to the LEAP sample, also in adults (autistic = 104, neurotypical = 125) a positive association was found between the interregional profile of differences in CT (autistic-neurotypical) and profiles of glutamate and GABA gene expression (t=2.38, q=0.023, d=0.74 and t=2.51, q=0.023, d=0.95, respectively). No significant associations were found in children.

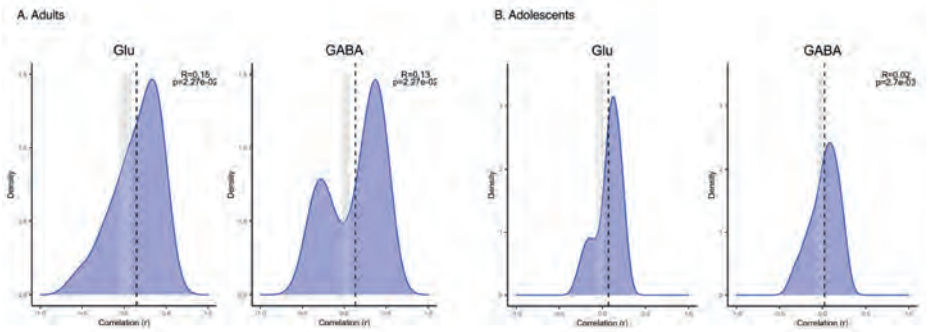

Figure S1: Correlation coefficients in ABIDE

Distributions of the inter-regional correlation coefficients between differences in cortical thickness (CT) and profiles of gene-expression in adults (A) and adolescents (B). The CT-difference profile was obtained from the ABIDE data, and the expression profiles from the Allen Human Brian Atlas (AHBA), in our glutamate- and GABA-pathway gene-sets. The x-axes show the correlation coefficient between CT-difference and expression profile for all genes in the gene-set; the y-axes show the estimated probability density for the correlation coefficients; the vertical dashed-lines indicates the average expression-CT difference correlation coefficient across all the genes in a gene-set; and the edges of the gray boxes indicates the 2.5% and 97.5%-critical values obtained from the empirical null distribution of the average expression-thickness correlation coefficient. If a vertical line sits outside the gray box, it implies that there is a significant association between gene-set and differences in CT at the unadjusted 5% significance level.

Sensory processing subgroups

The LEAP data was separated into low, moderate or severe sensory processing subgroups (3). Interregional CT profiles were calculated by taking the average CT across participants in each brain region, in each sensory subgroup separately. Interregional CT profiles were significantly associated with both glutamate and GABA pathway gene expression in all sensory processing subgroups (LOW: glutamate: t=3.02, q=0.004, Cohen's d = 0.94; GABA: t=3.19, q=0.004, Cohen's d=1.21; MODERATE: glutamate: t=3.18, q=0.003, Cohen's d = 0.99; GABA: t=3.30, q=0.003, Cohen's d=1.25; SEVERE: glutamate: t=3.03, q=0.004, Cohen's d = 0.95; GABA: t=3.18, q=0.004, Cohen's d=1.20), see Figure S2. Regions with increased expression of glutamate and GABA genes showed greater CT across all sensory subgroups.

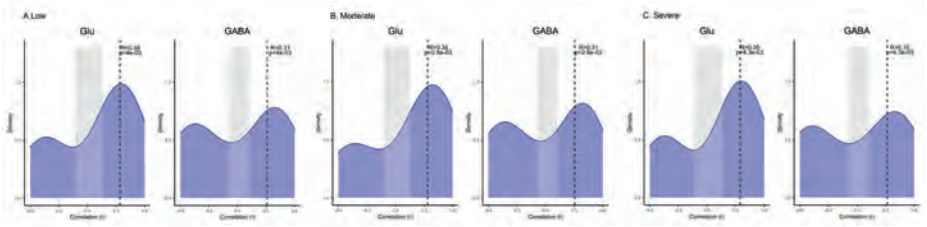

Figure S2: Correlation coefficients in sensory subgroups

Distributions of the inter-regional correlation coefficients between interregional profiles cortical thickness (CT) and profiles of gene-expression in separate sensory subgroups (A – sensory low, B – sensory moderate, C – sensory severe). The CT-difference profiles were obtained from our LEAP data, and the expression profiles from the Allen Human Brian Atlas (AHBA), in our glutamate-pathway and GABA-pathway gene-sets. The x-axes show the correlation coefficient between CT and expression profile for all genes in the gene-set; the y-axes show the estimated probability density for the correlation coefficients; the vertical dashed-lines indicates the average expression-CT correlation coefficient across all the marker genes in a gene-set; and the edges of the gray boxes indicates the 2.5% and 97.5%-critical values obtained from the empirical null distribution of the average expression-thickness correlation coefficient. If a vertical line sits outside the gray box, it implies that there is a significant association between gene-set and CT profiles at the unadjusted 5% significance level.

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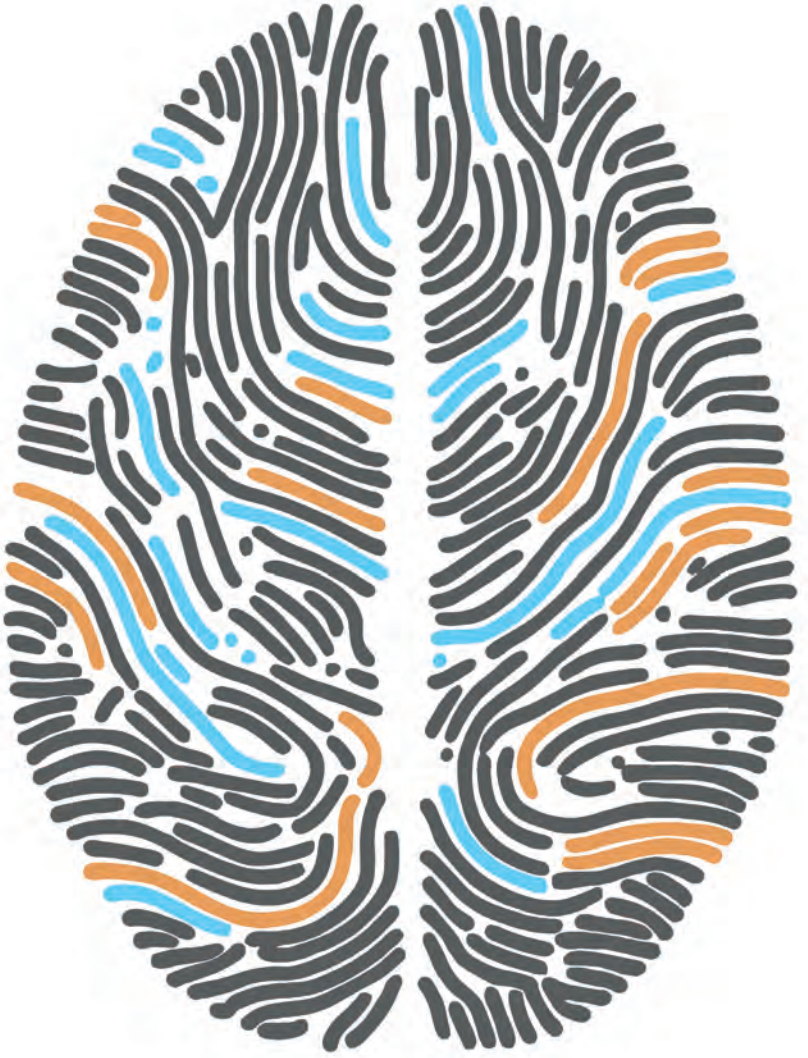

Chapter 4

Estimating differing causal roles of glutamate and GABA genes on brain and behavior in autism

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Abstract

The excitatory/inhibitory (E/I) imbalance theory suggests that an imbalance between excitation and inhibition underlies autism characteristics. The nature of this suggested imbalance, how it is mediated in the brain or leads to the behavioral characteristics of autism, is unclear. We aimed to address this by building causal models to estimate relationships between autism polygenic scores in excitation (glutamate) and inhibition (GABA) communication pathway genes and behavioral measures of core clinical behavioral characteristics of autism. Particular attention was put on the restricted-repetitive behavioral domain, as it is one of the most common autism traits, and may be reflected by functional activity (fMRI) measures during inhibitory control. We used Bayesian Constraint-based Causal Discovery (BCCD) algorithms to build causal models of the relationships between these data modalities in a discovery sample (LEAP cohort: n = 596, autistic = 343, neurotypical = 253) and two generalization cohorts with partially overlapping measures (TACTICS: n = 160, autistic = 60, neurotypical = 100; Simon Simplex Collection (SSC): n, autistic = 2756). While we did not find links between functional activity during the inhibitory control task and the other gene and behavior measures, we found causal links between genetics and behavior. Glutamate polygenic scores were estimated to causally underlie autism characteristics captured by the Autism Diagnostic Interview (ADI-R) in autistic participants. This was not replicated in another cohort, likely due to clinical and genetic differences between the LEAP and SSC cohorts, as indicated by post-hoc tests. In a generalization cohort including *in vivo* ^{1}H-MRS measures of glutamate, we also identified a causal link between GABA polygenic scores with ACC glutamate concentrations. Conclusively, glutamate and GABA genes seem to play different roles relating to behavioral autism traits, and these relationships differ between autistic and neurotypical individuals.

Introduction

Autism spectrum disorder (autism) is a heterogeneous neurodevelopmental condition characterized by difficulties in social interaction and communication, restricted repetitive behaviors and altered sensory processing (1). Autism is highly heritable and affected both by rare genetic variants and common genes, but its etiology is not yet well understood (2,3). One of the most influential theories of its underlying mechanisms is the excitatory/inhibitory (E/I) imbalance theory, which suggests that a chemical imbalance between excitatory (predominantly glutamate) and inhibitory (predominantly GABA (γ-aminobutyric acid)) neurotransmission underlies autism symptomatology (4). However, we do not know *how* alterations in excitation and inhibition may give rise to autism characteristics, and studies investigating glutamatergic and GABAergic functions in the brain have had inconsistent results. This is likely due to several factors, including the different aspects of E/I mechanisms studied across animal models, post-mortem and *in vivo* approaches, differences across study populations, and the possibility that various alterations in the brain may lead to similar clinical characteristics (5,6).

To date most studies have focused on either excitatory *or* inhibitory measures, rather than investigating both simultaneously, which ignores the complex interactions that may play a part in developing behavioral autistic characteristics. Thus, E/I imbalance(s) can arise in various ways, which in turn, underlie different expressions of autism characteristics (7). Excitation and inhibition are fundamental aspects of brain functioning, and E/I mechanisms exist and interact on a cellular level within individual neurons, between neurons within brain regions, and across the whole brain in communication networks. Genetic associations between glutamate and GABA communication pathways and behavioral autism characteristics have previously been found in both animal and human studies (8–11). Here we aimed to assess potential causal associations across several domains by combining genetic approximations of glutamate and GABA and core clinical characteristics of autism. As we also had access to functional activity during an inhibitory control task we put particular focus on the restricted-repetitive behavior domain.

Restricted-repetitive behaviors are amongst the most common and impactful autistic traits. A feature of repetitive behaviors is inhibitory control difficulties, where increased repetitive behaviors contribute to increased inhibitory control difficulties in autistic individuals (12–14). Inhibitory control can be captured in cognitive tasks such as flanker and stop-signal tasks. Fronto-striatal circuits are known to be involved in regulating inhibitory control, and *in vivo* measures of alterations

in glutamate concentrations have been associated with differences in inhibitory control performance in the anterior cingulate cortex (ACC) and striatum (15–17). Yet, studies investigating inhibitory control in autism have had inconsistent results, where some studies have found differences in performance, or functional brain activation, between autistic and neurotypical participants (12–14) while others have not (18–20). Some studies have found differences in functional activity during inhibitory control using functional magnetic resonance imaging (fMRI) despite an absence of behavioral differences (19,21,22). These inconsistencies could be due to several factors, including heterogeneity across autistic individuals, differences across study populations, and varying impact of E/I imbalance on inhibitory control performance and functional brain activity. Gaining a deeper understanding of how E/I imbalance relates to behavioral characteristics of autism, and the functional brain activity of such behaviors, will be beneficial for disentangling the etiologies of various autism traits.

All in all, links across genetic contributions, functional activity and behavioral characteristics in autism are not well understood. Findings to date have had inconsistent approaches, study populations, and results. Here we aimed to address this by using causal discovery models to evaluate links between these measures to identify the most likely causal relationships between genetics, brain, and behavior. This is a data driven approach that estimates the most likely causal structure between the data, which has the potential to direct future investigations more effectively. We also included the possibility that other commonly co-occurring traits such as ADHD, anxiety, age and sex affect these relationships.

More specifically, we used Bayesian Constraint-based Causal Discovery (BCCD), a state-of-the-art algorithm that learns causes and effects from observational data and detects whether the dependency between variables is direct or mediated through other variables (23). Genetic variation within glutamate and/or GABA pathways was estimated using gene-set autism polygenic scores, to aggregate the various contributions of these genes. These polygenic scores were evaluated for causal relationships with core behavioral characteristics of autism, and brain activity in selected regions of interest during inhibitory control. We used a large sample (n = 596, autistic = 343, neurotypical = 253) as our discovery sample, and two generalization samples (first sample: n = 160, autistic = 60, neurotypical = 100, second sample: n, autistic = 2756). These additional cohorts did not provide the same measures and age ranges as the discovery sample, but were used for generalization analyses. The first generalization sample additionally included Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) measures of *in vivo* glutamate

concentrations in the ACC and striatum, which allowed for inclusion of another level of E/I proxies to be evaluated with the genetic and fMRI based measures.

BCCD differs from commonly used regression analyses as it disentangles causal structures, while regression analyses test strengths of presupposed associations under the assumption that such relationships are true. By identifying the most plausible structures between data modalities, we could identify which relationships are most likely useful to focus on in further investigations.

Methods

Participants

Data from three separate cohorts were used, one as a discovery sample and two others as generalization samples. Our discovery sample was the Longitudinal European Autism Project (LEAP) cohort, part of the AIMS-2-TRIALS research programme (https://www.aims-2-trials.eu/) (24–26). We used data from 596 participants (autistic = 343, neurotypical = 253) aged 6-30 years, collected at six centers across europe (Institute of Psychiatry, Psychology and Neuroscience, King's College London (IoPPN/KCL, UK), Autism Research Centre, University of Cambridge (UCAM, UK), University Medical Centre Utrecht (UMCU, Netherlands), Radboud University Medical Centre (RUMC, Netherlands), Central Institute of Mental Health (CIMH, Germany), and the University Campus Bio-Medico (UCBM) in Rome, Italy).

The first generalization sample was from the European Union funded TACTICS cohort (27)(www.tactics-project.eu), where we included data from of 160 participants (autistic = 60, neurotypical = 100), aged 8-13 years old, collected from three centers across Europe (Radboud University Medical Centre, Nijmegen, The Netherlands; King's College London, London, United Kingdom; and Central Institute of Mental Health, Mannheim, Germany). Details regarding inclusion and exclusion criteria for both cohorts can be found in the supplement.

The second generalization sample included genetic and behavioral measures from the Simons Simplex Collection (SSC), where we used data from 2756 autistic participants between 4-18 years old, collected in the USA (28).

Phenotypic measures

The phenotypic measures in the LEAP cohort were part of a larger test battery (see (24)). We included three questionnaires capturing the core autism characteristics; social behaviors (Social Responsiveness Scale-Revised (SRS-2; (29)), repetitive behaviors (Repetitive Behavior Scale-Revised (RBS-R; (30) and sensory processing (Short Sensory Profile (SSP; (31)). In the autistic participants the autism scores on the Autism Diagnostic Observation Schedule Second Edition (ADOS-2, (32)) and Autism Diagnostic Interview - Revised (ADI-R, (33)) were available. The RBS-R and ADI-R were also available in the TACTICS cohort. Additionally, the Children's Social Behavior Questionnaire (CSBQ; (34)) in the TACTICS sample, similar to the SRS-2 used in LEAP, was included as a similar measure of social communicative behaviors. These questionnaires were either parent or self-report depending on age and diagnostic group. In the SSC cohort the SRS-2, RBS-R, ADOS-2 and ADI-R were available. An overview of what measures were used in which cohort can be found in supplementary Table S1.

We included measures of the most common co-occurring conditions to account for potential confounding or mediating effects between our measures of interest; ADHD (which also consistently show differences on inhibitory control tasks) (DSM-5 ADHD-Rating Scale), anxiety (Beck Anxiety Inventory (BAI; (35)) and depression (Beck Depression Inventory-II; (36)) in the LEAP cohort. In the TACTICS cohort, ADHD measures from a different rating scale were also available (Conners' Parent Rating Scale (CPRS-R; (37).

Genetics

Genotyping

Genotyping of the LEAP cohort was performed at the Centre National de Recherche en Génomique Humaine (CNRGH) using the Infinium OmniExpress-24v1 BeadChip Illumina. Genotyping of the TACTICS cohort was performed using the PsychChip_v1-1_15073391 platform in Bonn. For details on how these were performed, see the supplement.

Gene-set selection

The glutamate (n = 72) and GABA (n = 124) gene-sets have been used in several studies previously (9,38,39), and consist of genes encoding proteins involved in glutamatergic and GABAergic pathways in the brain. The gene selection was based on Ingenuity Pathway Analysis software (http://www.ingenuity.com), a database for genetic pathway analysis based on evidence from scientific literature and other

sources such as gene expression and annotation databases, assigning genes to groups and categories of functionally related genes. The complete lists of genes in each gene-set can be found in supplementary Tables S2 and S3.

Gene-set polygenic scores

We derived gene-set based polygenic scores (PGS) for our glutamate and GABA gene-sets using the PRSet function in PRSice-2 (40,41) with the summary statistics of the PGC ASD GWAS (Genome wide association study) (42). SNPs were clumped based on LD using PRSice default settings (bidirectional 250Kb-window and R2-threshold of 0.1), resulting in 103.045 LD-clumped SNPs in the LEAP cohort, 103.043 LD-clumped SNPs in the TACTICS cohort, and 174.617 LD-clumped SNPs in the SSC cohort. Glutamate and GABA gene-set PGS were calculated at a p-value threshold of 1, to include the whole gene-set in the PGS.

Neuroimaging

fMRI acquisition

In both the LEAP and TACTICS cohorts, all sites acquired data on 3T Magnetic Resonance (MR) scanners, obtaining functional MRI during an inhibitory control task (see below for details). Additional Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) data were acquired in the TACTICS cohort to measure glutamate concentrations in the ACC and striatum. The scanner and sequence details for both cohorts, as well as the processing details for the ^{1}H-MRS data, can be seen in the supplement and Tables S4-S5.

fMRI preprocessing

The data from both cohorts have been processed and analyzed previously, and were reused here for consistency with prior work. Task processing was performed identically in both cohorts (see below for further information). The LEAP data were preprocessed using the Statistical Parametric Mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Acquisition time correction was followed by two-step realignment procedure to the mean functional image, coregistration of the functional data to the individual anatomical scan, followed by unified segmentation and normalization to standard stereotactic space as defined by the Montreal Neurological Institute (MNI), and smoothing with a 8mm full-width-at-half-maximum Gaussian Kernel. For a subset of participants from Mannheim, preprocessing additionally included bias correction of the mean image during coregistration, to adjust for measurements performed without prescan normalize option.

The TACTICS data were preprocessed using FSL (https://fsl.fmrib.ox.ac.uk/fsl/). Head movement was corrected by realigning to the middle volume (MCFLIRT; (43). Grand mean scaling and spatial smoothing was done with a Gaussian kernel at FWHM of 6mm. ICA-AROMA was used to remove secondary-head motion, followed by nuisance regression to remove CSF and white matter signal, and high-pass filtering (100 s). The fMRI data was coregistered to each participant's anatomical scan using boundary-based registration by non-linear registration FSL-FNIRT (44). Lastly, coregistration to the MNI template was done using a 6 mm FWHM.

fMRI inhibitory control tasks

In the LEAP cohort the inhibitory control task was a modified version of a combined flanker-go/no-go task (45), where participants were asked to press a left or right button depending on the direction of an arrow presented at the center of the screen. This arrow was flanked by arrows pointing either in the same direction (congruent), opposite direction (incongruent) or flanked by x's (neutral) to the centrally presented arrow. If the arrow was flanked by x's the participant was asked to withhold a response (no-go). In the TACTICS cohort the inhibitory control task consisted of a stop-signal task (46), where one arrow was presented on a screen and participants were asked to press a left or right button depending on the direction of the arrow presented on the screen. In 20% of trials the arrow was followed by a stop cue (arrow pointing upwards) and the participant was asked to withhold a response. The time between the stimulus and stop-signal (stop-signal delay) was adaptive depending on the participants performance, ensuring successful inhibition in approximately 50% of stop-trials.

To allow comparisons between the two inhibitory control tasks across the LEAP and TACTICS cohorts, contrasts reflecting successful and failed inhibitory control were modified from standard contrasts as follows, in SPM12 (Statistical Parametric Mapping release 12, https://www.fil.ion.ucl.ac.uk/spm/). In the LEAP cohort, successful inhibitory control was defined as no-go trials - failed trials, failed inhibitory control was defined as failed trials - congruent or neutral trials. Note that failed trials comprised all committed errors including omission errors in no-go trials, interference errors to incongruent trials, and omission errors to congruent, incongruent and neutral trials. In the TACTICS cohort successful inhibitory control was defined as successful stop trials - failed stop, and failed inhibitory control was defined as failed stop - successful go trials. The second level analyses of these contrasts used full-factorial designs where t-contrasts were applied to the first level contrast maps. These contrasts were created to capture inhibitory control mechanisms as similarly as possible across the cohorts. For more details on these processing pipelines, see the supplement.

We extracted the mean beta weights, which were the estimated changes in BOLD activity during our inhibitory control contrasts, from the ACC and dorsal striatum. These regions were our task-relevant regions of interest for the inhibitory control task, and additionally had ^{1}H-MRS measures of glutamate from ACC and striatum in the TACTICS cohort. Registration between fMRI and ^{1}H-MRS was done with the MarsBar toolbox (47), using the voxel placement of the ^{1}H-MRS measures as the ROI in both cohorts. This resulted in four estimations of mean beta weight of functional activity for each participant; for each contrast (successful and failed inhibitory control), and in each brain region (ACC and striatum). The LEAP cohort has fMRI data available from 354 participants. The TACTICS cohort had fMRI data available from 44 participants who additionally had ^{1}H-MRS measures of glutamate concentrations in ACC and striatum.

Statistical analysis

BCCD analysis

We used the Bayesian constraint-based causal discovery (BCCD) algorithm to find direct and indirect (mediated by other variables) interactions (23). The benefits of the BCCD algorithm are its ability to handle a combination of continuous and discrete variables, while also handling missing data, which is dealt with when estimating the correlation matrix using expectation maximization algorithms (48). This method combines the strengths of constraint-based methods giving strong and clear causal relationships, and of score-based methods estimating confidence measures of inferred causal relationships. BCCD gives us reliable estimated causal relationships between variables, with an estimation of the likelihood of these relationships. It has been evaluated and confirmed to be an effective method in this context, and has been used on similar types of datasets investigating other neurodevelopmental conditions such as ADHD, and psychopathologies (49–53).

BCCD is a hypothesis free approach, based on a set of assumptions including the absence of cyclic dependencies (for more details, see (23,52)), and can therefore validate previously found associations between data modalities. It also provides additional information compared to regression-based approaches for casual interpretation: regression analysis assumes predefined relationships between variables, and is based on the decomposition of variance in the dependent variables. BCCD is very different as it explores evidence for causal probabilities between variables, and generates a causal model that best explains the observed structure between the data. The observational data fed into the BCCD is mapped onto a correlation matrix through a Gaussian transformation (23). This is followed

by an efficient search to obtain Bayesian reliability scores, resulting in weighted independence constraints. Lastly, the logical independence constraints are used with initially defined background knowledge (behavioral measures cannot cause polygenic scores, sex, or age) and creates an output model. Estimated causal links with a reliability of 60% or higher are considered robust, which are presented in Figures 1-5. BCCD does not provide effect sizes, unlike regression models which assume pre-defined relationships between variables and estimate the effect of those relationships. Instead, it provides likelihood estimations of the identified relationships, which can be found across all models in the supplementary Tables S9-S18. To investigate whether there are differences between autistic and neurotypical participants, we created separate models with autistic and neurotypical LEAP participants, and additionally created a model with all participants combined. As the SSC cohort only consist of autistic participants, separating by diagnostic group also allowed for a more direct comparison between the cohorts.

For each model (autism, neurotypical, and whole cohort), participants with >50% of the data missing were excluded to reduce the risk of unwanted imputation effects, resulting in 596 LEAP participants (autistic = 343, neurotypical = 253) and 2756 SSC participants. In the TACTICS cohort a large part of participants had >50% missing data, we therefore included participants with up to 60% missing data, resulting in 160 included participants (autistic = 60, neurotypical = 100). This did not affect the estimated causal structure in the model, but provided increased power for more accurate model estimation. For an overview of which measures were included in what cohort, see Table S1 in the supplement.

Comparing cohorts

Post-hoc tests were performed to compare gene-set PGS and ADI-R scores between the LEAP and SSC cohorts using standard two-sided t-tests in base R software (54).

Results

Demographics

Demographic and clinical characteristics of all cohorts are shown in Tables S6-S8 in the supplement. In the LEAP cohort, no differences were found between the diagnostic groups in age or sex, the autism group had a lower IQ compared to neurotypical participants (details can be seen in Table S6). In the TACTICS cohort there were no group differences in age, female-to-male ratio, or IQ. The SSC cohort only include autistic participants, therefore no group comparisons were performed.

Bayesian constraint-based causal discovery

The models output by the BCCD algorithm can be seen in the figures below, where variables of interest (nodes) are connected via lines (edges), representing an estimated causal relationship. The figures show edges with a causal link reliability of >= 60%. Exact values of all edges and estimated correlations between variables can be seen in supplementary Tables S9-S18.

LEAP

Starting with LEAP, Figure 1 shows the autism group, Figure 2 the neurotypical group and Figure 3 the whole cohort. Only Figure 1 includes the measures of ADOS-2 and ADI-R, as these were only measured in the autistic participants.

In the autism group (Figure 1), we observed a direct causal link of 95% reliability (Table S9) between the glutamate PGS and the ADI-R communication domain. Additionally, there were indirect links continuing to the ADI-R social and repetitive domains as well. The glutamate and GABA PGS were causally linked to each other with 97% reliability in the autism group, which was not present in the neurotypical group (Figure 2). As the glutamate and GABA PGS are both genetic scores we cannot infer directionality between them.

Across the whole cohort (Figure 3), as well as in the separate groups (Figures 1 and 2), we observed causal links between RBS-R, SRS-2 and SSP scores. These results show that what are typically referred to as the core clinical behaviors for autism (repetitive behaviors, social-communicative behaviors and sensory processing) are not just related within autistic individuals but that these behaviors affect each other across participants irrespective of diagnosis. In the autism group (Figure 1) we also observed links between SRS-2 and the ADI-R social domain, and between RBS-R and the ADI-R repetitive behavior domain, confirming that the SRS-2 and RBS-R questionnaires capture similar behavioral traits as the ADI-R social and repetitive domains.

The BOLD contrast measures of functional activity during successful and failed inhibitory control in the ACC and striatum were causally connected with at least 97% reliability, but were separate from the other measures in the models, both across the whole cohort and in the separate groups (Figures 1-3, Tables S9, S11, S13). In the neurotypical group however, there was a causal link of 93% reliability (Table S11) between IQ and failed inhibitory control BOLD activity in striatum, which was not present in the autism group. This link was also seen across the whole sample (Figure 3).

TACTICS

We did not have enough data to divide into diagnostic groups due to too much missingness, and therefore only used the whole sample (Figure 4). We did however replicate some of the causal relationships between the behavioral measures that overlap in the LEAP cohort. Firstly, the CSBQ, an equivalent measure to the SRS in LEAP, showed causal links to RBS and ADHD scores similar to the LEAP cohort (Figure 3), indicating that the causal links between social and repetitive behaviors captured by these questionnaires are robust. Secondly, we replicated the link between BOLD signal in striatum during failed inhibitory control and IQ as seen in the LEAP cohort, particularly in the neurotypical group. The BOLD activity during failed and successful inhibitory control was not causally linked in the same way as in the LEAP cohort, however, there was a mediating effect by age between successful inhibitory control in ACC and striatum which potentially point towards the differences in age ranges across the TACTICS and LEAP cohorts.

The addition of ^{1}H-MRS glutamate concentrations in this model showed that striatal glutamate concentrations had a causal link to striatal BOLD activity during successful inhibitory control with 96% reliability (Table S15). GABA PGS showed a causal link with 99% reliability to ACC glutamate (Table S15). To exclude that these results were introduced due to imputation effects, as we included participants with up to 60% missing data in this cohort, we confirmed that these patterns were also present in a model without imputation.

As the sample size in TACTICS was relatively small we wanted to attempt to replicate, or generalize, our findings in another cohort, specifically focusing on the gene-set PGS links to behavioral measures in the autism group in the LEAP sample. For this, we used the SSC; the demographic information of which is available in the supplementary Table S8.

SSC

Figure 5 shows causal links between the SRS-2 and ADI-R social domain, and with the RBS-R and ADI-R repetitive domain, replicating the behavioral links between these in the LEAP cohort. However, the in LEAP reported causal link between glutamate PGS to ADI-R domains were not captured here, and glutamate and GABA PGS were also not causally linked to each other, while they were in the autism group in the LEAP cohort.

Comparing cohorts

To disentangle why some estimated causal links did not generalize across cohorts, we compared behavioral and genetic profiles between the LEAP and SSC cohorts using t-tests between the autistic samples on the glutamate and GABA PGS and ADI-R domains. These tests showed that the overall cohorts differ from each other in their glutamate and GABA PGS, and the ADI-R measures (all p-values <0.001), indicating a difference in the genetic and clinical profile of the SSC cohort compared to our European LEAP cohort. These results can be seen in the supplementary Table S19, and individual data points can be seen in supplementary Figures S2-S5.

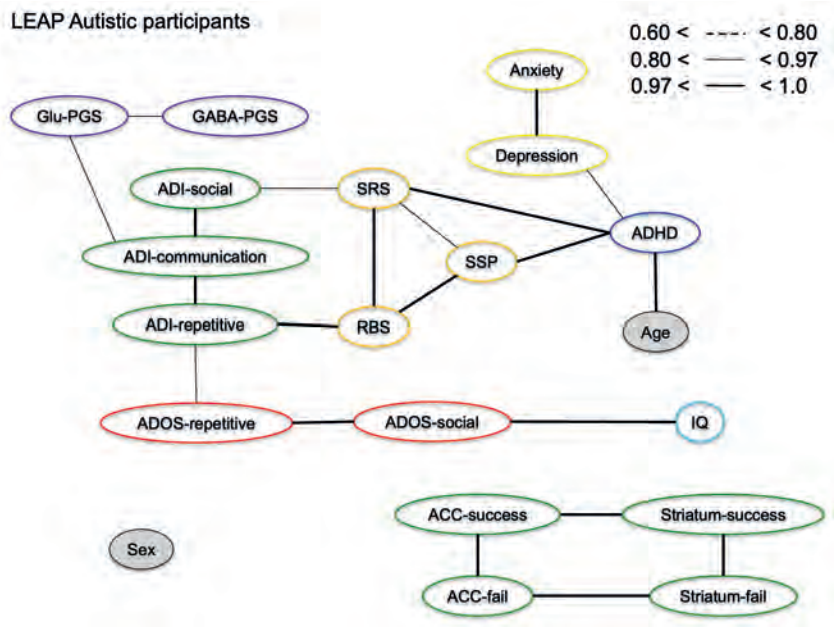

Figure 1: BCCD LEAP Autistic participants

Output causal model representing causal relationships between the genetic, task-based functional MRI and behavioral measures. Reliability estimates for edges shown here are depicted as ranges of percentages as defined in the figure. Glu-PGS, Glutamate polygenic score for autism; GABA-PGS, GABA polygenic score for autism; SRS-2, Social Responsiveness Scale-Revised; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile; ADI-social, Autism Diagnostic Interview-Revised Social domain; ADI-communication, Autism Diagnostic Interview-Revised Communication domain; ADI-repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors; ADOS-total, Autism Diagnostic Observation Schedule Second Edition Total score; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory; ADHD, DSM-V ADHD Rating Scale; ACC-success, BOLD signal in ACC during successful inhibitory control; Striatum-success, BOLD signal in striatum during successful inhibitory control; ACC-fail, BOLD signal in ACC during failed inhibitory control; Striatum-fail, BOLD signal in striatum during failed inhibitory control.

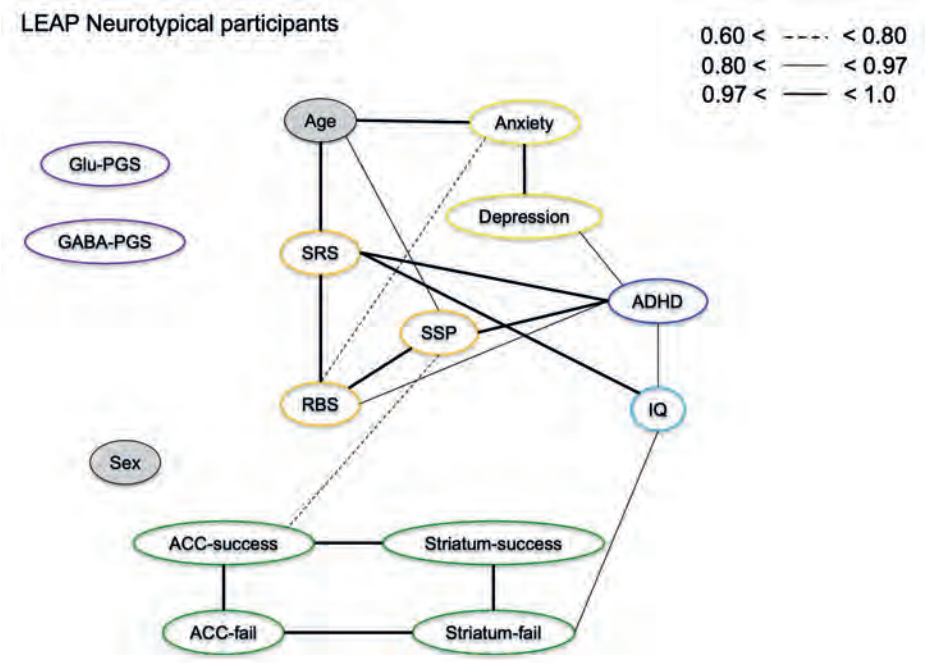

Figure 2: BCCD LEAP Neurotypical participants

Output causal model representing causal relationships between the genetic, task-based functional MRI and behavioral measures. Reliability estimates for edges shown here are depicted as ranges of percentages as defined in the figure. Glu-PGS, Glutamate polygenic score for autism; GABA-PGS, GABA polygenic score for autism; SRS-2, Social Responsiveness Scale-Revised; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory; ADHD, DSM-V ADHD Rating Scale; ACC-success, BOLD signal in ACC during successful inhibitory control; Striatum-success, BOLD signal in striatum during successful inhibitory control; ACC-fail, BOLD signal in ACC during failed inhibitory control; Striatum-fail, BOLD signal in striatum during failed inhibitory control.

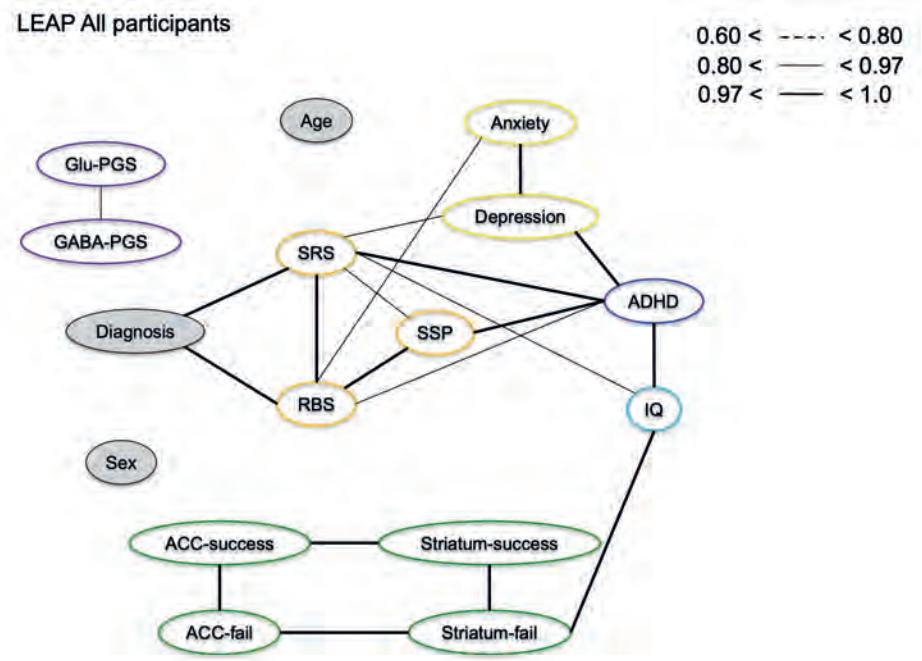

Figure 3: BCCD LEAP All participants

Output causal model representing causal relationships between the genetic, task-based functional MRI and behavioral measures. Reliability estimates for edges shown here are depicted as ranges of percentages as defined in the figure. Glu-PGS, Glutamate polygenic score for autism; GABA-PGS, GABA polygenic score for autism; SRS-2, Social Responsiveness Scale-Revised; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile; ADI-social, Autism Diagnostic Interview-Revised Social domain; ADI-communication, Autism Diagnostic Interview-Revised Communication domain; ADI-repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADHD, DSM-V ADHD Rating Scale; ACC-success, BOLD signal in ACC during successful inhibitory control; Striatum-success, BOLD signal in striatum during successful inhibitory control; ACC-fail, BOLD signal in ACC during failed inhibitory control; Striatum-fail, BOLD signal in striatum during failed inhibitory control.

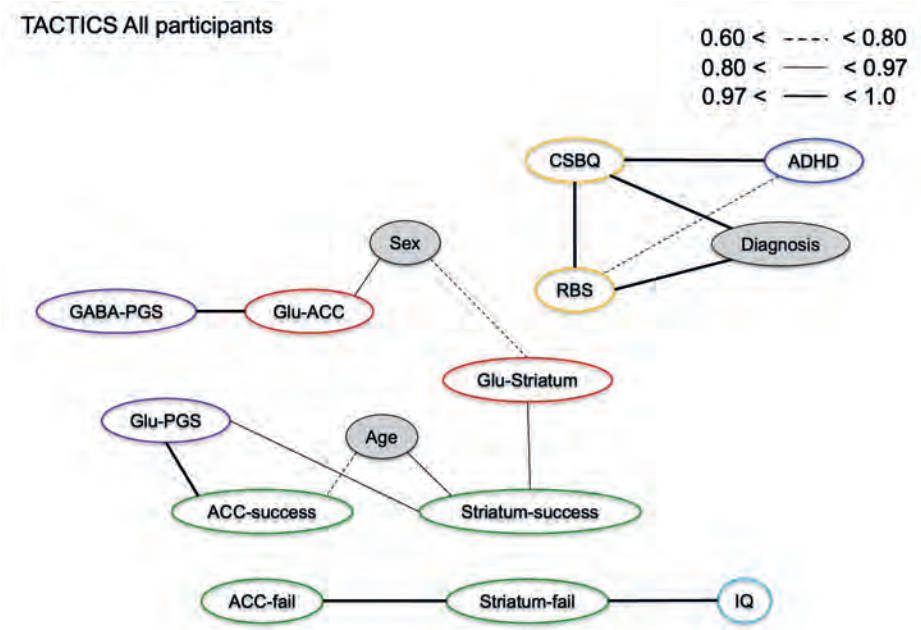

Figure 4: BCCD TACTICS All participants

Output causal model representing causal relationships between the genetic, task-based functional MRI, ^{1}H-MRS glutamate, and behavioral measures. Reliability estimates for edges shown here are depicted as ranges of percentages as defined in the figure. Glu-PGS, Glutamate polygenic score for autism; GABA-PGS, GABA polygenic score for autism; CSBQ, Children's Social Behavior Questionnaire; RBS-R, Repetitive Behavior Scale-Revised; SSP, Short Sensory Profile; ADI-social, Autism Diagnostic Interview-Revised Social domain; ADI-communication, Autism Diagnostic Interview-Revised Communication domain; ADI-repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADHD, Conners' Parent Rating Scale; ACC-success, BOLD signal in ACC during successful inhibitory control; Striatum-success, BOLD signal in striatum during successful inhibitory control; ACC-fail, BOLD signal in ACC during failed inhibitory control; Striatum-fail, BOLD signal in striatum during failed inhibitory control.

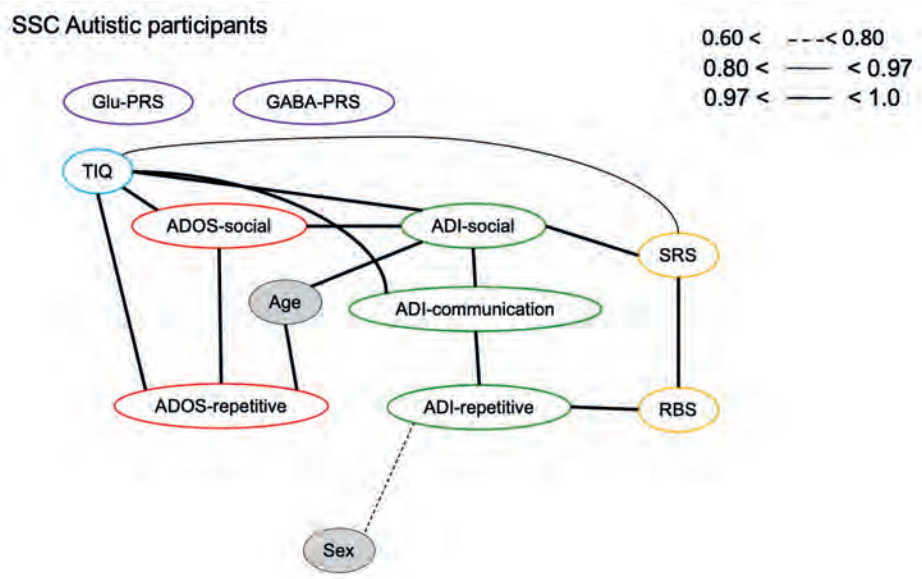

Figure 5: BCCD SSC Autistic participants

Output causal model representing causal relationships between the genetic and behavioral measures. Reliability estimates for edges shown here are depicted as ranges of percentages as defined in the figure. Glu-PGS, Glutamate polygenic score for autism; GABA-PGS, GABA polygenic score for autism; SRS-2, Social Responsiveness Scale-Revised; RBS-R, Repetitive Behavior Scale-Revised; ADI-social, Autism Diagnostic Interview-Revised Social domain; ADI-communication, Autism Diagnostic Interview-Revised Communication domain; ADI-repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors; ADOS-total, Autism Diagnostic Observation Schedule Second Edition Total score.

Discussion

We used BCCD to identify probable causal relationships between glutamate and GABA polygenic scores (PGS), behavioral measures of autism traits and functional MRI, in one discovery cohort and two generalization cohorts with partially overlapping measures. We did not observe links between functional activity during inhibitory control with genetic or behavioral measures, but we did identify plausible causal relationships between genetic and behavioral measures. We observed strong indications for a causal connection between glutamate (autism) PGS and ADI-R domains in the autism group of LEAP, which showed that there are shared genetics between autism polygenic scores and autism traits captured by the ADI-R. These findings confirm previously found associations between the glutamate system and the ADI-R using LEAP, then looking at aggregated genetic variation rather than PGS (9). Additionally, we found a causal link between the GABA (autism) PGS and ACC glutamate in TACTICS, which also serves as confirmation of earlier work on the TACTICS cohort that observed a larger decrease in ACC glutamate in the autistic participants (15). The causal links estimated between these measures and cohorts show that glutamate and GABA genes causally underlie autism traits in distinct ways, and is informative for future work to disentangle regional specificity in the brain, identify more specific biological underpinnings of these causal relationships, and stratify individual differences.

In the autism LEAP sample, glutamate and GABA PGS were causally linked. These links may reflect interactions between glutamate and GABA communication pathways affecting autism likelihood in autistic individuals. However, these findings were absent in the SSC which is discussed below. Glutamate and GABA are metabolically closely related and interact as part of neuronal functioning (55,56). Causal interactions between glutamate and GABA in the autism group specifically may therefore reflect compensatory mechanisms of excitatory and inhibitory functions attempting to maintain balance between them (5). Although the diagnostic groups are not compared directly, this suggests different relationships between the autism polygenic scores in these glutamate and GABA gene-sets between autistic and neurotypical participants.

The estimated causal links between genetic and behavioral measures found here are novel and important for understanding the etiology of autism. That said, they are relatively far removed from the mechanisms in the brain that we try to disentangle, as we do not pick up on e.g. ratios between excitation and inhibition or have regional specificity of where in the brain differences are expressed. In

future work it would be beneficial to include additional measures, such as ^{1}H-MRS concentrations of glutamate and GABA combined, to investigate how ratios between these excitatory/inhibitory measures may indicate (im)balances and how they relate to other brain, gene and behavior measures.

We started to bridge this gap by including glutamate ^{1}H-MRS measures in the TACTICS cohort, although we did not observe links mediating the relationships between genes and behavior, or to brain activity during inhibitory control. There were however links between the successful and failed inhibitory control contrasts. The BOLD contrast measures in the ACC and striatum during inhibitory control had strong links across both LEAP and TACTICS, although the structure of links between failed and successful inhibitory control were not identical across the cohorts. This is possibly due to LEAP and TACTICS using different inhibitory control tasks. While the contrasts of successful and failed inhibitory control were created to be as identical across the cohorts as possible, the potential differences across the tasks and contrasts constitute a limitation for attempted replication and generalization across the cohorts. Further, the ACC and striatum BOLD signals were relatively separate from the other measures (Figures 3-4) which suggests that autism predictors such as the gene-set autism PGS do not strongly influence these functional activity contrasts, at least in these brain regions. It should also be noted that the associations of glutamate genes could indicate both increased glutamate function (increasing excitability) and decreased function (decreasing excitability), but broadly shows that genetic disposition towards differences in glutamate impacts development of autistic traits and thus, that differences in glutamate function are causally driving autism characteristics. This is consistent with prior work showing that altered concentrations of both glutamate and GABA are associated with autism, although these neurotransmitters have rarely been investigated simultaneously (10,15,16,57–64).

Across behavioral measures, we found robust and consistent causal relationships between several behavioral measures that generalize across the LEAP, TACTICS and SSC cohorts. In particular, the SRS-2 had a strong causal relationship with the ADI-R social score, and the RBS-R with the ADI-R repetitive score in both LEAP and SSC. These findings confirm that these measures capture similar aspects of social and repetitive autism traits, reinforcing associations established previously (24) and validating them using a hypothesis-free, data driven approach. Identifying these relationships across the three cohorts also gave us strong confidence that the models themselves are robust and that other findings throughout this chapter could be considered reliable.

It is important to highlight that the glutamate PGS to ADI-R relationships were not replicated in the SSC cohort. Genetics, including polygenic scores, cannot fully explain complex behaviors as they aggregate the small effects of common genetic variants. They therefore do not capture all potential factors where genetics may affect autism likelihood or expression of specific traits. The glutamate PGS to ADI-R relationships may still exist in the SSC cohort, but be operationalized differently and mediated by factors not included in the model, such as epigenetic or environmental factors. The varying results may also be due to differences in genetic and clinical profiles of autism traits in the SSC compared to LEAP. Our post-hoc tests showed differences in the glutamate and GABA PGS. While the GWAS used to create the PGS is the largest available to date, it is based on a European cohort, which may be less accurate for the USA SSC data (65). Furthermore, the PRSet tool used to calculate the PGS is better powered in larger target sample sizes (40). SSC also differ in its clinical profile, as seen in the higher ADI-R scores. This is likely due to more stringent inclusion criteria in the SSC cohort, where ADI-R and ADOS-2 cutoff for diagnosis were used as inclusion criteria. The LEAP and TACTICS cohorts instead relied on prior clinical diagnosis, and used diagnostic scores for ADOS-2 and ADI-R as an additional validation rather than inclusion criterion, which potentially lead to subtle differences in recruitment of participants. However, we do replicate the causal relationships between the behavioral measures in the SSC cohort. The differences across these cohorts are relevant and warrant further investigation. They also highlight the need for caution when generalizing findings beyond datasets like these, particularly outside Europe and the USA, where additional cultural and clinical variations may exist. Such nuances, even between large cohorts like LEAP and SSC can have important impacts on results. It is clear that we cannot solely rely on large sample sizes to combat this.

In conclusion, we found reliable causal relationships between glutamate PGS for autism with behavioral autism traits as captured by the ADI-R, which were not seen with the GABA PGS. In another cohort (TACTICS), we identified a likely causal link between GABA PGS for autism with ACC glutamate concentrations. Glutamate and GABA genes show different roles underlying behavioral autistic characteristics, which is informative for future research disentangling more specific biological underpinnings of these relationships and how it underlies the behaviors and experiences of autistic individuals.

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Conflict of interest statements

Prof. Banaschewski served in an advisory or consultancy role for ADHS digital, Infectopharm, Lundbeck, Medice, Neurim Pharmaceuticals, Oberberg GmbH, Roche, and Takeda. He received conference support or speaker's fee by Medice and Takeda. He received royalties from Hogrefe, Kohlhammer, CIP Medien, Oxford University Press; the present work is unrelated to these relationships. Prof. Buitelaar has been in the past 3 years a consultant to / member of advisory board of / and/or speaker for Takeda, Roche, Medice, Angelini, Janssen, Boehringer-Ingelheim, and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. Dr. Poelmans is director and Dr. Ruisch. and Dr. de Witte are employees of Drug Target ID, Ltd., but their activities at this company do not constitute competing interests with regard to this paper. The remaining authors declare no potential conflict of interest.

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Supplement

Inclusion and exclusion criteria LEAP

Inclusion criteria for the autism group were a clinical diagnosis of autism and age between 6 and 30 years. Autism characteristics were assessed using the Autism Diagnostic Observation Schedule Second Edition (ADOS-2; (1)) and the Autism Diagnostic Interview-Revised (ADI-R; (2)). For the neurotypical participants exclusion criterion consisted of parent- or self-report of any psychiatric disorder. Individuals who had a normative T-score of 70 or higher on the Social Responsiveness Scale Second Edition (SRS-2; (3)) were excluded. Some individuals in the autism and neurotypical groups had intellectual disability (ID) (autism=53, neurotypical=25), defined as an IQ score between 40 and 74. For further details of the recruitment of participants in this cohort see (4,5).

Inclusion and exclusion criteria TACTICS

The inclusion criteria across groups were IQ > 70, ability to speak and comprehend the native language of the location of recruitment and being of Caucasian descent. To confirm a diagnosis in the autistic participants the ADI-R was used. Neurotypical participants were confirmed to not score in the clinical range for any DSM-IV axis I diagnoses using the Child Behavior Checklist (CBCL) and the Teacher Report Form (TRF) (6). For further details of the recruitment of participants in this cohort, see (7).

Genotyping

LEAP

Sample quality controls such as sex check (based on the X chromosome homozygosity rate or the median of the Log R ratio of the X and Y chromosomes), Mendelian errors (transmission errors within full trios) and Identity By State were performed using PLINK 1.90. Imputation of 17 million SNPs was performed using the 700k genotyped SNPs on the Michigan Imputation Server (8). The HRC r1.1 2016 reference panel for a European population was used, as the majority of individuals in the LEAP cohort were from European ancestry. Only autosomes were imputed. Linkage disequilibrium-based SNP pruning was done for SNPs with a MAF > 1% and SNPs with an R2 < 0.1 in windows of 500kb were selected. This resulted in 546 participants with genotypic data (n = 304 autistic, n = 242 neurotypical).

TACTICS

Standard GWAS quality control procedures (including filtering based on minor allele frequency (MAF), Hardy-Weinberg equilibrium (p-value > 1x10e-6), single nucleotide polymorphism (SNP) call rate (> 95%), subject call rate (> 90%), principal component analysis) and imputation (1000 Genomes reference panel) were performed based on RICOPILI (9). The imputed data underwent additional quality control, in which SNPs with an imputation information score (INFO) lower than 0.8 and MAF lower than 0.05 were excluded. After this step, 5.139.250 SNPs across the autosomal genome were retained (no X-chromosome data available). This resulted in 106 participants with genotypic data (n = 31 autistic, n = 75 neurotypical).

Table S1: Overview of available measures in all three cohorts

	LEAP	TACTICS	SSC	Parent/self report
Glutamate PGS	X	X	X	
GABA PGS	X	X	X	
ADI	X	X	X	Parent
ADOS	X		X	Self
RBS	X	X	X	Parent/self
SRS	X	CSBQ used as equivalent	X	Parent/self
SSP	X			Parent/self
ADHD	X	X		Parent/self
Depression	X			Parent/self
Anxiety	X			Parent/self
MRS glutamate (ACC, Striatum)		X		
fMRI successful inhibitory control (ACC, Striatum)	X	X		
fMRI failed inhibitory control (ACC, Striatum)	X	X		

Glutamate PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADI-social, Autism Diagnostic Interview-Revised Social domain; ADI-communication, Autism Diagnostic Interview-Revised Communication domain; ADI-repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors; ADOS-total, Autism Diagnostic Observation Schedule Second Edition Total score; RBS-R, Repetitive Behavior Scale-Revised; SRS-2, Social Responsiveness Scale-Revised; CSBQ, Children's Social Behavior Questionnaire; SSP, Short Sensory Profile; ADHD, DSM-V ADHD Rating Scale; Depression, Beck Depression Inventory; Anxiety, Beck Anxiety Inventory.

Table S2: Summary table of all genes in the glutamate gene-set

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ALDH5A1	7915	6	24495197	24537435	+	297
CALM1	801	14	90863327	90874619	+	47
CALML5	51806	10	5540658	5541533	-	6
CAMK4	814	5	110559947	110830584	+	1538
DLG4	1742	17	7093209	7123369	-	102
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GLS	2744	2	191745547	191830278	+	290
GLUD1	2746	10	88809959	88854776	-	186
GLUD2	2747	X	120181462	120183796	+	
GLUL	2752	1	182350839	182361341	-	55
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2	2783	7	100271363	100276792	+	19
GNB3	2784	12	6949375	6956564	+	34
GNB5	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041
GOT1	2805	10	101156627	101190530	-	146
GOT1L1	137362	8	37791799	37797664	-	17
GOT2	2806	16	58741035	58768246	-	229
GRIA1	2890	5	152870084	153193429	+	1819
GRIA2	2891	4	158141736	158287227	+	425
GRIA3	2892	X	122317996	122624766	+	
GRIA4	2893	11	105480800	105852819	+	1505
GRID1	2894	10	87359312	88126250	-	4622
GRID2	2895	4	93225453	94695707	+	7119
GRIK1	2897	21	30909254	31312282	-	2258
GRIK2	2898	6	101841584	102517958	+	3720
GRIK3	2899	1	37261128	37499844	-	963
GRIK4	2900	11	120382465	120859514	+	2775

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GRIK5	2901	19	42502468	42574278	-	138
GRIN1	2902	9	140033609	140063214	+	86
GRIN2A	2903	16	9847265	10276611	-	3419
GRIN2B	2904	12	13713684	14133022	-	2569
GRIN2C	2905	17	72838162	72856966	-	93
GRIN2D	2906	19	48898132	48948188	+	222
GRIN3A	116443	9	104331634	104500862	-	942
GRIN3B	116444	19	1000437	1009723	+	108
GRINA	2907	8	145064226	145067596	+	9
GRIP1	23426	12	66741178	67463014	-	4124
GRM1	2911	6	146286032	146758782	+	2121
GRM2	2912	3	51741081	51752629	+	16
GRM3	2913	7	86273230	86494193	+	1110
GRM4	2914	6	33989623	34123399	-	1020
GRM5	2915	11	88237256	88796846	-	3817
GRM6	2916	5	178405328	178422124	-	141
GRM7	2917	3	6902802	7783218	+	5656
GRM8*	2918	7	126078652	126892428	-	4521
HOMER1	9456	5	78669647	78809659	-	705
HOMER2	9455	15	83517729	83654905	-	736
HOMER3	9454	19	19040010	19052041	-	42
PICK1	9463	22	38453262	38471708	+	92
SLC17A1	6568	6	25783125	25832287	-	297
SLC17A2	10246	6	25912982	25930954	-	109
SLC17A6	57084	11	22359667	22401049	+	208
SLC17A7	57030	19	49932655	49945617	-	39
SLC17A8	246213	12	100750857	100815837	+	347
SLC1A1	6505	9	4490427	4587469	+	544
SLC1A2	6506	11	35272752	35441610	-	1155
SLC1A3	6507	5	36606457	36688436	+	420
SLC1A4	6509	2	65215579	65250999	+	145
SLC1A6	6511	19	15060845	15121455	-	503
SLC1A7	6512	1	53552855	53608304	-	472
SLC38A1	81539	12	46576838	46663208	-	441
SUCLG2	8801	3	67410884	67705038	-	1963

All genes in the table were included in the glutamate pathway gene-set. NSNPS, number of single nucleotide polymorphisms (SNPs).

Table S3: Summary table of all genes in the GABA gene-set.

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ADCY1	107	7	45614125	45762715	+	760
ADCY10	55811	1	167778357	167883608	-	659
ADCY2	108	5	7396343	7830194	+	2563
ADCY3	109	2	25042038	25142602	-	694
ADCY4	196883	14	24787555	24804277	-	81
ADCY5	111	3	123001143	123167924	-	858
ADCY6	112	12	49159975	49182820	-	81
ADCY7	113	16	50278830	50352046	+	333
ADCY8	114	8	131792546	132053012	-	1901
ADCY9	115	16	4012650	4166186	-	1082
ALDH5A1	7915	6	24495197	24537435	+	297
ALDH9A1	223	1	165631449	165667900	-	239
AP1B1	162	22	29723669	29784754	-	255
AP1G2	8906	14	24028777	24038754	-	14
AP2A1	160	19	50270180	50310369	+	165
AP2A2	161	11	925809	1012245	+	487
AP2B1	163	17	33913918	34053436	+	746
AP2M1	1173	3	183892634	183901879	+	53
AP2S1	1175	19	47341423	47354203	-	35
CACNA1A	773	19	13317256	13617274	-	1465
CACNA1B	774	9	140772241	141019076	+	880
CACNA1C	775	12	2079952	2807115	+	3692
CACNA1D	776	3	53529076	53847179	+	1844
CACNA1E	777	1	181452447	181775920	+	1671
CACNA1F	778	X	49061523	49089833	-	
CACNA1G	8913	17	48638429	48704835	+	310
CACNA1H	8912	16	1203241	1271772	+	422
CACNA1I	8911	22	39966758	40085740	+	591
CACNA1S	779	1	201008635	201081694	-	505
CACNA2D1	781	7	81575760	82073031	-	3150
CACNA2D2	9254	3	50400230	50540892	-	656
CACNA2D3	55799	3	54156620	55108584	+	5930
CACNA2D4	93589	12	1901123	2027870	-	775
CACNB1	782	17	37329709	37353956	-	89

Table S3: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
CACNB2	783	10	18429373	18830688	+	2968
CACNB3	784	12	49208215	49222726	+	46
CACNB4	785	2	152689285	152955593	-	1246
CACNG1	786	17	65040652	65052913	+	56
CACNG2	10369	22	36956916	37098690	-	720
CACNG3	10368	16	24266874	24373737	+	675
CACNG4	27092	17	64960980	65029518	+	432
CACNG5	27091	17	64831235	64881941	+	373
CACNG6	59285	19	54494403	54515920	+	115
CACNG7	59284	19	54412704	54447195	+	105
CACNG8	59283	19	54466290	54493469	+	111
CATSPER1	117144	11	65784223	65793988	-	45
CATSPER2	117155	15	43922772	43941039	-	63
CATSPER3	347732	5	134303596	134347397	+	207
CATSPER4	378807	1	26517119	26529033	+	107
DNM1	1759	9	130965634	131017528	+	223
GABARAP	11337	17	7143738	7145753	-	5
GABBR1	2550	6	29570005	29600962	-	219
GABBR2	9568	9	101050364	101471479	-	2637
GABRA1	2554	5	161274197	161326965	+	283
GABRA2	2555	4	46246470	46392056	-	727
GABRA3	2556	X	151334706	151619831	-	
GABRA4	2557	4	46920917	46996424	-	406
GABRA5	2558	15	27111866	27194357	+	158
GABRA6	2559	5	161112658	161129598	+	81
GABRB1	2560	4	47033295	47432801	+	2058
GABRB2	2561	5	160715426	160975130	-	1268
GABRB3	2562	15	26788693	27018935	-	1332
GABRD	2563	1	1950768	1962192	+	10
GABRE	2564	X	151121596	151143156	-	
GABRG1	2565	4	46037786	46126082	-	496
GABRG2	2566	5	161494648	161582545	+	435
GABRG3	2567	15	27216429	27778373	+	2556
GABRP	2568	5	170210723	170241051	+	193
GABRQ	55879	X	151806637	151821825	+	

Table S3: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GABRR1	2569	6	89887223	89941007	-	344
GABRR2	2570	6	89966840	90025018	-	405
GABRR3	200959	3	97705527	97754148	-	264
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GNA11	2767	19	3094408	3121468	+	144
GNA12	2768	7	2767739	2883963	-	883
GNA13	10672	17	63005407	63052920	-	84
GNA14	9630	9	80037995	80263232	-	1496
GNA15	2769	19	3136191	3163766	+	201
GNAI1	2770	7	79764140	79848725	+	383
GNAI2	2771	3	50264120	50296786	+	114
GNAI3	2773	1	110091186	110138465	+	181
GNAL	2774	18	11689014	11885684	+	1003
GNAO1	2775	16	56225251	56391356	+	866
GNAQ	2776	9	80335189	80646219	-	1344
GNAS	2778	20	57414756	57486250	+	323
GNAT1	2779	3	50229043	50235129	+	12
GNAT2	2780	1	110145889	110155705	-	45
GNAZ	2781	22	23412669	23467224	+	256
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2	2783	7	100271363	100276792	+	19
GNB3	2784	12	6949375	6956564	+	34
GNB4	59345	3	179113876	179169371	-	290
GNB5	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041

Table S3: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GPHN	10243	14	66974125	67648525	+	3011
GPR37	2861	7	124385655	124406079	-	81
KCNH2	3757	7	150642044	150675402	-	179
KCNN1	3780	19	18062111	18110133	+	207
KCNN2	3781	5	113698016	113832197	+	840
KCNN3	3782	1	154669938	154842754	-	925
KCNN4	3783	19	44270685	44286269	-	72
KCNQ2	3785	20	62031561	62103993	-	607
KCNQ3	3786	8	133133105	133493004	-	2095
MRAS	22808	3	138066490	138124377	+	307
NSF	4905	17	44668035	44834830	+	108
OPN1SW	611	7	128412543	128415844	-	20
RPS27A	6233	2	55459039	55462989	+	27
SLC32A1	140679	20	37353105	37358015	+	20
SLC6A1	6529	3	11034420	11080935	+	267
SLC6A11	6538	3	10857917	10980146	+	739
SLC6A12	6539	12	299243	323740	-	169
SLC6A13	6540	12	329787	372039	-	322
UBA52	7311	19	18674576	18688270	+	83
UBB	7314	17	16284367	16286059	+	7
UBC	7316	12	125396192	125399587	-	23
UBD	10537	6	29523389	29527702	-	42
UBQLN1	29979	9	86274878	86323168	-	265

All genes in the table were included in the GABA pathway gene-set. NSNPS, number of single nucleotide polymorphisms (SNPs).

Neuroimaging

LEAP

Structural brain images were acquired on 3T MRI scanners at all sites, with T1-weighted MPRAGE sequence, which were used for registration of the functional scans. Details on the structural and functional scan parameters can be found in Table S3.

TACTICS

Structural T1-weighted scans were acquired based on the ADNI GO protocols (10,11), which were used for registration of the functional scans and voxel placement for the ^{1}H-MRS. Spectra were acquired using a point resolved spectroscopy sequence (PRESS) with a chemically selective water suppression (CHESS) (12) from the midline pregenual ACC and the left dorsal striatum covering caudate and putamen with an 8 cm^3 voxel size (2 × 2 × 2). Voxel locations were adjusted to maximize the amount of gray matter (GM) and minimize the cerebrospinal fluid (CSF) content to keep the quality of the data as high as possible. Details on the structural, functional and ^{1}H-MRS scan parameters can be found in Table S4.

Proton Magnetic Resonance Spectroscopy. Glutamate concentrations were estimated using Linear Combination Model (LCModel), with water as reference (13,14). Tissue correction and partial volume effects was calculated using the formula:

$$Metabolite_{corrected} = Metabolite_{Raw} \times \left(\frac{(43\,300 \times f_{GM} + 35\,880 \times f_{WM} + 55\,556 \times f_{CSF})}{35\,880}\right) \times \left(\frac{1}{(1 - f_{CSF})}\right)$$

where 3300 is the water concentration in millimolar for gray-matter, 35880 for white-matter, and 55556 for cerebrospinal fluid (CSF), as described in the LCModel manual (13). Quality control criteria were the signal-to-noise ratio of ≥ 15, Cramér-Rao lower bounds ≤ 20% and FWHM ≤ 0.1 parts per million. This resulted in data available from 44 participants. Example spectra can be seen in Figure S1 and raw glutamate levels can be found in Table S5.

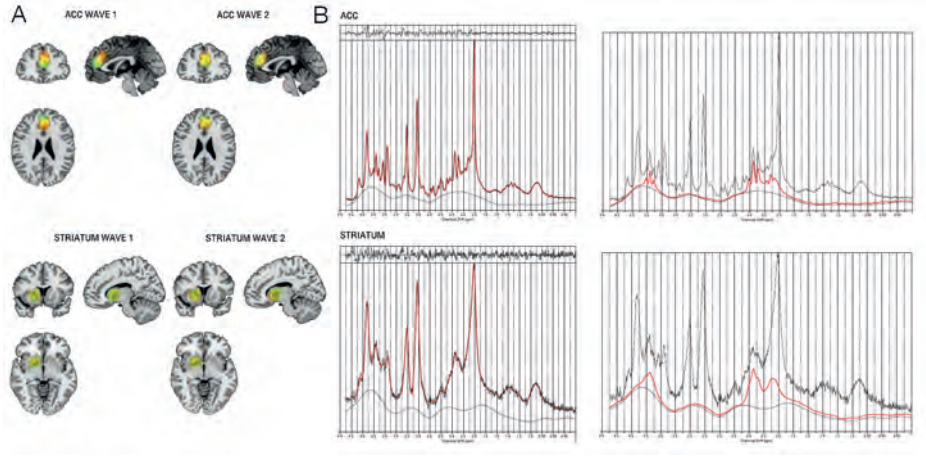


Figure S1. ^{1}H-MRS voxel placement in TACTICS cohort

A: Superposition on the MNI152 template of all individual voxel placements in ACC and striatum, for ASD (red), OCD (blue) and neurotypical (yellow). The placements were consistent across diagnoses, as seen by the large overlap of voxels. B: Example spectra of a 3T proton magnetic resonance spectroscopy (^{1}H-MRS) Linear Combination (LC) Model spectral fit in ACC and striatum from one of the control participants. The top of the images represents the residuals. The black line represents frequency-domain data, the red line is the LCModel fit. The right images show the fits for glutamate only.

Table S4: Scanner parameters LEAP

Site	Manufacturer	Model	Software version	Acquisition sequence	Slices	TR	TE	FA	Coverage	Thickness	Resolution	FOV
						[s]	[ms]	[°]		[mm]	[mm³]	
Cambridge	Siemens	Verio	Syngo MR B17	Tfl3d1_ns	176	2.3	2.95	9				
KCL	GE Medical systems	Discovery mr750	LX MR DV23.1_ V02_1317.c	SAG ADNI GO ACC SPGR	196	7.31	3.02	11				
Mannheim	Siemens	TimTrio	Syngo MR B17	MPRAGE ADNI	176	2.3	2.93	9	256*256	1.2	1.1*1.1*1.2	270
Nijmegen	Siemens	Skyra	Syngo MR D13	Tfl3d1_16ns	176	2.3	2.93	9				
Rome	GE Medical systems	Signa HDxt	24/LX/MR HD16.0_ V02_1131.a	SAG ADNI GO ACC SPGR	172	5.96	1.76	11				
Utrecht	Philips Medical Systems	Achieva/ Ingenia CX	3.2.3/3.2.3.1/ 5.1.9/5.1.9.1	ADNI GO 2	170	6.76	3.1	9				

Abbreviations: FA, flip angle; FOV, field of view; TE, echo time; TR, repetition time.

Table S5: Scanner parameters TACTICS

Sequence	Site	TR/TE/TI (ms)	Flip angle	Field of view (mm)	Matrix RL/AP/ slices	Voxel – size (mm)	Gap (%)	Parallel Imaging	Averages Water suppressed/ unsuppressed
T1	Nijmegen (Siemens)	2300*/2.98/900	9	256	212/256/176	1.0´1.0´1.2	NA	2	NA
	Mannheim (Siemens)	2300*/2.98/900	9	270	212/254/176	1.1´1.1´1.2	NA	2	NA
	London (GE)	7.31*/3.02/400	11	270	256/256/196	1.1´1.1´1.2	NA	1.75	NA
^{1}H-MRS PRESS	All	3000/30/-	NA	NA	NA	20´20´20	NA	NA	96/16
Functional MRI	All	2070/35/-	74	192	192/192/36	3.0´3.0´3.0	13	2	NA

*As provided by the manufacturer. GE defines a TR as the time between excitation pulses, while Siemens defines TR as the time between inversion recovery pulses.

Table S6: LEAP demographics

		Neurotypical (N=253)		Autism (N=343)		Test statistic	df	p-value
Sex, m/f		163/90		244/99		t = -1.73	525.71	0.08
	N	**Mean**	**SD**	**Mean**	**SD**			
Age		17.49	5.84	17.35	5.49	t = -0.31	523.76	0.75
IQ		105.48	19.96	99.05	17.27	t = -4.21	679.04	< 0.001
SRS-2	546	28.58	23.17	89.11	30.81	t = 26.20	543.12	< 0.001
RBS-R	432	2.53	8.43	16.36	13.96	t = 12.79	416.78	< 0.001
SSP	323	176.94	15.62	139.43	27.27	t = -15.72	320.46	< 0.001
ADI-R								
Social	335	-	-	16.67	6.69	-	-	-
Communication	335	-	-	13.27	5.59	-	-	-
Restricted repetitive	335	-	-	4.27	2.65	-	-	-
ADOS-2								
Social affect	336	-	-	6.19	2.58	-	-	-
Restrictive repetitive	336	-	-	4.65	2.69	-	-	-

SD, standard deviation; df, degrees of freedom; SRS-2, Social Responsiveness Scale 2nd edition; RBS-R, Repetitive Behavior Scale - Revised; SSP, Short Sensory Profile; ADI-R, Autism Diagnostic Interview-Revised; Restricted repetitive, Restrictive Repetitive Behaviors domain; Communication, ADI-R Communication domain; Social, ADI-R Social domain; ADOS-2, Autism Diagnostic Observation Schedule 2nd edition; Social affect, ADOS-2 Social Affect.

Table S7: TACTICS demographics

		Neurotypical (N=100)		Autism (N=60)		Test statistic	df	p-value
Sex, m/f		70/30		45/15		t =0.69	129.63	0.49
	N	**Mean**	**SD**	**Mean**	**SD**			
Age		10.76	1.24	10.81	1.52	t= -0.20	105.36	0.84
IQ		110.09	11.47	107.99	15.12	t = -0.93	99.70	0.36
RBS-R	159	0.95	1.88	22.25	20.12	t = 8.11	58.60	< 0.0001
ADI-R								
Social	55	-	-	18.24	5.33	-	-	-
Communication	56	-	-	13.38	3.73	-	-	-
Restricted repetitive	55	-	-	3.62	2.61	-	-	-

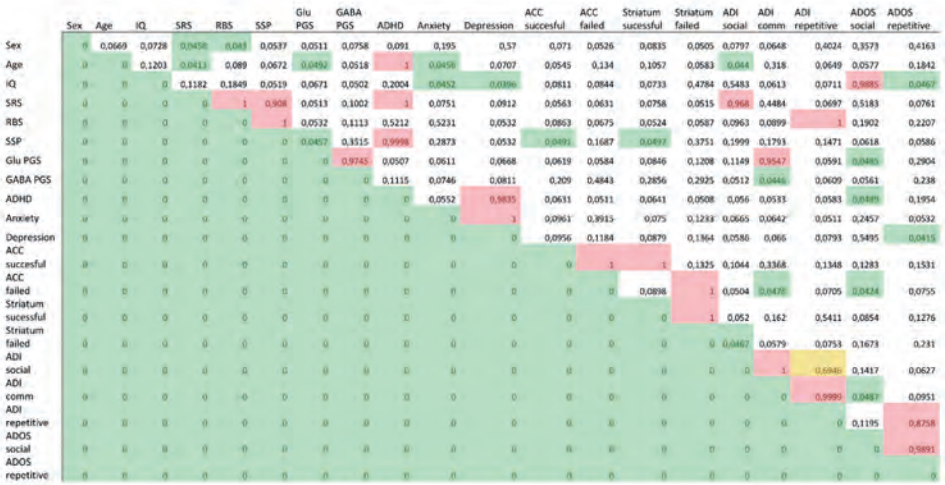
SD, standard deviation; df, degrees of freedom; RBS-R, Repetitive Behavior Scale - Revised; ADI-R, Autism Diagnostic Interview-Revised; Restricted repetitive, Restrictive Repetitive Behaviors domain; Communication, ADI-R Communication domain; Social, ADI-R Social domain.

Table S8: SSC demographics

		Autism (N=2756)	
Sex, m/f		2382/374	
	N	**Mean**	**SD**
Age		9.03	3.57
IQ		81.15	27.96
SRS-2	2747	98	27.01
RBS-R	2754	27.14	17.39
ADI-R			
Social	2755	20.34	5.71
Communication	2422	16.5	4.26
Restricted repetitive	2755	6.52	2.50
ADOS-2			
Social affect	2756	13.33	4.16
Restrictive repetitive	2756	3.96	2.06

SD, standard deviation; df, degrees of freedom; SRS-2, Social Responsiveness Scale 2nd edition; RBS-R, Repetitive Behavior Scale - Revised; ADI-R, Autism Diagnostic Interview-Revised; Restricted repetitive, Restrictive Repetitive Behaviors domain; Communication, ADI-R Communication domain; Social, ADI-R Social domain; ADOS-2, Autism Diagnostic Observation Schedule 2nd edition; Social affect, ADOS-2 Social Affect.

Table S9: LEAP Autistic participants, all edges



	Sex	Age	IQ	SRS	RBS	SSP	Glu PGS	GABA PGS	ADHD	Anxiety	Depression	ACC succesful	ACC failed	Striatum sucessful	Striatum failed	ADI social	ADI comm	ADI repetitive	ADOS social	ADOS repetitive
Sex	0	0,0669	0,0728	0,0458	0,043	0,0537	0,0511	0,0758	0,091	0,195	0,57	0,071	0,0526	0,0835	0,0505	0,0797	0,0648	0,4024	0,3573	0,4163
Age	0	0	0,1203	0,0413	0,089	0,0672	0,0492	0,0518	1	0,0458	0,0707	0,0545	0,134	0,1057	0,0583	0,044	0,318	0,0649	0,0577	0,1842
IQ	0	0	0	0,1182	0,1849	0,0519	0,0671	0,0502	0,2004	0,0452	0,0396	0,0811	0,0844	0,0733	0,4784	0,5483	0,0613	0,0711	0,9885	0,0467
SRS	0	0	0	0	1	0,908	0,0513	0,1002	1	0,0751	0,0912	0,0563	0,0631	0,0758	0,0515	0,968	0,4484	0,0697	0,5183	0,0761
RBS	0	0	0	0	0	1	0,0532	0,1113	0,5212	0,5231	0,0532	0,0863	0,0675	0,0524	0,0587	0,0963	0,0899	1	0,1902	0,2207
SSP	0	0	0	0	0	0	0,0457	0,3515	0,9998	0,2873	0,0532	0,0491	0,1687	0,0497	0,3751	0,1999	0,1793	0,1471	0,0618	0,0586
Glu PGS	0	0	0	0	0	0	0	0,9745	0,0507	0,0611	0,0668	0,0619	0,0584	0,0846	0,1208	0,1149	0,9547	0,0591	0,0485	0,2904
GABA PGS	0	0	0	0	0	0	0	0	0,1115	0,0746	0,0811	0,209	0,4843	0,2856	0,2925	0,0512	0,0446	0,0609	0,0561	0,238
ADHD	0	0	0	0	0	0	0	0	0	0,0552	0,9835	0,0631	0,0511	0,0641	0,0508	0,056	0,0533	0,0583	0,0491	0,1954
Anxiety	0	0	0	0	0	0	0	0	0	0	1	0,0961	0,3915	0,075	0,1233	0,0665	0,0642	0,0511	0,2457	0,0532
Depression	0	0	0	0	0	0	0	0	0	0	0	0,0956	0,1184	0,0879	0,1364	0,0586	0,065	0,0793	0,5495	0,0415
ACC succesful	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0,1325	0,1044	0,3368	0,1348	0,1283	0,1531
ACC failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0898	1	0,0504	0,0478	0,0705	0,0424	0,0755
Striatum sucessful	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0,052	0,162	0,5411	0,0854	0,1276
Striatum failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0467	0,0579	0,0753	0,1673	0,231
ADI social	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0,6946	0,1417	0,0627
ADI comm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,9999	0,0487	0,0951
ADI repetitive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,1195	0,8758
ADOS social	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,9891
ADOS repetitive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Red colors indicate edges of 80% reliability and above, yellow colors indicate edges between 60-80% reliability, green colors indicate below 5% reliability. Note that numbers are rounded and there may therefore be some threshold numbers with different colors. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control; ADI social, Autism Diagnostic Interview-Revised Social domain; ADI comm, Autism Diagnostic Interview-Revised Communication domain; ADI repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors.

Table S10: LEAP Autistic participants, all correlations

	Sex	Age	IQ	SRS	RBS	SSP	Glu PGS	GABA PGS	ADHD	Anxiety	Depression	ACC succesful	ACC failed	Striatum sucessful	Striatum failed	ADI social	ADI comm	ADI repetitive	ADOS social	ADOS repetitive
Sex	1	-0,043	-0,035	0,0086	-0,039	0,0215	0,0146	0,0426	-0,075	0,1807	0,1932	0,015	0,0634	0,0619	0,0008	-0,088	-0,076	-0,148	-0,166	-0,155
Age	-0,043	1	-0,065	-0,193	-0,236	0,2283	0,0478	-0,021	-0,447	-0,074	-0,064	0,0244	-0,051	-0,032	0,0217	-0,084	-0,181	-0,03	0,0306	0,0873
IQ	-0,035	-0,065	1	-0,224	-0,223	0,0652	-0,029	-0,025	-0,203	-0,067	-0,042	0,0329	0,0661	-0,006	0,1327	-0,211	-0,125	-0,004	-0,239	-0,079
SRS	0,0086	-0,193	-0,224	1	0,701	-0,598	-0,018	0,1007	0,5989	0,292	0,3019	-0,065	0,0015	-0,065	-0,041	0,4168	0,3857	0,3201	0,2047	0,1574
RBS	-0,039	-0,236	-0,223	0,701	1	-0,662	-0,023	0,1056	0,5507	0,2934	0,2009	-0,069	-0,061	-0,047	-0,055	0,3619	0,3295	0,4293	0,1844	0,1946
SSP	0,0215	0,2283	0,0652	-0,598	-0,662	1	0,0559	-0,152	0,558	-0,274	-0,186	-0,023	0,1381	-0,006	0,152	-0,344	-0,331	-0,34	-0,095	-0,124
Glu PGS	0,0146	0,0478	-0,029	-0,018	-0,023	0,0559	1	0,1903	0,0006	-0,011	0,0082	-0,025	0,0201	-0,05	0,0721	-0,066	-0,193	-0,089	-0,015	-0,12
GABA PGS	0,0426	-0,021	-0,025	0,1007	0,1056	-0,152	0,1903	1	0,1077	0,0747	0,0633	0,1725	-0,193	0,1752	-0,185	0,0425	-0,006	0,0611	0,0441	0,1143
ADHD	-0,075	-0,447	-0,203	0,5989	0,5507	-0,558	0,0006	0,1077	1	0,2228	0,4227	-0,035	0,0261	-0,048	-0,008	0,2077	0,2246	0,2079	0,0115	0,1647
Anxiety	0,1807	-0,074	-0,067	0,292	0,2934	-0,274	-0,011	0,0747	0,2228	1	0,688	-0,062	0,1951	-0,048	0,164	0,0344	0,0449	-0,009	-0,184	0,0083
Depression	0,1932	-0,064	-0,042	0,3019	0,2009	-0,186	0,0082	0,0633	0,4227	0,688	1	-0,149	0,1986	-0,129	0,1741	-0,041	-0,051	-0,08	-0,199	-0,048
ACC succesful	0,015	0,0244	0,0329	-0,065	-0,069	-0,023	-0,025	0,1725	-0,035	-0,062	-0,149	1	-0,63	0,7978	-0,509	-0,085	-0,124	-0,104	-0,068	0,0995
ACC failed	0,0634	-0,051	0,0661	0,0015	-0,061	0,1381	0,0201	-0,193	0,0261	0,1951	0,1986	-0,63	1	-0,455	0,7256	-0,046	-0,014	0,0225	-0,068	-0,067
Striatum sucessful	0,0619	-0,032	-0,006	-0,065	-0,047	-0,006	-0,05	0,1752	-0,048	-0,048	-0,129	0,7978	-0,455	1	-0,609	-0,03	-0,116	-0,143	-0,01	0,097
Striatum failed	0,0008	0,0217	-0,1327	-0,041	-0,055	0,152	0,0721	-0,185	-0,008	0,164	0,1741	-0,509	0,7256	-0,609	1	-0,032	0,0231	0,0549	-0,109	-0,126
ADI social	-0,088	-0,084	-0,211	0,4168	0,3619	-0,344	-0,066	0,0425	0,2077	0,0344	-0,041	-0,085	-0,046	-0,03	-0,032	1	0,6465	0,4053	0,1625	0,1378
ADI comm	-0,076	-0,181	-0,125	0,3857	0,3295	-0,331	-0,193	-0,006	0,2246	0,0449	-0,051	-0,124	-0,014	-0,116	0,0231	0,6465	1	0,4584	0,1164	0,1572
ADI repetitive	-0,148	-0,03	-0,004	0,3201	0,4293	-0,34	-0,089	0,0611	0,2079	-0,009	-0,08	-0,104	0,0225	-0,143	0,0549	0,4053	0,4584	1	0,1511	0,2245
ADOS social	-0,166	0,0306	-0,239	0,2047	0,1844	-0,095	-0,015	0,0441	0,0115	-0,184	-0,199	-0,068	-0,068	-0,01	-0,109	0,1625	0,1164	0,1511	1	0,2368
ADOS repetitive	-0,155	0,0873	-0,079	0,1574	0,1946	-0,124	-0,12	0,1143	0,1647	0,0083	-0,048	0,0995	-0,067	0,097	-0,126	0,1378	0,1572	0,2245	0,2368	1

Red colors indicate correlations 0.5 and above, yellow colors indicate correlations between 0.3-0.49, green colors indicate negative correlations from -0.3. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control; ADI social, Autism Diagnostic Interview-Revised Social domain; ADI comm, Autism Diagnostic Interview-Revised Communication domain; ADI repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors.

Table S11: LEAP Neurotypical participants, all edges

	Sex	Age	IQ	SRS	RBS	SSP	Glu PGS	GABA PGS	ADHD	Anxiety	Depression	ACC succesful	ACC failed	Striatum sucessful	Striatum failed
Sex	0	0,0577	0,0703	0,2163	0,0875	0,0621	0,1657	0,1483	0,1187	0,4384	0,1684	0,1424	0,176	0,0851	0,1087
Age	0	0	0,0546	0,999	0,0541	0,9132	0,0836	0,2097	0,0581	0,9875	0,0727	0,0761	0,0857	0,0637	0,0655
IQ	0	0	0	0,9994	0,0719	0,2514	0,1983	0,0975	0,9486	0,0869	0,0511	0,0763	0,0957	0,0734	0,9315
SRS	0	0	0	0	0,9999	0,166	0,0762	0,0903	0,9991	0,0624	0,203	0,0717	0,063	0,0836	0,063
RBS	0	0	0	0	0	1	0,2602	0,0631	0,9598	0,7824	0,11	0,0734	0,1732	0,067	0,0609
SSP	0	0	0	0	0	0	0,0825	0,0944	0,9687	0,1232	0,2234	0,7796	0,4046	0,0826	0,083
Glu PGS	0	0	0	0	0	0	0	0,119	0,0663	0,0964	0,1317	0,1864	0,1683	0,0772	0,0844
GABA PGS	0	0	0	0	0	0	0	0	0,2291	0,087	0,0879	0,0917	0,0748	0,1001	0,0832
ADHD	0	0	0	0	0	0	0	0	0	0,093	0,9184	0,0947	0,08	0,118	0,0573
Anxiety	0	0	0	0	0	0	0	0	0	0	1	0,2065	0,0925	0,1523	0,1148
Depression	0	0	0	0	0	0	0	0	0	0	0	0,0795	0,0705	0,1477	0,1155
ACC succesful	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0,1084
ACC failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0,1047	1
Striatum sucessful	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Striatum failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Red colors indicate edges of 80% reliability and above, yellow colors indicate edges between 60-80% reliability, green colors indicate below 5% reliability. Note that numbers are rounded and there may therefore be some threshold numbers with different colors. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control.

Table S12: LEAP Neurotypical participants, all correlations

	Sex	Age	IQ	SRS	RBS	SSP	Glu PGS	GABA PGS	ADHD	Anxiety	Depression	ACC succesful	ACC failed	Striatum sucessful	Striatum failed
Sex	1	-0,064	0,0537	-0,113	0,0807	0,0149	-0,091	0,082	-0,085	0,1507	0,1204	-0,041	0,1015	0,0468	0,0773
Age	-0,064	1	-0,08	0,2716	-0,006	0,2542	-0,045	-0,11	0,0865	-0,282	-0,161	-0,013	0,1467	0,0015	0,0743
IQ	0,0537	-0,08	1	-0,43	-0,273	0,3307	0,0924	0,0493	-0,397	-0,042	-0,147	-0,17	0,2121	-0,115	0,2709
SRS	-0,113	0,2716	-0,43	1	0,4815	-0,369	0,0371	-0,057	0,4866	0,1798	0,2885	0,1012	-0,091	0,1044	0,135
RBS	0,0807	-0,006	-0,273	0,4815	1	-0,498	-0,11	0,0048	0,4524	0,3308	0,3313	0,0567	-0,061	0,0334	-0,104
SSP	0,0149	0,2542	0,3307	-0,369	-0,498	1	0,0703	-0,067	-0,429	-0,263	-0,316	-0,369	0,3617	-0,281	0,2225
Glu PGS	-0,091	-0,045	0,0924	0,0371	-0,11	0,0703	1	0,067	-0,012	0,053	-0,09	-0,101	0,1043	-0,037	0,0348
GABA PGS	0,082	-0,11	0,0493	-0,057	0,0048	-0,067	0,067	1	0,1089	-0,037	0,0636	0,0525	-0,043	0,0602	-0,045
ADHD	-0,085	0,0865	-0,397	0,4866	0,4524	-0,429	-0,012	0,1089	1	0,2112	0,3392	0,0892	-0,078	0,082	-0,113
Anxiety	0,1507	-0,282	-0,042	0,1798	0,3308	-0,263	0,053	-0,037	0,2112	1	0,674	-0,016	0,0301	0,0937	-0,051
Depression	0,1204	-0,161	-0,147	0,2885	0,3313	-0,316	-0,09	0,0636	0,3392	0,674	1	0,0598	-0,014	0,1054	-0,064
ACC succesful	-0,041	-0,013	-0,17	0,1012	0,0567	-0,369	-0,101	0,0525	0,0892	-0,016	0,0598	1	-0,663	0,7532	-0,502
ACC failed	0,1015	0,1467	0,2121	-0,091	-0,061	0,3617	0,1043	-0,043	-0,078	0,0301	-0,014	-0,663	1	-0,453	0,6954
Striatum sucessful	0,0468	0,0015	-0,115	0,1044	0,0334	-0,281	-0,037	0,0602	0,082	0,0937	0,1054	0,7532	-0,453	1	-0,663
Striatum failed	0,0773	0,0743	0,2709	-0,135	-0,104	0,2225	0,0348	-0,045	-0,113	-0,051	-0,064	-0,502	0,6954	-0,663	1

Red colors indicate correlations 0.5 and above, yellow colors indicate correlations between 0.3-0.49, green colors indicate negative correlations from -0.3. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control.

Table S13: LEAP All participants, all edges

	Diagnosis	Sex	Age	IQ	SRS	RBS	SSP	PGS	PGS	ADHD	Anxiety	Depression	succesful	failed	sucessful	failed
Diagnosis	0	0.1228	0.0459	0,3654	1	1	0,0906	0,078	0,0556	0,0575	0,2075	0,451	0,1716	0,058	0,1005	0,0567
Sex	0	0	0,0488	0,0391	0,1852	0,1055	0,0655	0,0581	0,1339	0,2995	0,3105	0,2333	0,0551	0,1273	0,1592	0,0632
Age	0	0	0	0,131	0,08	0,0504	0,4689	0,0407	0,0489	0,2586	0,5424	0,0464	0,1626	0,0345	0,0551	0,0536
IQ	0	0	0	0	0,8831	0,0857	0,0493	0,0442	0,0364	0,9688	0,0358	0,0351	0,0481	0,0468	0,0564	0,9755
SRS	0	0	0	0	0	1	0,9433	0,0762	0,0624	1	0,095	0,9217	0,0843	0,0534	0,0713	0,0584
RBS	0	0	0	0	0	0	1	0,189	0,0709	0,8518	0,8571	0,059	0,0726	0,0654	0,0512	0,0544
SSP	0	0	0	0	0	0	0	0,201	0,4374	1	0,6939	0,0648	0,053	0,3753	0,05	0,1886
Glu PGS	0	0	0	0	0	0	0	0	0,9074	0,0632	0,0482	0,1346	0,1058	0,0911	0,0604	0,0679
GABA PGS	0	0	0	0	0	0	0	0	0	0,1189	0,0403	0,0595	0,0818	0,2913	0,1491	0,2747
ADHD	0	0	0	0	0	0	0	0	0	0	0,0657	1	0,0502	0,0427	0,0546	0,0439
Anxiety	0	0	0	0	0	0	0	0	0	0	0	1	0,0755	0,2376	0,1633	0,0751
Depression	0	0	0	0	0	0	0	0	0	0	0	0	0,0531	0,1009	0,0536	0,0671
ACC succesful	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0,0718
ACC failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0561	1
Striatum sucessful	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Striatum failed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Red colors indicate edges of 80% reliability and above, yellow colors indicate edges between 60-80% reliability, green colors indicate below 5% reliability. Note that numbers are rounded and there may therefore be some threshold numbers with different colors. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control.

Table S14: LEAP All participants, all correlations

	Diagnosis	Sex	Age	IQ	SRS	RBS	SSP	Glu PGS	GABA PGS	ADHD	Anxiety	Depression	ACC succesful	ACC failed	Striatum sucessful	Striatum failed
Diagnosis	1	-0,081	-0,009	-0,143	0,7328	0,7009	0,564	-0,056	0,0081	0,5127	0,4036	0,4428	0,0831	-0,062	0,0686	-0,066
Sex	-0,081	1	-0,047	0,0152	-0,09	-0,077	0,065	-0,028	0,0617	-0,1	0,1128	0,1008	-0,028	0,088	0,0383	0,0401
Age	-0,009	-0,047	1	-0,06	0,0161	-0,087	0,1555	0,0148	-0,042	-0,145	-0,153	-0,099	0,0382	0,0359	1E-03	0,0583
IQ	-0,143	0,0152	-0,06	1	-0,332	-0,302	0,2103	0,0182	-0,011	-0,333	-0,115	-0,156	-0,058	0,1174	-0,037	0,1699
SRS	0,7328	-0,09	0,0161	-0,332	1	0,8234	-0,719	-0,039	0,025	0,7045	0,4449	0,5129	0,0628	-0,064	0,0542	-0,086
RBS	0,7009	-0,077	-0,087	-0,302	0,8234	1	-0,777	-0,079	0,0503	0,687	0,4624	0,4652	0,0123	-0,065	0,0197	-0,078
SSP	-0,564	0,065	0,1555	0,2103	-0,719	-0,777	1	0,0835	-0,112	-0,68	-0,437	-0,43	-0,07	0,1311	-0,05	0,1342
Glu PGS	-0,056	-0,028	0,0148	0,0182	-0,039	-0,079	0,0835	1	0,1308	-0,042	-0,008	-0,063	-0,056	0,0553	-0,032	0,046
GABA PGS	0,0081	0,0617	-0,042	-0,011	0,025	0,0503	-0,112	0,1308	1	0,084	0,0158	0,0481	0,0732	-0,105	0,0838	-0,104
ADHD	0,5127	-0,1	-0,145	-0,333	0,7045	0,687	-0,68	-0,042	0,084	1	0,3841	0,4947	0,0456	-0,044	0,0334	-0,071
Anxiety	0,4036	0,1128	-0,153	-0,115	0,4449	0,4624	-0,437	-0,008	0,0158	0,3841	1	0,7415	-0,009	0,0865	0,0489	0,0224
Depression	0,4428	0,1008	-0,099	-0,156	0,5129	0,4652	-0,43	-0,063	0,0481	0,4947	0,7415	1	0,004	0,0667	0,0238	0,0315
ACC succesful	0,0831	-0,028	0,0382	-0,058	0,0628	0,0123	-0,07	-0,056	0,0732	0,0456	-0,009	0,004	1	-0,643	0,7756	-0,501
ACC failed	-0,062	0,088	0,0359	0,1174	-0,064	-0,065	0,1311	0,0553	-0,105	-0,044	0,0865	0,0667	-0,643	1	-0,458	0,7201
Striatum sucessful	0,0686	0,0383	1E-03	-0,037	0,0542	0,0197	-0,05	-0,032	0,0838	0,0334	0,0489	0,0238	0,7756	-0,458	1	-0,631
Striatum failed	-0,066	0,0401	0,0583	0,1699	-0,086	-0,078	0,1342	0,046	-0,104	-0,071	0,0224	0,0315	-0,501	0,7201	-0,631	1

Red colors indicate correlations 0.5 and above, yellow colors indicate correlations between 0.3-0.49, green colors indicate negative correlations from -0.3. SRS, Social Responsiveness Scale 2nd edition; RBS, Repetitive Behavior Scale - Revised; SSP, Social Responsiveness Scale-Revised; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism; ADHD, DSM-5 ADHD-Rating Scale; Anxiety, Beck Anxiety Inventory; Depression, Beck Depression Inventory-II; ACC successful, BOLD signal in ACC during successful inhibitory control; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control.

Table S15: TACTICS All participants, all edges

	Diagnosis	Sex	Age	IQ	RBS	Glu PGS	GABA PGS	ADHD	ACC failed	Striatum failed	ACC successful	Striatum successful	Glutamate ACC	Glutamate Striatum	CSBQ
Diagnosis	0	0,0952	0,0778	0,1463	1	0,1307	0,1401	0,2128	0,161	0,094	0,3549	0,0986	0,0768	0,4723	0,9999
Sex	0	0	0,0701	0,1038	0,1215	0,1339	0,1498	0,2312	0,3309	0,0747	0,0764	0,1244	0,9115	0,607	0,1602
Age	0	0	0	0,2923	0,1285	0,0925	0,2864	0,1996	0,2335	0,1316	0,6363	0,9528	0,4223	0,0728	0,0831
IQ	0	0	0	0	0,1441	0,4697	0,1853	0,0801	0,079	1	0,1049	0,0747	0,0779	0,0944	0,1774
RBS	0	0	0	0	0	0,1466	0,533	0,6643	0,1617	0,0912	0,1197	0,0868	0,0742	0,3069	1
Glu PGS	0	0	0	0	0	0	0,0775	0,2977	0,2957	0,3521	0,9986	0,9412	0,0872	0,4663	0,1404
GABA PGS	0	0	0	0	0	0	0	0,1596	0,1648	0,1214	0,1063	0,2574	0,9888	0,3208	0,1047
ADHD	0	0	0	0	0	0	0	0	0,0814	0,0756	0,0806	0,0731	0,1738	0,1418	0,9996
ACC failed	0	0	0	0	0	0	0	0	0	1	0,1344	0,0961	0,1986	0,1643	0,5141
Striatum failed	0	0	0	0	0	0	0	0	0	0	0,069	0,073	0,3013	0,26	0,1304
ACC successful	0	0	0	0	0	0	0	0	0	0	0	0,473	0,3709	0,0799	0,0829
Striatum successful	0	0	0	0	0	0	0	0	0	0	0	0	0,1669	0,9619	0,0848
Glutamate ACC	0	0	0	0	0	0	0	0	0	0	0	0	0	0,155	0,1327
Glutamate Striatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,2536
CSBQ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Red colors indicate edges of 80% reliability and above, yellow colors indicate edges between 60-80% reliability, green colors indicate below 5% reliability. Note that numbers are rounded and there may therefore be some threshold numbers with different colors. RBS, Repetitive Behavior Scale - Revised; Glu PGS, Glutamate polygenic score for autism, GABA PGS, GABA polygenic score for autism; ADHD, Conners' Parent Rating Scale; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control; ACC successful, BOLD signal in ACC during successful inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Glutamate ACC, estimated glutamate concentrations in the ACC using water reference; Glutamate Striatum, estimated glutamate concentrations in Striatum using water reference; CSBQ, Children's Social Behavior Questionnaire.

Table S16: TACTICS All participants, all correlations

	Diagnosis	Sex	Age	IQ	RBS	Glu PGS	GABA PGS	ADHD	ACC failed	Striatum failed	ACC successful	Striatum successful	Glutamate ACC	Glutamate Striatum	CSBQ
Diagnosis	1	-0,054	0,0312	-0,073	0,7917	-0,111	0,0774	0,2469	-0,158	-0,066	0,1671	0,1089	0,0221	-0,211	0,7959
Sex	-0,054	1	-0,021	-0,051	-0,091	0,1179	0,0945	0,1097	0,1637	0,0135	-0,047	-0,091	-0,251	-0,188	-0,122
Age	0,0312	-0,021	1	-0,147	-0,077	0,1383	-0,144	0,1321	0,1342	0,1021	0,2083	-0,269	0,215	-0,001	0,0443
IQ	-0,073	-0,051	-0,147	1	-0,111	0,1764	0,1147	-0,011	0,1439	0,4793	-0,117	-0,018	-0,083	-0,075	-0,175
RBS	0,7917	-0,091	-0,077	-0,111	1	-0,11	-0,052	0,2036	-0,221	-0,061	-0,032	-0,014	0,002	-0,021	0,8223
Glu PGS	-0,111	0,1179	0,1383	0,1764	-0,11	1	0,0693	0,1476	-0,204	-0,203	-0,396	-0,358	0,0716	-0,19	-0,116
GABA PGS	0,0774	0,0945	-0,144	0,1147	-0,052	0,0693	1	-0,111	0,0993	-0,102	-0,117	-0,168	0,2945	0,1733	0,0587
ADHD	0,2469	0,1097	0,1321	-0,011	0,2036	0,1476	-0,111	1	0,0183	-0,018	-0,044	0,0417	-0,116	-0,163	0,3851
ACC failed	-0,158	0,1637	0,1342	0,1439	-0,221	-0,204	0,0993	0,0183	1	0,5	0,1393	-0,077	-0,163	0,1741	-0,279
Striatum failed	-0,066	0,0135	0,1021	0,4793	-0,061	-0,203	-0,102	-0,018	0,5	1	0,0571	0,0122	-0,183	0,1743	-0,19
ACC successful	0,1671	-0,047	0,2083	-0,117	-0,032	-0,396	-0,117	-0,044	0,1393	0,0571	1	0,2862	0,175	0,0663	0,0509
Striatum successful	0,1089	-0,091	-0,269	-0,018	-0,014	-0,358	-0,168	0,0417	-0,077	0,0122	0,2862	1	-0,188	-0,273	0,0773
Glutamate ACC	0,0221	-0,251	0,215	-0,083	0,002	0,0716	0,2945	-0,116	-0,163	-0,183	0,175	-0,188	1	0,1545	-0,081
Glutamate Striatum	0,211	0,188	0,001	0,075	-0,021	0,19	0,1733	0,163	0,1741	0,1743	0,0663	0,273	0,1545	1	0,195
CSBQ	0,7959	-0,122	0,0443	-0,175	0,8223	-0,116	0,0587	0,3851	-0,279	-0,19	0,0509	0,0773	-0,081	-0,195	1

Red colors indicate correlations 0.5 and above, yellow colors indicate correlations between 0.3-0.49, green colors indicate negative correlations from -0.3. RBS, Repetitive Behavior Scale - Revised; Glu PGS, Glutamate polygenic score for autism, GABA PGS, GABA polygenic score for autism; ADHD, Conners' Parent Rating Scale; ACC failed, BOLD signal in ACC during failed inhibitory control; Striatum failed, BOLD signal in striatum during failed inhibitory control; ACC successful, BOLD signal in ACC during successful inhibitory control; Striatum successful, BOLD signal in striatum during successful inhibitory control; Glutamate ACC, estimated glutamate concentrations in the ACC using water reference; Glutamate Striatum; Estimated glutamate concentrations in Striatum using water reference; CSBQ, Children's Social Behavior Questionnaire.

Table S17: SSC All (autistic) participants, all edges

	ADI repetitive	ADI comm	ADI social	ADOS repetitive	ADOS social	RBS	Sex	IQ	SRS	Age	Glu PRS	GABA PRS
ADI repetitive	0	1	0,1185	0,1445	0,0195	1	0,804	0,0424	0,0389	0,02	0,0212	0,0224
ADI comm	0	0	1	0,582	0,096	0,0154	0,0144	0,996	0,025	0,183	0,1247	0,0276
ADI social	0	0	0	0,0247	0,9987	0,0263	0,0189	1	1	1	0,0711	0,0399
ADOS repetitive	0	0	0	0	1	0,0719	0,0186	1	0,0218	1	0,0399	0,0311
ADOS social	0	0	0	0	0	0,0179	0,0217	1	0,036	0,2449	0,0341	0,0236
RBS	0	0	0	0	0	0	0,0174	0,2614	1	0,1761	0,0283	0,0242
Sex	0	0	0	0	0	0	0	0	0,0178	0,0323	0,0491	0,0217
IQ	0	0	0	0	0	0	0	0	0,8759	0,0312	0,0425	0,0664
SRS	0	0	0	0	0	0	0	0	0	0,034	0,0244	0,0352
Age	0	0	0	0	0	0	0	0	0	0	0,0261	0,0224
Glu PRS	0	0	0	0	0	0	0	0	0	0	0	0,0693
GABA PRS	0	0	0	0	0	0	0	0	0	0	0	0

Red colors indicate edges of 80% reliability and above, yellow colors indicate edges between 60-80% reliability, green colors indicate below 5% reliability. Note that numbers are rounded and there may therefore be some threshold numbers with different colors. ADI repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADI comm, Autism Diagnostic Interview-Revised Communication domain; ADI social, Autism Diagnostic Interview-Revised Social domain; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; RBS, Repetitive Behavior Scale - Revised; SRS, Social Responsiveness Scale 2nd edition; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism.

Table S18: SSC All (autistic) participants, all correlations

	ADI repetitive	ADI comm	ADI social	ADOS repetitive	ADOS social	RBS	Sex	IQ	SRS	Age	Glu PRS	GABA PRS
ADI repetitive	1	0,2636	0,2245	0,1321	0,0604	0,4019	-0,061	-0,033	0,2452	0,0592	-0,001	-0,003
ADI comm	0,2636	1	0,6632	0,2539	0,2632	0,2493	0,0177	-0,34	0,3318	0,1057	-0,042	0,0167
ADI social	0,2245	0,6632	1	0,2298	0,2893	0,2426	0,0184	-0,381	0,4139	0,2384	-0,035	0,0241
ADOS repetitive	0,1321	0,2539	0,2298	1	0,4568	0,1514	0,003	-0,426	0,1491	-0,211	-0,026	0,0191
ADOS social	0,0604	0,2632	0,2893	0,4568	1	0,1084	0,0399	-0,421	0,1679	-0,13	-0,023	0,0104
RBS	0,4019	0,2493	0,2426	0,1514	0,1084	1	-0,003	-0,177	0,6027	-0,038	-0,018	0,0059
Sex	-0,061	0,0177	0,0184	0,003	0,0399	-0,003	1	-0,08	0,03	0,0175	0,0245	0,0053
IQ	-0,033	-0,34	-0,381	-0,426	-0,421	-0,177	-0,08	1	-0,239	0,0014	0,0271	-0,031
SRS	0,2452	0,3318	0,4139	0,1491	0,1679	0,6027	0,03	-0,239	1	0,1108	-0,013	0,0221
Age	0,0592	0,1057	0,2384	-0,211	-0,13	-0,038	0,0175	0,0014	0,1108	1	0,0127	-0,006
Glu PRS	-0,001	-0,042	-0,035	-0,026	-0,023	-0,018	0,0245	0,0271	-0,013	0,0127	1	0,0288
GABA PRS	-0,003	0,0167	0,0241	0,0191	0,0104	0,0059	0,0053	-0,031	0,0221	-0,006	0,0288	1

Red colors indicate correlations 0.5 and above, yellow colors indicate correlations between 0.3-0.49, green colors indicate negative correlations from -0.3. ADI repetitive, Autism Diagnostic Interview-Revised Restricted and Repetitive Behaviors domain; ADI comm, Autism Diagnostic Interview-Revised Communication domain; ADI social, Autism Diagnostic Interview-Revised Social domain; ADOS repetitive, Autism Diagnostic Observation Schedule Second Edition Restricted and Repetitive Behaviors; ADOS social, Autism Diagnostic Observation Schedule Second Edition Social affect; RBS, Repetitive Behavior Scale - Revised; SRS, Social Responsiveness Scale 2nd edition; Glu PGS, Glutamate polygenic score for autism; GABA PGS, GABA polygenic score for autism.

Table S19: Post-hoc tests of differences between cohorts

	Glutamate PGS	GABA PGS	ADI-R communication	ADI-R restricted repetitive	ADI-R social
LEAP - SSC	**t =-17.616 df = 382.02 p<2.2e-16**	**t = -7.1013 df = 375.56, p= 6.229e-12**	**t = 6.2974 df = 62.366 p = 3.408e-08**	**t = -14.728df = 423.4 p < 2.2e-16**	**t = 3.2538 df = 60.76 p = 0.001862**

t, t-score; df, degrees of freedom; Glutamate PGS, Glutamate polygenic score, GABA PGS, GABA polygenic score, ADI-R, Autism Diagnostic Interview-Revised; Restricted repetitive, Restrictive Repetitive Behaviors domain; Communication, ADI-R Communication domain; Social, ADI-R Social domain. LEAP, Longitudinal European Autism Project cohort, SSC, Simons Simplex Collection cohort. Significant results are marked in bold.

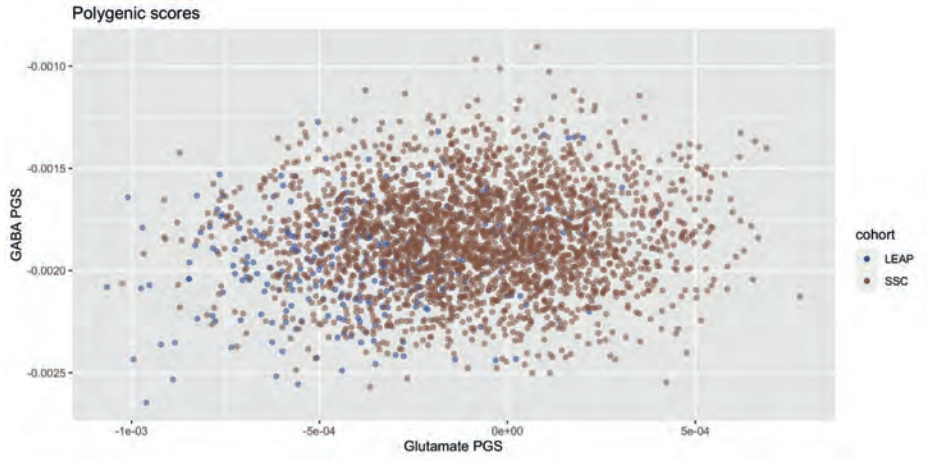


Figure S2. Polygenic scores

Glutamate and GABA polygenic scores. SSC, Simons Simplex Collection (brown); LEAP, Longitudinal European Autism Project (blue).

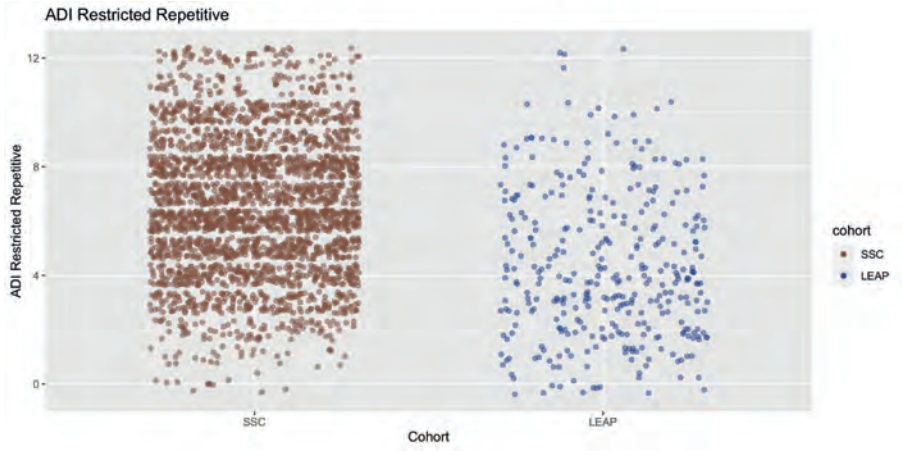


Figure S3. ADI-R Restricted Repetitive

ADI-R Restricted Repetitive domain scores. SSC, Simons Simplex Collection (brown); LEAP, Longitudinal European Autism Project (blue).

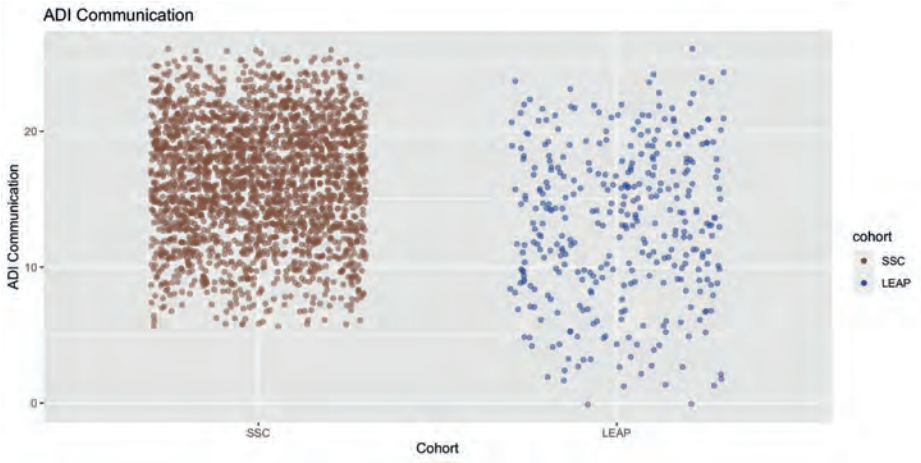


Figure S4. ADI-R Communication

ADI-R Communication domain scores. SSC, Simons Simplex Collection (brown); LEAP, Longitudinal European Autism Project (blue).

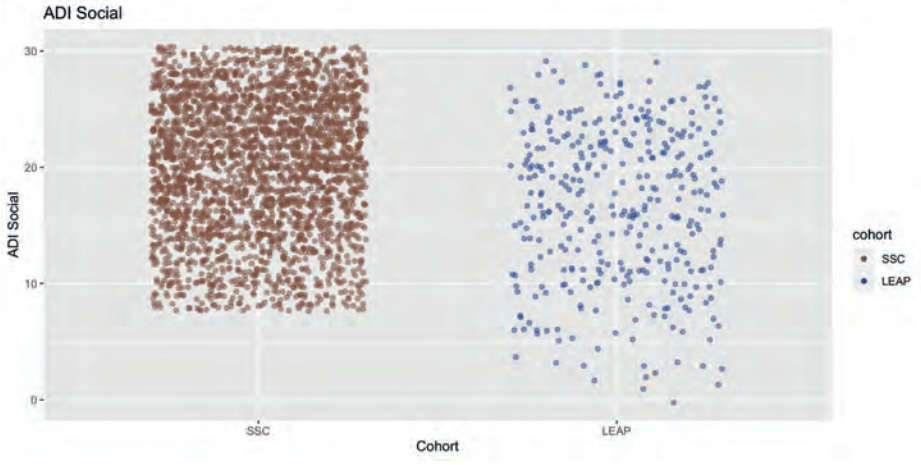


Figure S5. ADI-R Social

ADI-R Social domain scores. SSC, Simons Simplex Collection (brown); LEAP, Longitudinal European Autism Project (blue).

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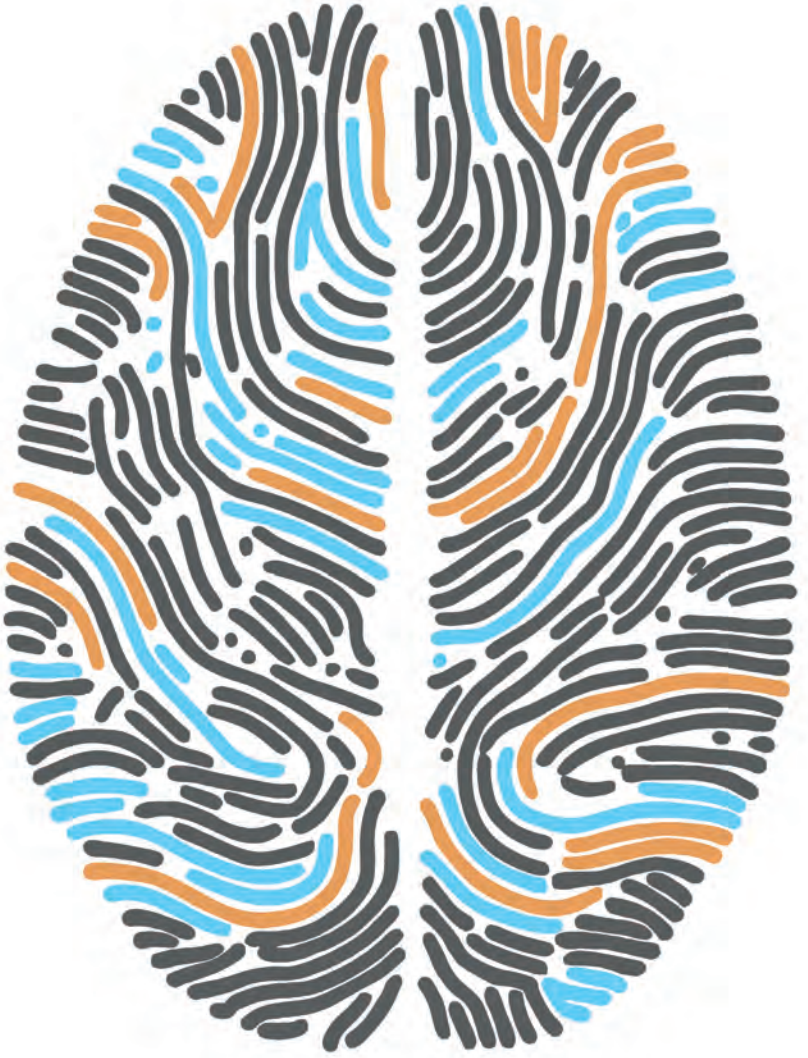

Chapter 5

Exploring the E/I imbalance theory of autism by combining genetic scores, concentrations of glutamate and GABA, and behavioral characteristics of autism

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Abstract

The excitatory/inhibitory (E/I) imbalance theory of autism suggests that an imbalance between excitatory and inhibitory mechanisms in the brain is underlying autism traits. Studies have mainly focused on either excitatory or inhibitory measures separately, using various isolated modalities, leading to inconsistent results. We attempted to bridge this gap by combining genetic and ^{1}H-MRS measures of glutamate and GABA, reflecting excitation and inhibition respectively, to examine their interaction, and association with behavioral autism characteristics. Participants were part of third wave of the AIMS-2-TRIALS LEAP cohort (166 participants (autistic = 103, male/female = 79/24; neurotypical = 63, male/female = 42/21), aged between 13-36 years. Using MAGMA for competitive gene-set analysis, we investigated associations of aggregated genetic variation of glutamate and GABA gene-sets with ^{1}H-MRS measures of glutamate and GABA in anterior cingulate cortex (ACC) and thalamus. We used linear models to associate glutamate and GABA polygenic scores (PGS) for autism and glutamate and GABA concentrations in the ACC and thalamus with core clinical autism traits. Genetic variation of glutamate genes were associated with GABA concentrations in the thalamus, and GABA genes with glutamate concentrations in the thalamus. ACC glutamate interacted with glutamate PGS in influencing social-communicative and sensory behaviors, and autism traits captured by Autism Diagnostic Observation Schedule-2 (ADOS-2). Glutamate/GABA ratios in the thalamus with gene-set PGS also had interaction effects on social-communicative behaviors and ADOS-2 scores. These results show that interactions of glutamate and GABA genes and their estimated metabolite concentrations are related to several behavioral autism characteristics. Genetic measures of glutamate and GABA may therefore mechanistically influence autism behaviors by affecting glutamate and GABA metabolites. These results also highlight the importance of investigating excitatory and inhibitory measures together, using multimodal data, to truly capture variations in E/I imbalance and how it may relate to autism characteristics.

Introduction

Autism spectrum disorder (autism) is characterized by difficulties in social communication and interactions, restricted and repetitive behaviors and differences in sensory processing (1). It is a heterogeneous and highly heritable neurodevelopmental condition that has been associated with many common genetic variants, and most of these genes are involved in excitatory and inhibitory functions in the brain (2,3). An influential theory about its underlying mechanisms suggest an imbalance between excitatory and inhibitory mechanisms in the brain leading to over/under excitation and/or over/under inhibition (4,5). However, research aiming to disentangle the nature of this suggested imbalance has reported inconsistent findings. This is likely due to clinical and biological heterogeneity of autism and differences across brain regions and development, leading to either increased or decreased ratios between excitation and inhibition. Studies have historically focused on either excitation *or* inhibition and rarely investigated both simultaneously. This combined with studies typically focusing on one measure of excitation or inhibition ignores the complexity of excitatory and inhibitory mechanisms in the brain. Here we took a multimodal approach along several clinical symptom dimensions to address these inconsistencies by assessing both genetic and *in vivo* markers of excitation and inhibition, and investigated how they interact and relate to behavioral autism characteristics.

An excitatory/inhibitory (E/I) imbalance may be due to alterations in excitatory and/or inhibitory neurotransmission, and previous work has found support for both (6–8). Glutamate, the most abundant excitatory neurotransmitter, and GABA (γ-aminobutyric acid), the most abundant inhibitory neurotransmitter, can be quantified *in vivo* using Proton Magnetic Resonance Spectroscopy (^{1}H-MRS). A recent review and meta-analysis of ^{1}H-MRS studies on autism found lower average concentrations of GABA in autistic children, particularly in limbic regions including the ACC (9). The meta-analysis showed limited evidence for glutamate differences. However, previous work using ^{1}H-MRS, post-mortem, pharmacological studies and animal model approaches to excitation and inhibition has shown convincing links between both glutamate and GABA to brain and behavior differences in autism (10–20). In contrast, other studies have found no group differences in metabolites of neither glutamate nor GABA (21–23), and pharmacological studies of interventions to alter glutamatergic or GABAergic mechanisms have reported inconsistent results (11,24–29). These inconsistencies point towards different alterations of glutamate and GABA, across brain regions but also across individuals and ages, which in turn could explain autism heterogeneity (8,30).

Given the fundamental roles of glutamate and GABA in excitation and inhibition and the strong genetic links to both excitatory and inhibitory functions and autism, we investigated the associations between genetic markers of glutamate and GABA with *in vivo* ^{1}H-MRS measures in the ACC and thalamus. These regions were selected based on their roles in functions crucial for traits associated with autism: thalamus in particular for its role relaying sensory information, and ACC for its many roles in higher cognitive functions and emotional control. Data were acquired in the largest autism dataset available to date with these measures available, spanning cross-sectionally from adolescence into adulthood. We applied competitive gene-set analysis using MAGMA to associate aggregated genetic variation of glutamate and GABA genes to *in vivo* glutamate and GABA concentrations in ACC and thalamus. To link these E/I markers to behavioral characteristics of autism we investigated the associations between glutamate and GABA polygenic scores for autism and metabolite concentrations and behavioral characteristics of autism. Based on previous findings we expected differential associations of glutamate and GABA measures to behaviors, particularly that associations to sensory processing may differ from associations to other autism characteristics. Given that there is no previous work simultaneously investigating glutamate and GABA gene-sets with ^{1}H-MRS markers of glutamate and GABA, let alone ratios between them, we did not have a priori expectations for specific findings or their directions.

Methods

Participants

Participants were part of the Longitudinal European Autism Project (LEAP), within the AIMS-2-TRIALS clinical research programme (www.aims-2-trials.eu/) (31–33). We used data from the third, most recent, wave of data collection which consisted of 166 participants (autistic = 103, neurotypical = 63) aged between 13-36, where ^{1}H-MRS data that passed quality control was available. Data were collected across three study centers across Europe; Institute of Psychiatry, Psychology and Neuroscience, King's College London (IoPPN/KCL, UK), Radboud University Medical Centre (RUMC, Netherlands), and Central Institute of Mental Health (CIMH, Germany). For autistic participants, inclusion criteria at the first wave of measurement (32) were an existing clinical diagnosis of autism, confirmed using the Autism Diagnostic Observation Schedule Second Edition (ADOS-2, (34)) and the Autism Diagnostic Interview - Revised (ADI-R, (34)), for more details see (31). For the neurotypical participants, exclusion criteria were reports of any psychiatric disorder. All participants or their legal guardian (where applicable) provided written

informed consent. For further details of the recruitment of participants in the LEAP study, see (32).

Phenotypic measures

The phenotypic measures used were selected from a larger test battery (see (31)). We included three questionnaires that capture the core autism characteristics; social communicative behaviors (Social Responsiveness Scale-Revised (SRS-2; (35)), repetitive behaviors (Repetitive Behavior Scale-Revised (RBS-R; (36) and sensory processing (Short Sensory Profile (SSP; (37)). These questionnaires use self- or parent-report ratings depending on age and diagnostic group. For the autistic participants scores on the ADOS-2 were also available.

Genetics

Genotyping

Genotyping was performed at the Centre National de Recherche en Génomique Humaine (CNRGH) using the Infinium OmniExpress-24v1 BeadChip Illumina. Sample quality controls such as sex check (based on the X chromosome homozygosity rate or the median of the Log R ratio of the X and Y chromosomes), Mendelian errors (transmission errors within full trios) and Identity By State were performed using PLINK 1.90. Imputation of 17 million SNPs was performed using the 700k genotyped SNPs on the Michigan Imputation Server (38). The HRC r1.1 2016 reference panel for a European population was used, as the majority of individuals in the LEAP cohort were from European ancestry. Only autosomes were imputed. Linkage disequilibrium-based SNP pruning was done for SNPs with a MAF > 1% and SNPs with an R2 < 0.1 in windows of 500 kb were selected.

Gene-set selection

The glutamate and GABA gene-sets have been used in several previous studies (17,19,39) and was based on ingenuity pathway analysis software (www.ingenuity.com), which is a database for genetic pathway analysis. The gene-sets consist of genes encoding proteins involved in glutamatergic and GABAergic communication pathways in the brain. Complete lists of genes in each gene-set can be found in Tables S1-S2 in the supplement.

Polygenic scores

Gene-set polygenic scores (PGS) for the glutamate and GABA gene-sets were calculated using the PRSet function in PRSice-2 (40,41), using the summary statistics of the PGS ASD GWAS (genome wide association study) (2). SNPs were

clumped based on LD using PRSice default settings (bidirectional 250Kb-window and R2-threshold of 0.1), resulting in 103.045 LD-clumped SNPs. Glutamate and GABA gene-set PGS are calculated at a p-value threshold of 1, to include the whole gene-set in the PGS.

Neuroimaging

Imaging acquisition

Structural brain images were acquired on 3T MRI scanners at all sites, with T1-weighted MPRAGE sequence. ^{1}H-MRS was acquired using an unedited Point Resolved Spectroscopy Sequence (PRESS), and an edited Hadamard Encoding and Reconstruction of Mega-Edited Spectroscopy (HERMES). The thalamus voxel was 26x40x24 mm^3, and placed with thalamus bi-laterally centered on the midline, with the superior edge of the voxel aligned with the third ventricle. The ACC voxel was 35x30x25 mm^3 at all sites (except the London site, where it was 30x35x25 mm^3), and was placed anteriorly, centered along the midline with the bottom of the voxel aligning with the front of the corpus callosum. Voxel locations were adjusted to maximize the amount of gray matter (GM) and minimize the amount of cerebrospinal fluid (CSF). An overlay of all voxel placements is shown in Figure S1 in the supplement. For a summary of scanner details and acquisition parameters at each site, see Table S3 in the supplement.

Imaging processing

^{1}H-MRS data was processed and quantified using Osprey, (version 2.4.0 (42)), an open source automated software tool for ^{1}H-MRS analysis based in Matlab (version 2022a). GABA was estimated from the HERMES scan (HERMES difference spectrum was used (GABA-edit ON - GABA edit OFF)) and glutamate was estimated from the PRESS scan. At 3T it is not possible to fully separate the glutamate signal from the glutamine signal, as they are neurochemically very similar. The estimated glutamate concentrations, while mostly consisting of glutamate, may therefore partially include some glutamine. The GABA signal also contains co-edited macromolecules, and this signal is therefore often referred to as a GABA+. Here, for consistency across the genetic and *in vivo* measures, we refer to the GABA+ signal as GABA throughout this chapter.

The PRESS water-unsuppressed transients were used for quantification whereas the HERMES water-unsuppressed scans were used for eddy current correction as per consensus recommendations (43). Following standard pre-processing and linear combination modeling, the Osprey co-registration module (via SPM version 12)

was used to register the ^{1}H-MRS data to the T1-weighted images acquired at the scan and segment the voxel volume into gray matter fraction, white matter fraction and cerebrospinal fluid (CSF) fraction. Segmented T1 images were used to obtain tissue composition corrected water-scaled estimates of metabolite concentrations (i.u), whereby metabolite concentrations are scaled according to the assumption that metabolite concentrations in CSF are negligible (44,45). Metabolite T1 and T2 relaxation effects were also accounted for (tissue water and metabolite; (42,44,46)). Finally, 'alpha correction' of GABA concentrations was performed in Osprey, with the assumption that GABA concentrations are two times greater in GM compared to WM (45,47). For HERMES, sub-spectra were aligned using residual water peaks or the 2.01 ppm NAA peak before sub-spectra were misused or combined to calculate the GABA DIFF (A + B – C – D) and SUM (A + B + C + D) spectra. Averaged PRESS and HERMES spectra were modeled with a TE-specific simulated basis set and a flexible spline baseline based on MRS vendor and scan sequence parameters (generated in the MATLAB toolbox FID-A; (42,48). Basis sets for macromolecule and lipid contributions were integrated as gaussian basis functions (42). All spectra were modeled between 0.5 ppm and 4 ppm with linear baseline correction and a knot spacing of 0.55 ppm according to the Osprey model algorithm (42). Modeling was performed for 19 metabolites (ascorbic acid, aspartic acid, total Creatine, creatine methylene, GABA, glycerophosphocholine, glutathione, glutamine, glutamate, myo-inositol, lactate, total N-acetylaspartate, n-acetylaspartylglutamate, total choline, phosphocholine, phosphocreatine, phosphatidylethanolamine, scyllo-inositol, taurine), five macromolecules and three lipids (MM09, MM12, MM14, MM17, MM20, Lip09, Lip13, Lip20) for all spectra. The Osprey co-registration module (via SPM version 12) was used to register the MRS voxel to the T1-weighted images acquired at the scan. Segmented T1 images were used to obtain tissue-corrected water-scaled (molar) estimates of metabolite concentrations (i.u), whereby water-reference-ratio metabolite concentrations are scaled according to the assumption that metabolite concentrations in CSF are negligible (44,45). Further corrections for tissue specific water concentrations (gray matter (GM), white matter (WM) and CSF), and tissue specific water and metabolite longitudinal and transverse relaxation were performed (44), as outlined in recent consensus papers. We focus on the estimated concentrations relative to water (in institutional units, i.u.), as there are age differences in creatine concentrations across the age span of our participants (9). For transparency (43) all subsequent analyses were additionally performed with creatine as reference which can be found in the supplement.

Spectra were visually inspected by an experienced ^{1}H-MRS data user blind to participant age and diagnosis. ^{1}H-MRS spectra with significant artifacts due to

motion and/or scanner drift and/or out of voxel echo and indistinguishable GABA peaks at 3.02 ppm were excluded. As an additional quality metric, signal to noise ratio (SNR) threshold of >5 was used to further validate the visually excluded data. This led to 31 excluded datasets in the ACC glutamate measures, 45 in the ACC GABA measures, 8 in the thalamus glutamate measures, and 38 in the thalamus GABA measures, from the total of 166 included participants with at least one ^{1}H-MRS measure available. Ratios between glutamate and GABA concentrations in each region of interest were estimated by taking the glutamate concentrations over the GABA concentrations.

Statistical analyses

Competitive gene-set analysis

To investigate associations between aggregated genetic variation of the glutamate and GABA gene-sets with the ^{1}H-MRS measures, MAGMA (multi-marker Analysis of GenoMic Annotation) competitive gene-set analysis was used (version 1.10 (49)). This tests whether the aggregated association of the genes in the gene-set with the phenotype (^{1}H-MRS measured glutamate or GABA) is stronger than all other genes in the genome. This is done in two steps; first gene-based p-values are calculated for all genes in the genome (excluding genes located on the X-chromosome, see supplement Tables S1-S2) on the phenotypes of interest, which here is the ^{1}H-MRS metabolite concentrations of glutamate or GABA in each region of interest (ACC or thalamus), using a multiple linear principal components regression using F-tests. The second step tests the association of the gene-set, aggregating the gene-based p-values using competitive analysis. This gene-set analysis is done with an intercept-only linear regression model for the gene-set, which tests whether the aggregated genetic variation of the genes in a gene-set is more strongly associated with the phenotype of interest than all other genes in the genome. Age, age^{2} (to account for non-linear effects of age), and site were added as covariates.

Linear models

We investigated linear effects of ^{1}H-MRS concentrations and gene-set autism polygenic scores (PGS) on behavioral measures using linear models in the base R-software package (50). Sex, age, age^{2}, and scan site were included as covariates in all analyses. Each model investigated effects of one ^{1}H-MRS concentration (glutamate, GABA, or ratio between them) in one region of interest (ACC or thalamus), combined with a gene-set PGS (glutamate or GABA) and the gene-set PGS^{2} to account for non-linear effects of the polygenic score. This resulted in 48 models, which are listed in Table S4 in the supplement. Additionally, the

same models were run with creatine referenced ^{1}H-MRS data, which are found in the supplement. Differences between autistic and neurotypical participants in glutamate and GABA concentrations in ACC and thalamus, along with glutamate and GABA PGS, were assessed using linear models with the diagnostic group as dependent variable.

Results

Groups did not differ in gray matter, white matter nor cerebrospinal fluid (CSF) composition.

Demographics

Demographic and clinical characteristics are shown in Table 1. No sex differences were found between the autism and neurotypical groups, but the autism group had a higher average age compared to the neurotypical group. As expected, the groups differed in the SRS-2, RBS-R and SSP (where lower scores indicate higher sensory sensitivity) scores.

Table 1: Demographic and clinical characteristics

		NT (N = 63)		Autism (N = 103)		Test statistic		p-value
Sex, m/f		42/21		79/24		KWχ^2=1.98		0.16
	N	**Mean**	**SD**	**Mean**	**SD**		**df**	
Age		20.45	4.81	22.26	5.28	t = -2.24	138.81	0.03
SRS-2	147	30.75	18.4	68.5	27.77	t = -9.85	142.91	< 0.001
RBS-R	87	1.0	3.3	112.71	11.70	t = -7.27	84.42	< 0.001
SSP	73	184.81	5.55	152.71	27..44	t = 8.19	66.86	< 0.001
ADOS-2 total	95	-	-	8.6	6.37	-	-	-

NT, neurotypical; autism, Autism Spectrum Disorder; SD, standard deviation; df, degrees of freedom; SRS-2, Social Responsiveness Scale 2nd edition; RBS-R, Repetitive Behavior Scale - Revised; SSP, Short Sensory Profile; ADOS-2, Autism Diagnostic Observation Schedule 2nd edition; KWχ^2, Kruskal-Wallis Chi-Square.

Competitive gene-set analysis

Aggregated genetic variation within the glutamate gene-set (n=72 genes) was associated with GABA concentrations in thalamus ($\beta = 0.19$, SE = 0.1, $p = 0.03$), and aggregated genetic variation within the GABA gene-set (n = 124) was associated

with glutamate concentrations in thalamus (β = 0.23, SE = 0.12, p = 0.02), for more details see Table 2. These associations did not survive FDR correction.

Aggregated genetic variation with the glutamate and GABA gene-sets using creatine referenced ^{1}H-MRS concentrations showed similar results with the GABA genetic variation and thalamus glutamate concentrations, but did not show the glutamate gene-set with GABA thalamus association, which can be seen in the supplementary Table S5.

Table 2: Glutamate and GABA and ^{1}H-MRS competitive gene-set analysis results

Glutamate: Pathway gene-set (N=72)MRS concentrations (i.u.):	**BETA**	**P**	**P_{FDR}**	**SE**
GABA ACC	-0.25965	0.99679	0.996790	0.095246
GABA Thalamus	**0.19183**	**0.027735**	0.110940	**0.10016**
Glutamate ACC	0.14876	0.077301	0.154602	0.1045
Glutamate Thalamus	0.17472	0.11679	0.155720	0.14667
GABA: Pathway gene-set (N=124)MRS concentrations (i.u.):	**BETA**	**P**	**P_{FDR}**	**SE**
GABA ACC	0.01068	0.44465	0.5928667	0.076733
GABA Thalamus	0.0245	0.38	0.5928667	0.080197
Glutamate ACC	-0.13136	0.94151	0.9415100	0.083794
Glutamate Thalamus	**0.23309**	**0.023866**	0.0954640	**0.11773**

N, number of genes in analysis. P_{FDR} p-value corrected using False discovery rate (FDR) which was performed for each gene-set; SE, standard error of the regression coefficient. Significant results ($p<0.05$) are marked in bold.

Linear models

There were no significant associations between diagnostic group (autistic versus neurotypical) and ^{1}H-MRS glutamate or GABA concentrations, which can be seen in Figure 1. In one model (Table S6) there was a main effect of glutamate PGS on diagnostic group (β = -0.19, SE = 0.09, t = -2.09, p = 0.04), indicating that the glutamate PGS were higher in the autism group. However, this link was assessed in 6 models but were only significant in one, and with a small effect size, indicating that this may not be a reliable finding. No effects of GABA PGS, or any interactions between these measures were found. All diagnostic group models can be seen in Table S6 in the supplement.

There were several interaction effects between metabolite concentrations and gene-set polygenic scores (PGS) on behavioral measures; all linear model outputs

can be seen in supplementary Tables S7-S12. ACC glutamate concentrations and glutamate PGS had interaction effects on SRS-2 (β = 0.28, SE = 0.11, t = 2.52, p = 0.01), SSP (β = -0.3, SE = 0.14, t = -2.16, p = 0.04) and ADOS-2 scores (β = 9.25, SE = 0.1, t = 2.4, p = 0.02), see Table S7.

Thalamus glutamate/GABA ratios and GABA PGS had interaction effects on SRS-2 (β = 7.71, SE = 3.66, t = 2.11, p = 0.04) and ADOS-2 scores (β = 7.78, SE = 3.12, t = 2.5, p = 0.02) (Table S12), the latter which was also seen with the creatine referenced ^{1}H-MRS data, see Table S18. Thalamus glutamate/GABA ratios and glutamate PGS also showed an interaction effect on SRS-2 scores (β = 13.89, SE = 6.17, t = 2.25, p = 0.03) (Table S9).

Additionally, in one model there was a main effect of age on ADOS-2 scores (Table S7), and in another there were main effects of GABA PGS and GABA PGS^{2} on ADOS-2 scores as well (Table S11). These results, respectively, indicate lower ADOS-2 scores in older (autistic) participants and both linear and non-linear negative effects of GABA PGS on ADOS-2 scores. However, these associations were tested in several models but were only significant in one, indicating that this may not be a reliable finding. Sex was associated with ADOS-2 scores in almost all linear models (see supplementary Tables S7-S12), where males had higher ADOS-2 scores than females. Most of the findings were not replicated with the creatine referenced ^{1}H-MRS data, results of which can be seen in the supplementary Tables S12-S18. ACC creatine concentrations (i.u.) were trending toward significant differences between groups (t = -1.91, df = 97.95, p = 0.06), and thalamus creatine concentrations (i.u.) were significantly different between groups (t = -2.35, df = 120.07, p = 0.02), where the autism group had lower creatine concentrations than the neurotypical group in both regions.

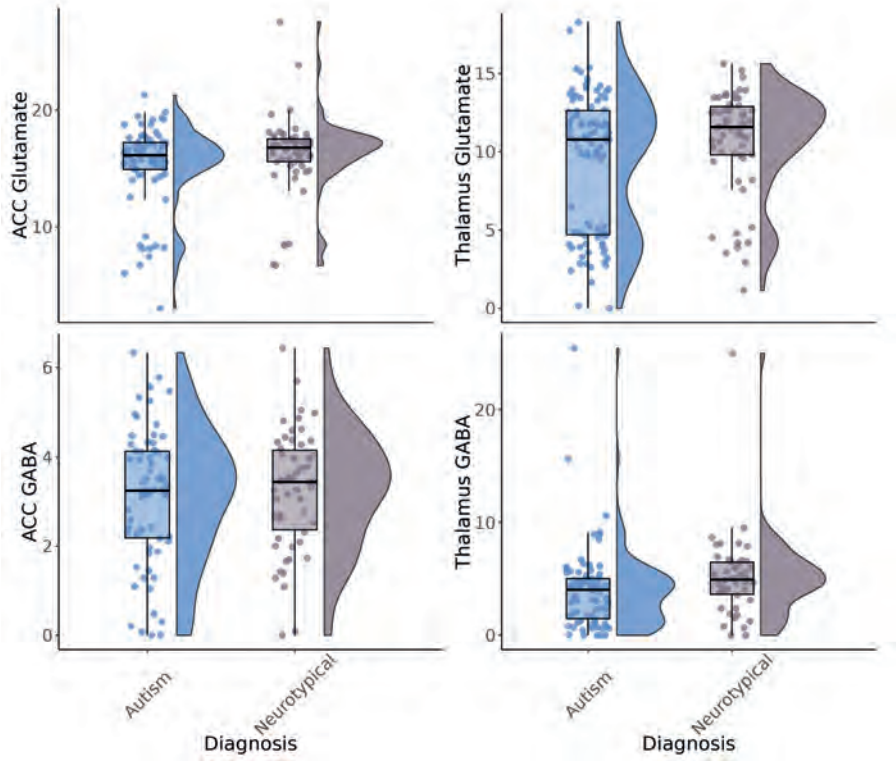

Figure 1: Group comparisons ^{1}H-MRS glutamate and GABA concentrations

Glutamate and GABA concentrations in ACC and thalamus, shown in institutional units (i.u.). There are no group-level differences between the autism and neurotypical groups in either metabolite in any region.

Discussion

To the best of our knowledge, this is the first study to explore how these metabolites, their ratios, and genetic markers of glutamate and GABA function together may underpin clinical traits and behaviors in autism. We found that interactions between genetic and metabolite measures of glutamate and GABA are associated with various behavioral traits, suggesting that genetic variations in glutamate and GABA pathways may modulate metabolite concentrations, ultimately affecting these behaviors. Further, genetic variation of glutamate genes affected GABA concentrations in thalamus, while genetic variation of GABA genes was associated with glutamate concentrations in the same region. These results indicate that glutamate/GABA ratios in the thalamus, a region highly involved in consolidation of sensory processing, cognition, and learning, are affected by both glutamate

and GABA genes. Our findings demonstrate the need to investigate glutamate and GABA measures together to fully understand the complex underpinnings of autism.

Social responsiveness as measured by the SRS-2 was affected by interactions of glutamate PGS with glutamate concentrations in ACC, glutamate PGS with glutamate/GABA ratios in thalamus, as well as GABA PGS with glutamate/GABA ratios in thalamus. Previous studies have found associations between glutamate and GABA, then looking at GABA/creatine in the ACC and glutamate/GABA ratios in cerebellum, with social behaviors (51,52). Our findings add to previous results by demonstrating that interactions between these metabolites, and interactions between genetic measures and metabolites, affect these behaviors in several brain regions. These results improves our mechanistic understanding of how genetic factors may affect differing social behaviors in autism, by altering glutamate/GABA metabolite concentrations. This has implications for future work aiming to disentangle markers for targeted therapeutic options, as targeting specific behavioral domains of autism characteristics would be most effective by understanding what kind of E/I alterations, where in the brain, affects which behaviors.

The ADOS-2 captures both social and restricted and repetitive behaviors, and we observed significant interactions of the glutamate PGS with ACC glutamate concentrations, as well as GABA PGS with glutamate/GABA ratios in thalamus. Previous work using data from the same cohort showed associations between aggregated genetic variation of these glutamate and GABA gene-sets and ADOS-2 scores (see Chapter 3, (17)). We also previously found links between glutamate PGS and the autism diagnostic interview (ADI-R, (53)), which captures similar behaviors to ADOS-2 but during childhood development (see Chapter 4). Collectively, these findings indicate that polygenic scores of genes encoding for glutamate and GABA functions in the brain interact with glutamate/GABA ratios in the thalamus to affect autism behaviors, which suggests a crucial role for the thalamus in the expression of autism traits. The MAGMA analyses further support this notion, as they showed that both thalamic glutamatergic and GABAergic concentrations were affected by genetic variations of the opposite metabolite, as seen in Table 2. These results indicate that interacting alterations in glutamatergic and GABAergic metabolism and neurotransmission occur in the thalamus, as captured by both gene-set PGS and aggregated genetic variation analyses. The thalamus is involved in many important functions including cognition, attention, and relaying sensory information to other regions such as the ACC (54–58), and these findings suggest that E/I alterations have downstream effects on these behaviors, which are relevant to autism. For example, sensory processing differences in autism are often described as hyper- or hypo-

sensitivity to certain stimuli, which can be attributed to increased noise surrounding those incoming stimuli (59). Increased noise in sensory input will impair the signal-to-noise ratio and make certain environments overwhelming (hypersensitivity), or make it more difficult to disentangle relevant input (hyposensitivity). The behavioral measure of sensory processing used here, the SSP, did not show interaction effects of PGS and metabolite concentrations in the thalamus (but did in the ACC). This may due to the role of thalamus in relaying initial sensory input, while the SSP captures more integrated sensory experiences processed in downstream brain regions such as the ACC. To increase the understanding of potential implications of the important role of thalamus functioning on autism characteristics suggested by our findings, future work should investigate whether similar or differing links are present in other brain regions. The interplay between glutamate and GABA could potentially explain not only the heterogeneous findings in previous studies, but also heterogeneous expressions of autism traits.

The results in this study should be considered in the context of its limitations. Firstly, there were more male than female participants. Although the ratios between them did not differ between diagnostic groups, there should be caution when generalizing results across sexes without having more equal distribution of data across the groups. Secondly, we were not able to attempt replication analyses, as there is currently no comparable dataset that combines glutamate and GABA ^{1}H-MRS measures, as well as genetic measures, particularly in both autistic and neurotypical individuals. While replication is a crucial part of not only validating results, but also to allow generalization across broader populations, we are confident that the results within this study are reliable and there will be replication attempts in the future as more large multimodal datasets become available. Another limitation pertains to the genetic data, as participants in this study were all of European ancestry. This further limits the ability to generalize results across diverse populations. Further, there are intrinsic limitations of ^{1}H-MRS measures. Glutamate concentrations measured by ^{1}H-MRS also contain some glutamine, while the signal captured by GABA also contains some macromolecules (64). Additionally, glutamine is a precursor for synthesis of both glutamate and GABA (65). This means that while our glutamate and GABA measures capture some other molecules in their signals, their measures are also not independent. It is important to keep in mind that ^{1}H-MRS measures do not directly reflect neurotransmission but also capture metabolite concentrations that, while involved in neuronal communication, also have other roles in the brain and its metabolism. Thus, while ^{1}H-MRS measures of glutamate and GABA are our most readily available measures of *in vivo* concentrations of these metabolites, these measures also reflect more general glutamate and GABA functions in the brain.

Most of our results were not replicated using creatine ratios rather than water as reference for the ^{1}H-MRS data. This does not invalidate our results, as we found group differences in creatine concentrations in these regions. Instead, it indicates that using creatine ratios is less suitable when investigating clinical populations, such as those with autism. Future work should also involve additional regions of ^{1}H-MRS measurements and broader age ranges of participants. Further, multimodal analysis including other co-occurring conditions in autism would be informative for disentangling underlying mechanisms through the lens of E/I imbalance and how they may lead to even more heterogeneous expressions of autism.

To summarize, we found that interactions between both ^{1}H-MRS and genetic markers, as well as ratios between glutamate and GABA concentrations in the thalamus, affect autism behaviors. Ultimately, these findings highlight the complex relationships between genes, brain and behavior, as genetic predispositions to autism of glutamate and GABA genes may influence autistic behaviors, by altering dynamics between glutamate and GABA metabolites in the brain. These findings also emphasize the importance of investigating the interaction between glutamate and GABA, in a multimodal fashion, to properly address how E/I imbalance may affect autism.

Conflict of interest statements

Jan Buitelaar has been in the past 3 years a consultant to / member of advisory board of / and/or speaker for Takeda, Roche, Medice, Angelini, Neuraxpharm, and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties.

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Supplement

Table S1: Summary table of all genes in the glutamate gene-set

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ALDH5A1	7915	6	24495197	24537435	+	297
CALM1	801	14	90863327	90874619	+	47
CALML5	51806	10	5540658	5541533	-	6
CAMK4	814	5	110559947	110830584	+	1538
DLG4	1742	17	7093209	7123369	-	102
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GLS	2744	2	191745547	191830278	+	290
GLUD1	2746	10	88809959	88854776	-	186
GLUD2	2747	X	120181462	120183796	+	
GLUL	2752	1	182350839	182361341	-	55
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2	2783	7	100271363	100276792	+	19
GNB3	2784	12	6949375	6956564	+	34
GNB5	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041
GOT1	2805	10	101156627	101190530	-	146
GOT1L1	137362	8	37791799	37797664	-	17
GOT2	2806	16	58741035	58768246	-	229
GRIA1	2890	5	152870084	153193429	+	1819
GRIA2	2891	4	158141736	158287227	+	425
GRIA3	2892	X	122317996	122624766	+	
GRIA4	2893	11	105480800	105852819	+	1505
GRID1	2894	10	87359312	88126250	-	4622
GRID2	2895	4	93225453	94695707	+	7119
GRIK1	2897	21	30909254	31312282	-	2258

Table S1: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GRIK2	2898	6	101841584	102517958	+	3720
GRIK3	2899	1	37261128	37499844	-	963
GRIK4	2900	11	120382465	120859514	+	2775
GRIK5	2901	19	42502468	42574278	-	138
GRIN1	2902	9	140033609	140063214	+	86
GRIN2A	2903	16	9847265	10276611	-	3419
GRIN2B	2904	12	13713684	14133022	-	2569
GRIN2C	2905	17	72838162	72856966	-	93
GRIN2D	2906	19	48898132	48948188	+	222
GRIN3A	116443	9	104331634	104500862	-	942
GRIN3B	116444	19	1000437	1009723	+	108
GRINA	2907	8	145064226	145067596	+	9
GRIP1	23426	12	66741178	67463014	-	4124
GRM1	2911	6	146286032	146758782	+	2121
GRM2	2912	3	51741081	51752629	+	16
GRM3	2913	7	86273230	86494193	+	1110
GRM4	2914	6	33989623	34123399	-	1020
GRM5	2915	11	88237256	88796846	-	3817
GRM6	2916	5	178405328	178422124	-	141
GRM7	2917	3	6902802	7783218	+	5656
GRM8*	2918	7	126078652	126892428	-	4521
HOMER1	9456	5	78669647	78809659	-	705
HOMER2	9455	15	83517729	83654905	-	736
HOMER3	9454	19	19040010	19052041	-	42
PICK1	9463	22	38453262	38471708	+	92
SLC17A1	6568	6	25783125	25832287	-	297
SLC17A2	10246	6	25912982	25930954	-	109
SLC17A6	57084	11	22359667	22401049	+	208
SLC17A7	57030	19	49932655	49945617	-	39
SLC17A8	246213	12	100750857	100815837	+	347
SLC1A1	6505	9	4490427	4587469	+	544
SLC1A2	6506	11	35272752	35441610	-	1155
SLC1A3	6507	5	36606457	36688436	+	420
SLC1A4	6509	2	65215579	65250999	+	145
SLC1A6	6511	19	15060845	15121455	-	503
SLC1A7	6512	1	53552855	53608304	-	472
SLC38A1	81539	12	46576838	46663208	-	441
SUCLG2	8801	3	67410884	67705038	-	1963

All genes in the table were included in the glutamate pathway gene-set. NSNPS, number of single nucleotide polymorphisms (SNPs).

Table S2: Summary table of all genes in the GABA gene-set.

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
ABAT	18	16	8768444	8878432	+	1013
ADCY1	107	7	45614125	45762715	+	760
ADCY10	55811	1	167778357	167883608	-	659
ADCY2	108	5	7396343	7830194	+	2563
ADCY3	109	2	25042038	25142602	-	694
ADCY4	196883	14	24787555	24804277	-	81
ADCY5	111	3	123001143	123167924	-	858
ADCY6	112	12	49159975	49182820	-	81
ADCY7	113	16	50278830	50352046	+	333
ADCY8	114	8	131792546	132053012	-	1901
ADCY9	115	16	4012650	4166186	-	1082
ALDH5A1	7915	6	24495197	24537435	+	297
ALDH9A1	223	1	165631449	165667900	-	239
AP1B1	162	22	29723669	29784754	-	255
AP1G2	8906	14	24028777	24038754	-	14
AP2A1	160	19	50270180	50310369	+	165
AP2A2	161	11	925809	1012245	+	487
AP2B1	163	17	33913918	34053436	+	746
AP2M1	1173	3	183892634	183901879	+	53
AP2S1	1175	19	47341423	47354203	-	35
CACNA1A	773	19	13317256	13617274	-	1465
CACNA1B	774	9	140772241	141019076	+	880
CACNA1C	775	12	2079952	2807115	+	3692
CACNA1D	776	3	53529076	53847179	+	1844
CACNA1E	777	1	181452447	181775920	+	1671
CACNA1F	778	X	49061523	49089833	-	
CACNA1G	8913	17	48638429	48704835	+	310
CACNA1H	8912	16	1203241	1271772	+	422
CACNA1I	8911	22	39966758	40085740	+	591
CACNA1S	779	1	201008635	201081694	-	505
CACNA2D1	781	7	81575760	82073031	-	3150
CACNA2D2	9254	3	50400230	50540892	-	656
CACNA2D3	55799	3	54156620	55108584	+	5930
CACNA2D4	93589	12	1901123	2027870	-	775
CACNB1	782	17	37329709	37353956	-	89
CACNB2	783	10	18429373	18830688	+	2968
CACNB3	784	12	49208215	49222726	+	46
CACNB4	785	2	152689285	152955593	-	1246
CACNG1	786	17	65040652	65052913	+	56

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
CACNG2	10369	22	36956916	37098690	-	720
CACNG3	10368	16	24266874	24373737	+	675
CACNG4	27092	17	64960980	65029518	+	432
CACNG5	27091	17	64831235	64881941	+	373
CACNG6	59285	19	54494403	54515920	+	115
CACNG7	59284	19	54412704	54447195	+	105
CACNG8	59283	19	54466290	54493469	+	111
CATSPER1	117144	11	65784223	65793988	-	45
CATSPER2	117155	15	43922772	43941039	-	63
CATSPER3	347732	5	134303596	134347397	+	207
CATSPER4	378807	1	26517119	26529033	+	107
DNM1	1759	9	130965634	131017528	+	223
GABARAP	11337	17	7143738	7145753	-	5
GABBR1	2550	6	29570005	29600962	-	219
GABBR2	9568	9	101050364	101471479	-	2637
GABRA1	2554	5	161274197	161326965	+	283
GABRA2	2555	4	46246470	46392056	-	727
GABRA3	2556	X	151334706	151619831	-	
GABRA4	2557	4	46920917	46996424	-	406
GABRA5	2558	15	27111866	27194357	+	158
GABRA6	2559	5	161112658	161129598	+	81
GABRB1	2560	4	47033295	47432801	+	2058
GABRB2	2561	5	160715426	160975130	-	1268
GABRB3	2562	15	26788693	27018935	-	1332
GABRD	2563	1	1950768	1962192	+	10
GABRE	2564	X	151121596	151143156	-	
GABRG1	2565	4	46037786	46126082	-	496
GABRG2	2566	5	161494648	161582545	+	435
GABRG3	2567	15	27216429	27778373	+	2556
GABRP	2568	5	170210723	170241051	+	193
GABRQ	55879	X	151806637	151821825	+	
GABRR1	2569	6	89887223	89941007	-	344
GABRR2	2570	6	89966840	90025018	-	405
GABRR3	200959	3	97705527	97754148	-	264
GAD1	2571	2	171673200	171717661	+	172
GAD2	2572	10	26505236	26593491	+	579
GNA11	2767	19	3094408	3121468	+	144
GNA12	2768	7	2767739	2883963	-	883
GNA13	10672	17	63005407	63052920	-	84

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
GNA14	9630	9	80037995	80263232	-	1496
GNA15	2769	19	3136191	3163766	+	201
GNAI1	2770	7	79764140	79848725	+	383
GNAI2	2771	3	50264120	50296786	+	114
GNAI3	2773	1	110091186	110138465	+	181
GNAL	2774	18	11689014	11885684	+	1003
GNAO1	2775	16	56225251	56391356	+	866
GNAQ	2776	9	80335189	80646219	-	1344
GNAS	2778	20	57414756	57486250	+	323
GNAT1	2779	3	50229043	50235129	+	12
GNAT2	2780	1	110145889	110155705	-	45
GNAZ	2781	22	23412669	23467224	+	256
GNB1	2782	1	1716725	1822552	-	250
GNB1L	54584	22	19775932	19842462	-	369
GNB2	2783	7	100271363	100276792	+	19
GNB3	2784	12	6949375	6956564	+	34
GNB4	59345	3	179113876	179169371	-	290
GNB5	10681	15	52413123	52483565	-	486
GNG10	2790	9	114423851	114432526	+	50
GNG11	2791	7	93551016	93555826	+	32
GNG12	55970	1	68167149	68299436	-	702
GNG13	51764	16	848041	850733	-	33
GNG2	54331	14	52327022	52436518	+	794
GNG3	2785	11	62475066	62476678	+	5
GNG4	2786	1	235710985	235814054	-	543
GNG5	2787	1	84964006	84972262	-	37
GNG7	2788	19	2511218	2702746	-	1041
GPHN	10243	14	66974125	67648525	+	3011
GPR37	2861	7	124385655	124406079	-	81
KCNH2	3757	7	150642044	150675402	-	179
KCNN1	3780	19	18062111	18110133	+	207
KCNN2	3781	5	113698016	113832197	+	840
KCNN3	3782	1	154669938	154842754	-	925
KCNN4	3783	19	44270685	44286269	-	72
KCNQ2	3785	20	62031561	62103993	-	607
KCNQ3	3786	8	133133105	133493004	-	2095
MRAS	22808	3	138066490	138124377	+	307
NSF	4905	17	44668035	44834830	+	108
OPN1SW	611	7	128412543	128415844	-	20

Table S2: Continued

Gene name	Entrez gene ID	Chromosome	Start position	End position	strand	NSNPS
RPS27A	6233	2	55459039	55462989	+	27
SLC32A1	140679	20	37353105	37358015	+	20
SLC6A1	6529	3	11034420	11080935	+	267
SLC6A11	6538	3	10857917	10980146	+	739
SLC6A12	6539	12	299243	323740	-	169
SLC6A13	6540	12	329787	372039	-	322
UBA52	7311	19	18674576	18688270	+	83
UBB	7314	17	16284367	16286059	+	7
UBC	7316	12	125396192	125399587	-	23
UBD	10537	6	29523389	29527702	-	42
UBQLN1	29979	9	86274878	86323168	-	265

All genes in the table were included in the GABA pathway gene-set. NSNPS, number of single nucleotide polymorphisms (SNPs).

Figure S1: Voxel overlays across sites

Superposition on the MNI152 template of voxel placements in the thalamus and anterior cingulate cortex (ACC), for all sites (London, blue; Mannheim, yellow; Nijmegen, green). The placements are consistent across and within sites.

Table S3: Scanner parameters across sites

Sequence / scanner details	Parameter	KCL	Mannheim	Nijmegen
Manufacturer		GE Medical systems	Siemens	Siemens
Model		Discovery mr750	TrimTrio	Skyra
T1w	TR/TE/TI (ms)	7.31/3.02/400	2300/2,95/900	2300 / 3 / 900
	FOV(mm)	270	270	
	Base res (mm)	256x256	1.1x1.1	1.1 x 1.1
	Slice thickness (mm)/number	1.2/196	1.2/176	1.2 / 176
	Flip angle (degrees)	11	9	9
	TA (minutes)	4:53	5:30	5:12
MRS	Voxel dims (mm) Thalamus ACC	26 x 40 x 24 30 x 35 x 25	26x40x24 35x30x25	26 x 40 x 24 35 x 30 x 25
	Flip angle (degrees)	90	90	90
PRESS	TR/TE (ms)	3000/30	2000/35	2000 / 35
	Averages sup/ unsup	64	64/16	64 / 16
	TA (minutes) sup/unsup	4:24		2:18 / 0:42
HERMES	TR/TE (ms)	2000/80	2000/80	2000 / 80
	Averages sup/ unsup	240	240/16	240 / 16
	TA (minutes) sup/unsup	8:40	8:08/0:40	8:08 / 0:40

Abbreviations: FA, flip angle; FOV, field of view; TE, echo time; TR, repetition time.

Table S4: All linear models

RBS-R	~ Glutamate ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SRS-2	~ Glutamate ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SSP	~ Glutamate ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
ADOS-2	~ Glutamate ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
RBS-R	~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS^2 + Age + Sex * Site
SRS-2	~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS^2 + Age + Sex * Site
SSP	~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS^2 + Age + Sex * Site
ADOS-2	~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS^2 + Age + Sex * Site
RBS-R	~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SRS-2	~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SSP	~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
ADOS-2	~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
RBS-R	~ GABA Thalamus * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SRS-2	~ GABA Thalamus * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site
SSP	~ GABA Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
ADOS-2	~ GABA Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
RBS-R	~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
SRS-2	~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
SSP	~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
ADOS-2	~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
RBS-R	~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
SRS-2	~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
SSP	~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
ADOS-2	~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS2 + Age * Sex + Site
RBS-R	~ Glutamate ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
SRS-2	~ Glutamate ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
SSP	~ Glutamate ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
ADOS-2	~ Glutamate ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
RBS-R	~ Glutamate Thalamus * GABA PGS + GABA PGS2 + Age + Sex * Site
SRS-2	~ Glutamate Thalamus * GABA PGS + GABA PGS2 + Age + Sex * Site
SSP	~ Glutamate Thalamus * GABA PGS + GABA PGS2 + Age + Sex * Site
ADOS-2	~ Glutamate Thalamus * GABA PGS + GABA PGS2 + Age + Sex * Site
RBS-R	~ GABA ACC * GABA PGS + GABA PGS2 + Age + Sex * Site
SRS-2	~ GABA ACC * GABA PGS + GABA PGS2 + Age + Sex * Site
SSP	~ GABA ACC * GABA PGS + GABA PGS2 + Age + Sex * Site
ADOS-2	~ GABA ACC * GABA PGS + GABA PGS2 + Age + Sex * Site
RBS-R	~ GABA Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site

Table S4: Continued

SRS-2	~ GABA Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
SSP	~ GABA Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
ADOS	~ GABA Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
RBS-R	~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
SRS-2	~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
SSP	~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
ADOS-2	~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS2 + Age * Sex + Site
RBS-R	~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
SRS-2	~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
SSP	~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS2 + Age * Sex + Site
ADOS-2	~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS^2 + Age * Sex + Site

RBS-R, Repetitive Behavior Scale-Revised; SRS-2, Social Responsiveness Scale, Second Edition; SSP, Short Sensory Profile; ADOS-2, Autism Diagnostic Observation Schedule 2nd edition."~" indicates that the variables on the right side are associated with the dependent variable on the left hand side. The "*" between the variables of interest indicates that the model assesses these variables both independently and their interaction effects.

Table S5: Glutamate and GABA and ^{1}H-MRS creatine-referenced competitive gene-set analysis results

Glutamate: Pathway gene-set (N=72) MRS concentrations (/creatine):	**BETA**	**P**	**P_{FDR}**	**SE**
GABA ACC	-0.093316	0.11011	0.80163	0.90869
GABA Thalamus	-0.26942	0.20215	0.90869	0.90869
Glutamate ACC	-0.11696	0.10413	0.86933	0.90869
Glutamate Thalamus	-0.17781	0.2091	0.80243	0.90869
GABA: Pathway gene-set (N=124) MRS concentrations (/creatine):	**BETA**	**P**	**P_{FDR}**	**SE**
GABA ACC	0.13307	0.088684	0.066754	0.1335080
GABA Thalamus	0.045058	0.16186	0.39036	0.5204800
Glutamate ACC	-0.17525	0.083488	0.98209	0.9820900
Glutamate Thalamus	**0.44562**	**0.16782**	**0.0039647**	**0.0158588**

N, number of genes in analysis. P_{FDR} p-value corrected using False discovery rate (FDR) which was performed for each gene-set; SE, standard error of the regression coefficient. Significant results ($p<0.05$) are marked in bold.

Table S6: Diagnostic group linear models

GLUTAMATE PGS				
Diagnosis ~ ACC Glutamate* Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.32077	0.34042	3.880	0.000186
Glu ACC	-0.11147	0.12990	-0.858	0.392864
Glu PGS	**-0.18515**	**0.08898**	**-2.081**	**0.039983**
Glu PGS²	-0.12024	0.09155	-1.313	0.192026
Site2	0.17484	0.38721	0.452	0.652567
Site3	0.34211	0.34436	0.993	0.322862
Sex	-0.03742	0.11336	-0.330	0.742038
Age	-0.12476	0.17977	-0.694	0.489265
Glu ACC:Glu PGS	0.04783	0.06552	0.730	0.467042
Sex:Age	0.15274	0.14018	1.090	0.278508
Residual standard error	0.4946 (on 101 degrees of freedom)			
Multiple R squared	0.09311			
Adjusted R squared	0.0123			
Diagnosis ~ Thalamus Glutamate* Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.82808	0.21467	8.516	6.55e-14
Glu Thalamus	0.06084	0.14917	0.408	0.6841
Glu PGS	-0.10094	0.08096	-1.247	0.2150
Glu PGS²	-0.06076	0.08385	-0.725	0.4701
Site2	-0.41078	0.23835	-1.723	0.0875
Site3	-0.26627	0.22203	-1.199	0.2329
Sex	0.00185	0.10033	0.018	0.9853
Age	-0.15568	0.16205	-0.961	0.3387
Glu Thalamus:Glu PGS	-0.04582	0.07013	-0.653	0.5148
Sex:Age	0.19453	0.12611	1.542	0.1257
Residual standard error	0.486 (on 117 degrees of freedom)			
Multiple R squared	0.09465			
Adjusted R squared	0.02501			
Diagnosis ~ ACC GABA* Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.66175	0.24436	6.800	1.07e-09
GABA ACC	0.01095	0.10128	0.108	0.9141
Glu PGS	-0.16151	0.09643	-1.675	0.0974
Glu PGS²	-0.09930	0.09845	-1.009	0.3159
Site2	-0.25400	0.25787	-0.985	0.3272

GABA PGS				
Diagnosis ~ ACC Glutamate* GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.329189	0.340846	3.900	0.000174
Glu ACC	-0.106270	0.133678	-0.795	0.428497
GABA PGS	0.232590	0.637112	0.365	0.715823
GABA PGS²	0.290952	0.628825	0.463	0.644581
Site2	0.073286	0.390458	0.188	0.851494
Site3	0.252188	0.341307	0.739	0.461688
Sex	0.026797	0.114538	0.234	0.815492
Age	-0.126856	0.187542	-0.676	0.500325
Glu ACC:GABA PGS	-0.001242	0.062595	-0.020	0.984203
Sex:Age	0.168903	0.147150	1.148	0.253750
Residual standard error	0.5031 (on 101 degrees of freedom)			
Multiple R squared	0.06162			
Adjusted R squared	-0.022			
Diagnosis ~ Thalamus Glutamate* GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.832899	0.217015	8.446	9.49e-14
Glu Thalamus	0.089210	0.149329	0.597	0.5514
GABA PGS	0.097052	0.516924	0.188	0.8514
GABA PGS²	0.132701	0.511615	0.259	0.7958
Site2	-0.451175	0.235730	-1.914	0.0581
Site3	-0.293932	0.223667	-1.314	0.1914
Sex	0.016102	0.101776	0.158	0.8746
Age	-0.161376	0.166128	-0.971	0.3334
Glu Thalamus:GABA PGS	-0.002641	0.058561	-0.045	0.9641
Sex:Age	0.199501	0.129571	1.540	0.1263
Residual standard error	0.489 (on 117 degrees of freedom)			
Multiple R squared	0.08346			
Adjusted R squared	0.01296			
Diagnosis ~ ACC GABA* GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.61543	0.25033	6.453	5.21e-09
GABA ACC	0.03248	0.10080	0.322	0.748
GABA PGS	-0.03474	0.66056	-0.053	0.958
GABA PGS²	0.02352	0.65482	0.036	0.971
Site2	-0.31885	0.26141	-1.220	0.226

Table S6: Continued

Site3	-0.08842	0.21426	-0.413	0.6808
Sex	0.01232	0.11803	0.104	0.9171
Age	-0.14375	0.18162	-0.791	0.4307
GABA ACC:Glu PGS	0.03619	0.09446	0.383	0.7025
Sex:Age	0.17278	0.14272	1.211	0.2292
Residual standard error	0.5 (on 91 degrees of freedom)			
Multiple R squared	0.08386			
Adjusted R squared	-0.00675			
Diagnosis ~ Thalamus GABA* Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.75336	0.19850	8.833	6.02e-14
GABA Thalamus	-0.05712	0.09508	-0.601	0.5494
Glu PGS	-0.12276	0.10064	-1.220	0.2256
Glu PGS²	-0.07475	0.10548	-0.709	0.4803
Site2	-0.44355	0.20117	-2.205	0.0299
Site3	-0.24276	0.14316	-1.696	0.0933
Sex	0.08820	0.11004	0.802	0.4249
Age	-0.20207	0.17241	-1.172	0.2442
GABA Thalamus:Glu PGS	-0.09219	0.09303	-0.991	0.3243
Sex:Age	0.22476	0.13410	1.676	0.0971
Residual standard error	0.471 (on 93 degrees of freedom)			
Multiple R squared	0.1397			
Adjusted R squared	0.05643			
Diagnosis ~ Glutamate/GABA ratio ACC* Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.64938	0.22474	7.339	7.58e-11
Glu/GABA ACC	0.14031	0.14165	0.990	0.3245
Glu PGS	-0.16465	0.09595	-1.716	0.0895
Glu PGS²	-0.07986	0.10287	-0.776	0.4395
Site2	-0.29263	0.24288	-1.205	0.2313
Site3	-0.03331	0.19155	-0.174	0.8623
Sex	0.01017	0.11442	0.089	0.9294
Age	-0.07893	0.18132	-0.435	0.6644
Glu/GABA ACC:Glu PGS	-0.10681	0.22005	-0.485	0.6285
Sex:Age	0.15075	0.13954	1.080	0.2827
Residual standard error	0.4857 (on 94 degrees of freedom)			
Multiple R squared	0.1206			
Adjusted R squared	0.03645			

Site3	-0.11550	0.21412	-0.539	0.591
Sex	0.07386	0.12075	0.612	0.542
Age	-0.19230	0.19152	-1.004	0.318
GABA ACC: GABA PGS	-0.02742	0.10079	-0.272	0.786
Sex:Age	0.22121	0.15149	1.460	0.148
Residual standard error	0.5068 (on 91 degrees of freedom)			
Multiple R squared	0.05872			
Adjusted R squared	-0.03437			
Diagnosis ~ Thalamus GABA* GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.742892	0.202673	8.600	1.87e-13
GABA Thalamus	-0.053396	0.096961	-0.551	0.583
GABA PGS	-0.023101	0.596273	-0.039	0.969
GABA PGS²	-0.003344	0.589672	-0.006	0.995
Site2	-0.435379	0.205706	-2.117	0.037
Site3	-0.235405	0.145890	-1.614	0.110
Sex	0.089604	0.115383	0.777	0.439
Age	-0.176640	0.181878	-0.971	0.334
GABA Thalamus: GABA PGS	0.022983	0.093431	0.246	0.806
Sex:Age	0.205972	0.142792	1.442	0.153
Residual standard error	0.4786 (on 93 degrees of freedom)			
Multiple R squared	0.1118			
Adjusted R squared	0.02589			
Diagnosis ~ Glutamate/GABA ratio ACC* GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.329966	0.292475	4.547	1.73e-05
Glu/GABA ACC	0.085878	0.801506	0.107	0.915
GABA PGS	-0.158751	0.648100	-0.245	0.807
GABA PGS²	-0.066698	0.641029	-0.104	0.917
Site2	-0.158598	0.275715	-0.575	0.567
Site3	0.143154	0.238906	0.599	0.551
Sex	0.109052	0.118582	0.920	0.360
Age	-0.109563	0.191058	-0.573	0.568
Glu/GABA ACC:GABA PGS	0.001527	0.602180	0.003	0.998
Sex:Age	0.197965	0.147611	1.341	0.183
Residual standard error	0.4963 (on 88 degrees of freedom)			
Multiple R squared	0.1018			
Adjusted R squared	0.00997			

Table S6: Continued

Diagnosis ~ Glutamate/GABA ratio Thalamus* Glutamate PGS + Glutamate PGS2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.81291	0.16008	11.325	<2e-16
Glu/GABA Thalamus	0.52133	0.45518	1.145	0.2546
Glu PGS	-0.04874	0.11046	-0.441	0.6599
Glu PGS^2	-0.09133	0.08231	-1.109	0.2697
Site2	-0.37087	0.18314	-2.025	0.0454
Site3	-0.22888	0.11064	-2.069	0.0410
Sex	0.05583	0.10214	0.547	0.5858
Age	-0.19396	0.16389	-1.184	0.2393
Glu/GABA Thalamus:Glu PGS	0.85126	0.76120	1.118	0.2660
Sex:Age	0.22837	0.12919	1.768	0.0800
Residual standard error	0.4695 (on 106 degrees of freedom)			
Multiple R squared	0.1364			
Adjusted R squared	0.0631			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Table S7: Linear model outputs ^{1}H-MRS glutamate and glutamate PGS

ACC GLUTAMATE & GLUTAMATE PGS				
RBS ~ Glutamate ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.07444	0.13548	-0.549	0.585
Glu ACC	-0.13762	0.26334	-0.523	0.604
Glu PGS	-0.12131	0.20339	-0.596	0.554
Glu PGS^2	-0.13716	0.19478	-0.704	0.485
Site2	-0.53704	0.73159	-0.734	0.466
Site3	-0.20335	0.79424	-0.256	0.799
Sex	-0.22186	0.25256	-0.878	0.384
Age	-0.04667	0.13846	-0.337	0.737
Glu ACC:Glu PGS	0.04872	0.13165	0.370	0.713
Sex:Age	0.05459	0.29755	0.183	0.855
Residual standard error	0.7509 (on 51 degrees of freedom)			
Multiple R squared	0.03638			
Adjusted R squared	-0.1337			

Diagnosis ~ Glutamate/GABA ratio Thalamus* GABA PGS + GABA PGS2 + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.84606	0.21545	8.569	9.18e-14
Glu/GABA Thalamus	0.96589	1.52230	0.634	0.5271
GABA PGS	0.02588	0.55423	0.047	0.9628
GABA PGS^2	-0.07093	0.52483	-0.135	0.8928
Site2	-0.41339	0.18406	-2.246	0.0268
Site3	-0.26347	0.11267	-2.338	0.0212
Sex	0.09045	0.10544	0.858	0.3929
Age	-0.18117	0.17097	-1.060	0.2917
Glu/GABA Thalamus:GABA PGS	1.21822	1.95226	0.624	0.5340
Sex:Age	0.21468	0.13418	1.600	0.1126
Residual standard error	0.4777 (on 106 degrees of freedom)			
Multiple R squared	0.1062			
Adjusted R squared	0.03027			

THALAMUS GLUTAMATE & GLUTAMATE PGS

RBS ~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.02302	0.15997	-0.144	0.886
Glu Thalamus	-0.08521	0.29636	-0.288	0.775
Glu PGS	-0.07021	0.20453	-0.343	0.733
Glu PGS^2	-0.11150	0.19644	-0.568	0.572
Site2	-0.10348	0.43048	-0.240	0.811
Site3	-0.04130	0.85433	-0.048	0.962
Sex	-0.26822	0.24742	-1.084	0.283
Age	0.10317	0.12948	0.797	0.429
Glu Thalamus:Glu PGS	0.12617	0.15573	0.810	0.421
Sex:Age	-0.10972	0.28872	-0.380	0.705
Residual standard error	0.8192 (on 62 degrees of freedom)			
Multiple R squared	0.05564			
Adjusted R squared	-0.08145			

Table S7: Continued

SRS ~ Glutamate ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.38775	0.10526	-3.684	0.000384
Glu ACC	**-0.66408**	**0.21226**	**-3.129**	**0.002339**
Glu PGS	-0.20440	0.14054	-1.454	0.149162
Glu PGS²	-0.18675	0.14550	-1.284	0.202470
Site2	**-1.71666**	**0.55914**	**-3.070**	**0.002797**
Site3	0.08032	0.31539	0.255	0.799546
Sex	0.13109	0.18555	0.707	0.481615
Age	-0.14264	0.10593	-1.347	0.181357
Glu ACC:Glu PGS	**0.28107**	**0.11126**	**2.526**	**0.013198**
Sex:Age	0.17845	0.22402	0.797	0.427702
Residual standard error	0.7755 (on 94 degrees of freedom)			
Multiple R squared	0.1703			
Adjusted R squared	0.09083			
SSP ~ Glutamate ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.44058	0.16002	2.753	0.00891
Glutamate ACC	0.19096	0.28386	0.673	0.50510
Glutamate PGS	0.29607	0.36165	0.819	0.41795
Glu PGS²	0.09452	0.31440	0.301	0.76529
Site2	0.56087	0.79937	0.702	0.48707
Site3	**-1.92418**	**0.82399**	**-2.335**	**0.02477**
Sex	0.21431	0.27337	0.784	0.43781
Age	0.20986	0.18119	1.158	0.25383
Glu ACC:Glu PGS	**-0.30229**	**0.14002**	**-2.159**	**0.03707**
Sex:Age	-0.09319	0.33849	-0.275	0.78454
Residual standard error	0.7661 (on 39 degrees of freedom)			
Multiple R squared	0.3236			
Adjusted R squared	0.1675			
ADOS ~ Glutamate ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.31841	0.12645	-2.518	0.01448
Glu ACC	**-0.68690**	**0.22210**	**-3.093**	**0.00301**
Glu PGS	0.25466	0.38654	0.659	0.51253
Glu PGS²	0.34074	0.31902	1.068	0.28976
Site2	**-1.39150**	**0.58305**	**-2.387**	**0.02017**
Site3	**0.83417**	**0.36928**	**2.259**	**0.02753**

SRS ~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.43996	0.14526	-3.029	0.00308
Glu Thalamus	-0.14442	0.28029	-0.515	0.60745
Glu PGS	-0.10771	0.15068	-0.715	0.47626
Glu PGS²	-0.07158	0.15904	-0.450	0.65354
Site2	0.23053	0.41775	0.552	0.58221
Site3	0.04058	0.36398	0.111	0.91144
Sex	0.10429	0.19618	0.532	0.59610
Age	-0.01706	0.11070	-0.154	0.87783
Glu Thalamus:Glu PGS	0.06754	0.13809	0.489	0.62577
Sex:Age	0.19543	0.23994	0.814	0.41717
Residual standard error	0.895 (on 107 degrees of freedom)			
Multiple R squared	0.05933			
Adjusted R squared	-0.01979			
SSP ~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.46542	0.21362	2.179	0.0344
Glutamate Thalamus	-0.21028	0.36128	-0.582	0.5633
Glutamate PGS	-0.01316	0.31652	-0.042	0.9670
Glu PGS²	-0.14477	0.28754	-0.503	0.6170
Site2	-0.50999	0.51472	-0.991	0.3269
Site3	**-2.00970**	**0.93134**	**-2.158**	**0.0361**
Sex	0.16594	0.28651	0.579	0.5652
Age	0.17115	0.18043	0.949	0.3477
Glu Thalamus:Glu PGS	-0.05405	0.17764	-0.304	0.7623
Sex:Age	-0.14276	0.35030	-0.408	0.6855
Residual standard error	0.8861 (on 47 degrees of freedom)			
Multiple R squared	0.1683			
Adjusted R squared	0.009009			
ADOS ~ Glutamate Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.15933	0.17107	-0.931	0.35467
Glu Thalamus	-0.55726	0.32931	-1.692	0.09475
Glu PGS	0.31398	0.22780	1.378	0.17220
Glu PGS²	0.39769	0.20889	1.904	0.06077
Site2	-0.17137	0.48708	-0.352	0.72595
Site3	**0.65572**	**0.38880**	**1.687**	**0.09585**

Table S7: Continued

Sex	**-0.61907**	**0.20836**	**-2.971**	**0.00426**
Age	**-0.26178**	**0.11812**	**-2.216**	**0.03048**
Glu ACC:Glu PGS	**0.24533**	**0.10217**	**2.401**	**0.01945**
Sex:Age	0.06137	0.26212	0.234	0.81568
Residual standard error	0.7035 (on 60 degrees of freedom)			
Multiple R squared	0.3917			
Adjusted R squared	0.3004			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Table S8: Linear model outputs ^{1}H-MRS GABA and glutamate PGS

ACC GABA & GLUTAMATE PGS				
RBS ~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.029093	0.138421	-0.210	0.834
GABA ACC	-0.346640	0.233125	-1.487	0.144
Glu PGS	0.067405	0.341267	0.198	0.844
Glu PGS^2	0.004802	0.311928	0.015	0.988
Site2	-0.474524	0.431677	-1.099	0.278
Site3	-0.401479	0.868891	-0.462	0.646
Sex	-0.256229	0.266152	-0.963	0.341
Age	0.002045	0.130672	0.016	0.988
GABA ACC:Glu PGS	0.279099	0.256631	1.088	0.283
Sex:Age	0.037277	0.307486	0.121	0.904
Residual standard error	0.7745 (on 45 degrees of freedom)			
Multiple R squared	0.09188			
Adjusted R squared	-0.08974			
SRS ~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.58210	0.10665	-5.458	4.78e-07
GABA ACC	-0.08747	0.16458	-0.531	0.596
Glu PGS	-0.21349	0.15627	-1.366	0.176
Glu PGS^2	-0.22931	0.16554	-1.385	0.170
Site2	-0.31205	0.35993	-0.867	0.388
Site3	0.12134	0.32148	0.377	0.707

Sex	**-0.65407**	**0.18818**	**-3.476**	**0.00085**
Age	-0.07163	0.10592	-0.676	0.50091
Glu Thalamus:Glu PGS	0.24899	0.12834	1.940	0.05614
Sex:Age	0.05796	0.23825	0.243	0.80845
Residual standard error	0.7366 (on 75 degrees of freedom)			
Multiple R squared	0.394			
Adjusted R squared	0.3213			

THALAMUS GABA & GLUTAMATE PGS				
RBS ~ GABA Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.006169	0.177258	0.035	0.972
GABA Thalamus	-0.167225	0.286289	-0.584	0.562
Glu PGS	-0.114804	0.238096	-0.482	0.632
Glu PGS²	-0.129849	0.250248	-0.519	0.606
Site2	-0.034824	0.394920	-0.088	0.930
Site3	-0.166152	0.996753	-0.167	0.868
Sex	-0.257305	0.320106	-0.804	0.426
Age	0.082894	0.161179	0.514	0.609
GABA Thalamus:Glu PGS	-0.007296	0.289922	-0.025	0.980
Sex:Age	-0.036584	0.368032	-0.099	0.921
Residual standard error	0.9358 (on 47 degrees of freedom)			
Multiple R squared	0.05345			
Adjusted R squared	-0.1278			
SRS ~ GABA Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.46850	0.12550	-3.733	0.000343
GABA Thalamus	0.19653	0.19688	0.998	0.321027
Glu PGS	-0.12683	0.19089	-0.664	0.508226
Glu PGS²	-0.16087	0.20432	-0.787	0.433300
Site2	**0.83145**	**0.29509**	**2.818**	**0.006031**
Site3	0.03193	0.36158	0.088	0.929834

Table S8: Continued

Sex	0.31967	0.19595	1.631	0.107
Age	-0.02485	0.10479	-0.237	0.813
GABA ACC:Glu PGS	-0.06408	0.15660	-0.409	0.683
Sex:Age	0.19827	0.23202	0.855	0.395
Residual standard error	0.7937 (on 84 degrees of freedom)			
Multiple R squared	0.06961			
Adjusted R squared	-0.03008			
SSP ~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.44114	0.14999	2.941	0.00603
GABA ACC	0.31094	0.24785	1.255	0.21872
Glu PGS	0.63494	0.45827	1.386	0.17548
Glu PGS^2	0.51465	0.38362	1.342	0.18918
Site2	0.89725	0.49368	1.817	0.07852
Site3	-1.40250	0.80682	-1.738	0.09177
Sex	0.11901	0.25050	0.475	0.63794
Age	0.08271	0.14543	0.569	0.57350
GABA ACC:Glu PGS	0.25977	0.27190	0.955	0.34654
Sex:Age	-0.21637	0.30262	-0.715	0.47979
Residual standard error	0.6676 (on 32 degrees of freedom)			
Multiple R squared	0.3551			
Adjusted R squared	0.1737			
ADOS ~ GABA ACC * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.58840	0.13435	-4.380	5.78e-05
GABA ACC	-0.21996	0.18787	-1.171	0.2470
Glu PGS	-0.22944	0.43697	-0.525	0.6018
Glu PGS^2	-0.02023	0.35263	-0.057	0.9545
Site2	0.17072	0.32963	0.518	0.6067
Site3	**0.95413**	**0.38240**	**2.495**	**0.0158**
Sex	**-0.45433**	**0.22124**	**-2.054**	**0.0451**
Age	-0.07514	0.11896	-0.632	0.5304
GABA ACC:Glu PGS	0.10263	0.22758	0.451	0.6539
Sex:Age	-0.04512	0.27451	-0.164	0.8701

Sex	0.13829	0.21750	0.636	0.526638
Age	-0.10385	0.11834	-0.878	0.382700
GABA Thalamus:Glu PGS	-0.20871	0.18619	-1.121	0.265511
Sex:Age	0.42116	0.26037	1.618	0.109511
Residual standard error	0.8801 (on 84 degrees of freedom)			
Multiple R squared	0.1211			
Adjusted R squared	0.0269			
SSP ~ GABA Thalamus * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.51085	0.16064	3.180	0.00308
GABA Thalamus	0.25444	0.25907	0.982	0.33277
Glu PGS	0.16454	0.24183	0.680	0.50072
Glu PGS^2	0.06269	0.23965	0.262	0.79518
Site2	-0.32335	0.36110	-0.895	0.37665
Site3	**-1.68911**	**0.78934**	**-2.140**	**0.03941**
Sex	-0.17159	0.26670	-0.643	0.52418
Age	0.22773	0.15797	1.442	0.15830
GABA Thalamus:Glu PGS	0.37811	0.26334	1.436	0.15992
Sex:Age	-0.38886	0.31930	-1.218	0.23143
Residual standard error	0.7244 (on 35 degrees of freedom)			
Multiple R squared	0.3364			
Adjusted R squared	0.1657			
ADOS ~ GABA Thalamus * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.41867	0.13431	-3.117	0.002821
GABA Thalamus	0.11401	0.19881	0.573	0.568513
Glu PGS	0.18845	0.24292	0.776	0.440994
Glu PGS^2	0.21579	0.22853	0.944	0.348909
Site2	**0.91088**	**0.25517**	**3.570**	**0.000718**
Site3	**0.86535**	**0.40144**	**2.156**	**0.035206**
Sex	**-0.53983**	**0.22448**	**-2.405**	**0.019340**
Age	-0.05454	0.11924	-0.457	0.649049
GABA Thalamus:Glu PGS	0.14561	0.19222	0.758	0.451754
Sex:Age	0.05878	0.27590	0.213	0.832028

Table S8: Continued

Residual standard error	0.7172 (on 52 degrees of freedom)
Multiple R squared	0.2207
Adjusted R squared	0.08578

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Table S9: Linear model outputs ^{1}H-MRS glutamate/GABA ratios and glutamate PGS

ACC GLUTAMATE/GABA RATIO & GLUTAMATE PGS				
RBS ~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.039318	0.188815	0.208	0.836
Glu/GABA ACC	1.207007	1.209774	0.998	0.324
Glu PGS	0.010388	0.444461	0.023	0.981
Glu PGS²	0.018278	0.416119	0.044	0.965
Site2	-0.033036	0.399497	-0.083	0.934
Site3	-9.625649	10.770084	-0.894	0.376
Sex	-0.195345	0.265264	-0.736	0.465
Age	-0.003943	0.136441	-0.029	0.977
Glu/GABA ACC:Glu PGS	-0.587683	0.732280	-0.803	0.426
Sex:Age	0.012591	0.304155	0.041	0.967
Residual standard error	0.7758 (on 47 degrees of freedom)			
Multiple R squared	0.04877			
Adjusted R squared	-0.1334			
SRS ~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.48906	0.11018	-4.439	2.64e-05
Glu/GABA ACC	0.21131	0.23607	0.895	0.373
Glu PGS	-0.16816	0.16106	-1.044	0.299
Glu PGS²	-0.13334	0.17454	-0.764	0.447
Site2	0.23797	0.33287	0.715	0.477
Site3	-0.26768	0.34725	-0.771	0.443
Sex	0.24750	0.19710	1.256	0.213
Age	0.01801	0.11442	0.157	0.875
Glu/GABA ACC:Glu PGS	0.01133	0.36609	0.031	0.975
Sex:Age	0.15901	0.23583	0.674	0.502

Residual standard error	0.7593 (on 59 degrees of freedom)
Multiple R squared	0.3188
Adjusted R squared	0.2149

THALAMUS GLUTAMATE/GABA RATIO & GLUTAMATE PGS				
RBS ~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.10757	0.29752	-0.362	0.719
Glu/GABA Thalamus	-0.65450	2.61072	-0.251	0.803
Glu PGS	0.77032	3.36012	0.229	0.820
Glu PGS²	-0.11947	0.21787	-0.548	0.586
Site2	0.04308	0.25464	0.169	0.866
Site3	-0.03028	0.90191	-0.034	0.973
Sex	-0.23693	0.28138	-0.842	0.403
Age	0.09219	0.14167	0.651	0.518
Glu/GABA Thalamus:Glu PGS	8.44856	32.45610	0.260	0.796
Sex:Age	-0.03771	0.33895	-0.111	0.912
Residual standard error	0.8663 (on 56 degrees of freedom)			
Multiple R squared	0.04116			
Adjusted R squared	-0.1129			
SRS ~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.7207	0.1727	-4.174	6.54e-05
Glu/GABA Thalamus	-1.8730	1.1450	-1.636	0.10511
Glu PGS	1.2558	0.6423	1.955	0.05345
Glu PGS²	-0.1441	0.1544	-0.934	0.35285
Site2	**0.6087**	**0.2122**	**2.868**	**0.00507**
Site3	0.2729	0.3697	0.738	0.46211
Sex	0.2187	0.1969	1.111	0.26950
Age	-0.0488	0.1070	-0.456	0.64948
Glu/GABA Thalamus:Glu PGS	**13.8936**	**6.1730**	**2.251**	**0.02666**
Sex:Age	0.3272	0.2421	1.351	0.17973

Table S9: Continued

Residual standard error	0.8045 (on 87 degrees of freedom)			
Multiple R squared	0.1135			
Adjusted R squared	0.02178			
SSP ~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.109294	0.228851	0.478	0.6359
Glu/GABA ACC	-3.183628	1.487154	-2.141	0.0393
Glu PGS	0.588080	0.590025	0.997	0.3257
Glu PGS²	0.065146	0.458355	0.142	0.8878
Site2	-0.515970	0.455171	-1.134	0.2647
Site3	14.249175	12.543825	1.136	0.2637
Sex	0.214775	0.285488	0.752	0.4569
Age	-0.003405	0.175536	-0.019	0.9846
Glu/GABA ACC:Glu PGS	2.820044	2.195412	1.285	0.2074
Sex:Age	0.127080	0.342765	0.371	0.7131
Residual standard error	0.7876 (on 35 degrees of freedom)			
Multiple R squared	0.3257			
Adjusted R squared	0.1523			
ADOS ~ Glutamate/GABA ratio ACC * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.4214	0.1529	-2.755	0.00789
Glu/GABA ACC	0.2535	0.6735	0.376	0.70805
Glu PGS	0.2759	0.4656	0.593	0.55588
Glu PGS²	0.4289	0.3735	1.148	0.25574
Site2	**0.6786**	**0.3266**	**2.078**	**0.04233**
Site3	0.8294	0.4706	1.762	0.08344
Sex	-0.4765	0.2524	-1.888	0.06418
Age	-0.1727	0.1410	-1.225	0.22565
Glu/GABA ACC:Glu PGS	-0.4064	1.1205	-0.363	0.71817
Sex:Age	0.1115	0.3046	0.366	0.71567
Residual standard error	0.7593 (on 56 degrees of freedom)			
Multiple R squared	0.2571			
Adjusted R squared	0.1377			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Residual standard error	0.85 (on 97 degrees of freedom)			
Multiple R squared	0.1412			
Adjusted R squared	0.0615			
SSP ~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.80807	0.30201	2.676	0.0106
Glu/GABA Thalamus	3.19982	2.52044	1.270	0.2112
Glu PGS	-3.29060	3.23847	-1.016	0.3154
Glu PGS²	0.03253	0.27210	0.120	0.9054
Site2	-0.30610	0.26519	-1.154	0.2549
Site3	**-1.88481**	**0.84731**	**-2.224**	**0.0315**
Sex	-0.12054	0.28523	-0.423	0.6747
Age	0.24595	0.16603	1.481	0.1460
Glu/GABA Thalamus:Glu PGS	-33.81191	31.47737	-1.074	0.2889
Sex:Age	-0.37247	0.35067	-1.062	0.2942
Residual standard error	0.8042 (on 42 degrees of freedom)			
Multiple R squared	0.2159			
Adjusted R squared	0.04783			
ADOS ~ Glutamate/GABA ratio Thalamus * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.47915	0.15412	-3.109	0.002741
Glu/GABA Thalamus	-0.16919	0.77221	-0.219	0.827233
Glu PGS	0.18786	0.27318	0.688	0.494007
Glu PGS²	0.27522	0.22307	1.234	0.221525
Site2	**0.75048**	**0.20245**	**3.707**	**0.000424**
Site3	**0.84055**	**0.39262**	**2.141**	**0.035872**
Sex	**-0.47245**	**0.20931**	**-2.257**	**0.027215**
Age	-0.02759	0.11538	-0.239	0.811730
Glu/GABA Thalamus:Glu PGS	-0.32523	1.29112	-0.252	0.801881
Sex:Age	0.05436	0.27544	0.197	0.844137
Residual standard error	0.7753 (on 68 degrees of freedom)			
Multiple R squared	0.286			
Adjusted R squared	0.1915			

Table S10: Linear model outputs ^{1}H-MRS glutamate and GABA PGS

ACC GLUTAMATE & GABA PGS				
RBS ~ Glutamate ACC * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.098390	0.136365	-0.722	0.474
Glu ACC	-0.084538	0.264648	-0.319	0.751
GABA PGS	-0.851298	1.294867	-0.657	0.514
GABA PGS^2	-0.766796	1.274291	-0.602	0.550
Site2	-0.388186	0.710804	-0.546	0.587
Site3	-0.000955	0.802096	-0.001	0.999
Sex	-0.138537	0.257109	-0.539	0.592
Age	-0.053262	0.135763	-0.392	0.696
Glu ACC:GABA PGS	0.013057	0.117699	0.111	0.912
Sex:Age	0.135146	0.311645	0.434	0.666
Residual standard error	0.7484 (on 51 degrees of freedom)			
Multiple R squared	0.0427			
Adjusted R squared	-0.1262			
SRS ~ Glutamate ACC * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.417584	0.108730	-3.841	0.000223
Glu ACC	**-0.654562**	**0.222641**	**-2.940**	**0.004131**
GABA PGS	0.141584	1.085383	0.130	0.896492
GABA PGS^2	0.107461	1.078030	0.100	0.920808
Site2	**-1.446589**	**0.574471**	**-2.518**	**0.013489**
Site3	0.181102	0.327529	0.553	0.581621
Sex	0.209955	0.190610	1.101	0.273497
Age	-0.088272	0.110478	-0.799	0.426307
Glu ACC:GABA PGS	-0.007441	0.106956	-0.070	0.944680
Sex:Age	0.129032	0.241530	0.534	0.594445
Residual standard error	0.8077 (on 94 degrees of freedom)			
Multiple R squared	0.0998			
Adjusted R squared	0.01361			
SSP ~ Glutamate ACC * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.43635	0.17180	2.540	0.0152
Glu ACC	0.03500	0.30367	0.115	0.9088
GABA PGS	2.62763	1.53340	1.714	0.0945
GABA PGS^2	2.59053	1.52202	1.702	0.0967

THALAMUS GLUTAMATE & GABA PGS				
RBS ~ Glutamate Thalamus * GABA PGS + GABA PGS² + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.03951	0.15157	-0.261	0.795
Glu Thalamus	-0.04780	0.27448	-0.174	0.862
GABA PGS	0.98425	1.06337	0.926	0.358
GABA PGS²	1.08873	1.05009	1.037	0.304
Site2	-0.13135	0.40052	-0.328	0.744
Site3	0.06754	0.81861	0.083	0.935
Sex	-0.29695	0.24106	-1.232	0.223
Age	0.07684	0.12409	0.619	0.538
Glu Thalamus:GABA PGS	0.16801	0.11591	1.450	0.152
Sex:Age	-0.17064	0.28059	-0.608	0.545
Residual standard error	0.7807 (on 62 degrees of freedom)			
Multiple R squared	0.1424			
Adjusted R squared	0.0179			
SRS ~ Glutamate Thalamus * GABA PGS + GABA PGS² + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.46266	0.14414	-3.210	0.00175
Glu Thalamus	-0.09083	0.27529	-0.330	0.74209
GABA PGS	0.33391	1.00704	0.332	0.74086
GABA PGS²	0.38141	0.99881	0.382	0.70332
Site2	0.28557	0.41290	0.692	0.49067
Site3	0.10648	0.36055	0.295	0.76831
Sex	0.10555	0.19359	0.545	0.58674
Age	-0.01714	0.11034	-0.155	0.87685
Glu Thalamus:GABA PGS	0.17196	0.11277	1.525	0.13024
Sex:Age	0.15074	0.24185	0.623	0.53442
Residual standard error	0.8842 (on 107 degrees of freedom)			
Multiple R squared	0.08195			
Adjusted R squared	0.004726			
SSP ~ Glutamate Thalamus * GABA PGS + GABA PGS² + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.43392	0.20567	2.110	0.0402
Glu Thalamus	-0.15990	0.33768	-0.474	0.6380
GABA PGS	2.22057	1.40134	1.585	0.1198
GABA PGS²	2.08466	1.39629	1.493	0.1421

Table S10: Continued

Site2	-0.26820	0.82379	-0.326	0.7465
Site3	**-2.22855**	**0.88122**	**-2.529**	**0.0156**
Sex	0.16613	0.29276	0.567	0.5737
Age	0.05270	0.18627	0.283	0.7787
Glu ACC:GABA PGS	-0.02358	0.13582	-0.174	0.8631
Sex:Age	-0.08257	0.37245	-0.222	0.8257
Residual standard error	0.8112 (on 39 degrees of freedom)			
Multiple R squared	0.2416			
Adjusted R squared	0.06662			
ADOS ~ Glutamate ACC * GABA PGS + GABA PGS² + Age * Sex + Sit				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.38675	0.12714	-3.042	0.00348
Glu ACC	**-0.57421**	**0.23627**	**-2.430**	**0.01809**
GABA PGS	-1.77801	1.30859	-1.359	0.17932
GABA PGS²	-1.67398	1.27914	-1.309	0.19563
Site2	-0.75214	0.59811	-1.258	0.21344
Site3	**0.89217**	**0.39186**	**2.277**	**0.02638**
Sex	**-0.57770**	**0.21806**	**-2.649**	**0.01030**
Age	-0.24937	0.12909	-1.932	0.05812
Glu ACC:GABA PGS	0.03329	0.10588	0.314	0.75431
Sex:Age	0.11067	0.28044	0.395	0.69452
Residual standard error	0.7592 (on 60 degrees of freedom)			
Multiple R squared	0.2917			
Adjusted R squared	0.1854			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Table S11: Linear model outputs ^{1}H-MRS GABA and GABA PGS

ACC GABA & GABA PGS				
RBS ~ GABA ACC * GABA PGS + GABA PGS² + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.07171	0.13661	-0.525	0.602
GABA ACC	-0.26120	0.22194	-1.177	0.245
GABA PGS	-1.51624	1.37691	-1.101	0.277
GABA PGS²	-1.47725	1.37746	-1.072	0.289

Site2	-0.48447	0.49046	-0.988	0.3283
Site3	**-2.15679**	**0.91122**	**-2.367**	**0.0221**
Sex	0.07809	0.28351	0.275	0.7842
Age	0.18139	0.18055	1.005	0.3202
Glu Thalamus:GABA PGS	-0.08492	0.16324	-0.520	0.6053
Sex:Age	-0.30882	0.35953	-0.859	0.3947
Residual standard error	0.8626 (on 47 degrees of freedom)			
Multiple R squared	0.2118			
Adjusted R squared	0.06085			

ADOS ~ Glutamate Thalamus * GABA PGS + GABA PGS² + Age + Sex * Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.23279	0.17474	-1.332	0.1868
Glu Thalamus	-0.55981	0.32866	-1.703	0.0926
GABA PGS	-1.66283	1.02209	-1.627	0.1080
GABA PGS²	-1.57836	1.00113	-1.577	0.1191
Site2	0.01959	0.49084	0.040	0.9683
Site3	0.56193	0.40822	1.377	0.1728
Sex	**-0.51587**	**0.19651**	**-2.625**	**0.0105**
Age	-0.08129	0.11122	-0.731	0.4671
Glu Thalamus:GABA PGS	0.01853	0.09996	0.185	0.8535
Sex:Age	0.20303	0.25059	0.810	0.4204
Residual standard error	0.7684 (on 75 degrees of freedom)			
Multiple R squared	0.3406			
Adjusted R squared	0.2615			

THALAMUS GABA & GABA PGS

RBS ~ GABA Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	Pr(>|t|)
(Intercept)	-0.009470	0.161527	-0.059	0.953
GABA Thalamus	0.023150	0.269918	0.086	0.932
GABA PGS	1.812234	1.407928	1.287	0.204
GABA PGS²	2.068648	1.395329	1.483	0.145

Table S11: Continued

Site2	-0.34791	0.41944	-0.829	0.411
Site3	0.14019	0.90866	0.154	0.878
Sex	-0.26863	0.28476	-0.943	0.351
Age	-0.04986	0.13459	-0.370	0.713
GABA ACC:GABA PGS	0.30200	0.21601	1.398	0.169
Sex:Age	0.06055	0.33034	0.183	0.855
Residual standard error	0.7688 (on 45 degrees of freedom)			
Multiple R squared	0.1053			
Adjusted R squared	-0.07363			
SRS ~ GABA ACC * GABA PGS + GABA PGS^2 + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.58455	0.10564	-5.534	3.49e-07
GABA ACC	-0.06688	0.16147	-0.414	0.6798
GABA PGS	-0.20327	1.10578	-0.184	0.8546
GABA PGS^2	-0.20632	1.10365	-0.187	0.8522
Site2	-0.26275	0.36118	-0.727	0.4690
Site3	0.05620	0.32966	0.170	0.8651
Sex	0.36827	0.19725	1.867	0.0654
Age	-0.01494	0.10700	-0.140	0.8893
GABA ACC:GABA PGS	-0.16871	0.16073	-1.050	0.2969
Sex:Age	0.22644	0.24358	0.930	0.3552
Residual standard error	0.7974 (on 84 degrees of freedom)			
Multiple R squared	0.06092			
Adjusted R squared	-0.0397			
SSP ~ GABA ACC * GABA PGS + GABA PGS^2 + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.43205	0.14955	2.889	0.00688
GABA ACC	0.25493	0.25906	0.984	0.33246
GABA PGS	0.94660	1.33527	0.709	0.48351
GABA PGS^2	0.86835	1.35599	0.640	0.52649
Site2	0.80589	0.49470	1.629	0.11311
Site3	-1.28454	0.82009	-1.566	0.12711
Sex	-0.07126	0.27471	-0.259	0.79700
Age	0.12453	0.15142	0.822	0.41693
GABA ACC:GABA PGS	0.28099	0.22781	1.233	0.22640
Sex:Age	-0.39073	0.32043	-1.219	0.23160
Residual standard error	0.6638 (on 32 degrees of freedom)			
Multiple R squared	0.3625			
Adjusted R squared	0.1832			

Site2	0.075905	0.362251	0.210	0.835
Site3	0.208161	0.929305	0.224	0.824
Sex	-0.348318	0.308975	-1.127	0.265
Age	-0.005015	0.148857	-0.034	0.973
GABA Thalamus:GABA PGS	0.218094	0.226474	0.963	0.340
Sex:Age	0.009398	0.358028	0.026	0.979
Residual standard error	0.8551 (on 47 degrees of freedom)			
Multiple R squared	0.2096			
Adjusted R squared	0.0582			

SRS ~ GABA Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.46474	0.12669	-3.668	0.000428
GABA Thalamus	0.18295	0.19745	0.927	0.356803
GABA PGS	1.37568	1.19752	1.149	0.253910
GABA PGS²	1.42988	1.18862	1.203	0.232367
Site2	**0.81919**	**0.29574**	**2.770**	**0.006899**
Site3	0.12256	0.36210	0.338	0.735856
Sex	0.11380	0.22172	0.513	0.609124
Age	-0.08294	0.11950	-0.694	0.489565
GABA Thalamus:GABA PGS	-0.09513	0.18414	-0.517	0.606773
Sex:Age	0.31699	0.27031	1.173	0.244235
Residual standard error	0.882 (on 84 degrees of freedom)			
Multiple R squared	0.1174			
Adjusted R squared	0.02285			

SSP ~ GABA Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.4831	0.1572	3.072	0.0041
GABA Thalamus	0.2729	0.2657	1.027	0.3115
GABA PGS	1.9949	1.5866	1.257	0.2170
GABA PGS²	1.7996	1.6252	1.107	0.2757
Site2	-0.3105	0.3519	-0.882	0.3836
Site3	**-1.8762**	**0.7861**	**-2.387**	**0.0225**
Sex	-0.2263	0.2762	-0.819	0.4182
Age	0.3055	0.1558	1.961	0.0578
GABA Thalamus:GABA PGS	0.1227	0.2271	0.540	0.5926
Sex:Age	-0.5671	0.3299	-1.719	0.0944
Residual standard error	0.7045 (on 35 degrees of freedom)			
Multiple R squared	0.3723			
Adjusted R squared	0.2109			

Table S11: Continued

ADOS ~ GABA ACC * GABA PGS + GABA PGS² + Age + Sex * Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.5528	0.1182	-4.678	2.1e-05
GABA ACC	-0.3267	0.1907	-1.714	0.0925
GABA PGS	**-3.2619**	**1.3692**	**-2.382**	**0.0209**
GABA PGS²	**-3.1424**	**1.3435**	**-2.339**	**0.0232**
Site2	0.1532	0.3174	0.483	0.6313
Site3	**0.8795**	**0.3723**	**2.362**	**0.0219**
Sex	-0.3968	0.2116	-1.875	0.0663
Age	-0.1395	0.1165	-1.198	0.2364
GABA ACC:GABA PGS	0.1117	0.1821	0.614	0.5422
Sex:Age	0.1214	0.2631	0.462	0.6463
Residual standard error	0.6987 (on 52 degrees of freedom)			
Multiple R squared	0.2604			
Adjusted R squared	0.1324			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Table S12: Linear model outputs ^{1}H-MRS glutamate/GABA ratios and GABA PGS

ACC GLUTAMATE/GABA RATIO & GABA PGS				
RBS ~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS² + Age * Sex + Sit				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.03956	0.14305	-0.277	0.783
Glu/GABA ACC	0.72397	0.75127	0.964	0.340
GABA PGS	-0.93541	1.22195	-0.766	0.448
GABA PGS²	-0.67031	1.20324	-0.557	0.580
Site2	-0.11978	0.37335	-0.321	0.750
Site3	16.56623	13.36896	1.239	0.221
Sex	-0.14863	0.26772	-0.555	0.581
Age	-0.03632	0.13581	-0.267	0.790
Glu/GABA ACC:GABA PGS	-1.65187	1.07699	-1.534	0.132
Sex:Age	0.09742	0.31748	0.307	0.760
Residual standard error	0.7607 (on 47 degrees of freedom)			
Multiple R squared	0.08541			
Adjusted R squared	-0.08972			

ADOS ~ GABA Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.42827	0.13495	-3.174	0.002392
GABA Thalamus	0.11596	0.20129	0.576	0.566728
GABA PGS	-0.59424	1.22462	-0.485	0.629299
GABA PGS²	-0.54430	1.20453	-0.452	0.653014
Site2	**0.93488**	**0.25841**	**3.618**	**0.000617**
Site3	0.72000	0.39235	1.835	0.071534
Sex	**-0.49347**	**0.23937**	**-2.062**	**0.043662**
Age	-0.07199	0.12164	-0.592	0.556247
GABA Thalamus:GABA PGS	0.04527	0.17627	0.257	0.798202
Sex:Age	0.19514	0.27894	0.700	0.486949
Residual standard error	0.7671 (on 59 degrees of freedom)			
Multiple R squared	0.3046			
Adjusted R squared	0.1985			

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THALAMUS GLUTAMATE/GABA RATIO & GABA PGS				
RBS ~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	3.84531	2.81096	1.368	0.177
Glu/GABA Thalamus	37.51861	27.04164	1.387	0.171
GABA PGS	5.35030	3.26693	1.638	0.107
GABA PGS²	1.24381	1.17935	1.055	0.296
Site2	-0.14415	0.25317	-0.569	0.571
Site3	0.09539	0.86137	0.111	0.912
Sex	-0.20847	0.27929	-0.746	0.459
Age	0.07455	0.13311	0.560	0.578
Glu/GABA Thalamus:GABA PGS	41.76261	30.03897	1.390	0.170
Sex:Age	0.06769	0.34760	0.195	0.846
Residual standard error	0.8218 (on 56 degrees of freedom)			
Multiple R squared	0.1371			
Adjusted R squared	-0.001589			

Table S12: Continued

SRS ~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS² + Age * Sex + Sit				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.39897	0.12606	-3.165	0.00214
Glu/GABA ACC	1.21406	0.77149	1.574	0.11920
GABA PGS	-0.61496	1.04496	-0.589	0.55772
GABA PGS2	-0.52210	1.04128	-0.501	0.61735
Site2	0.29446	0.32605	0.903	0.36897
Site3	-0.22910	0.35048	-0.654	0.51505
Sex	0.30454	0.19653	1.550	0.12487
Age	0.02617	0.11411	0.229	0.81913
Glu/GABA ACC:GABA PGS	-0.75355	0.58098	-1.297	0.19805
Sex:Age	0.16410	0.24310	0.675	0.50144
Residual standard error	0.8004 (on 87 degrees of freedom)			
Multiple R squared	0.1224			
Adjusted R squared	0.03159			
SSP ~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS² + Age * Sex + Sit				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.04744	0.30755	-0.154	0.8783
Glu/GABA ACC	-4.08870	2.30159	-1.776	0.0843
GABA PGS	1.44441	1.48887	0.970	0.3386
GABA PGS²	2.50158	1.30581	1.916	0.0636
Site2	-0.71157	0.43139	-1.649	0.1080
Site3	167.36629	122.00325	1.372	0.1789
Sex	0.13417	0.29445	0.456	0.6514
Age	0.02351	0.17397	0.135	0.8933
Glu/GABA ACC:GABA PGS	-8.30356	6.61627	-1.255	0.2178
Sex:Age	-0.07419	0.35420	-0.209	0.8353
Residual standard error	0.7702 (on 35 degrees of freedom)			
Multiple R squared	0.3551			
Adjusted R squared	0.1892			
ADOS ~ Glutamate/GABA ratio ACC * GABA PGS + GABA PGS² + Age * Sex + Sit				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.4526	0.1511	-2.996	0.00407
Glu/GABA ACC	0.3673	0.8560	0.429	0.66950
GABA PGS	-1.8126	1.4230	-1.274	0.20800
GABA PGS²	-1.6717	1.3913	-1.202	0.23459
Site2	**0.8188**	**0.3178**	**2.576**	**0.01265**

SRS ~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.09895	0.34755	0.285	0.77647
Glu/GABA Thalamus	6.10516	3.29821	1.851	0.06720
GABA PGS	0.94465	1.05942	0.892	0.37478
GABA PGS²	0.22246	1.00572	0.221	0.82541
Site2	**0.58605**	**0.21652**	**2.707**	**0.00803**
Site3	0.29052	0.37015	0.785	0.43444
Sex	0.22718	0.19931	1.140	0.25715
Age	-0.03939	0.10888	-0.362	0.71833
Glu/GABA Thalamus:GABA PGS	**7.70866**	**3.65629**	**2.108**	**0.03758**
Sex:Age	0.32869	0.24947	1.318	0.19075
Residual standard error	0.8555 (on 97 degrees of freedom)			
Multiple R squared	0.1302			
Adjusted R squared	0.04945			
SSP ~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.2175	2.8751	-0.076	0.9401
Glu/GABA Thalamus	-6.7052	27.5113	-0.244	0.8086
GABA PGS	1.7771	3.5383	0.502	0.6181
GABA PGS²	2.4712	1.3183	1.874	0.0678
Site2	-0.4027	0.2728	-1.476	0.1474
Site3	**-2.1194**	**0.8133**	**-2.606**	**0.0126**
Sex	-0.2307	0.2771	-0.833	0.4097
Age	0.2745	0.1579	1.738	0.0895
Glu/GABA Thalamus:GABA PGS	-8.4843	30.5389	-0.278	0.7825
Sex:Age	-0.5504	0.3581	-1.537	0.1319
Residual standard error	0.7688 (on 42 degrees of freedom)			
Multiple R squared	0.2834			
Adjusted R squared	0.1298			
ADOS ~ Glutamate/GABA ratio Thalamus * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.08674	0.25577	0.339	0.7355
Glu/GABA Thalamus	6.07228	2.43215	2.497	0.0150
GABA PGS	-1.04866	1.08286	-0.968	0.3363
GABA PGS²	-1.75107	1.04729	-1.672	0.0991
Site2	**0.88474**	**0.19533**	**4.529**	**2.45e-05**

Table S12: Continued

Site3	0.7795	0.4752	1.641	0.10649
Sex	-0.4528	0.2485	-1.822	0.07381
Age	-0.2060	0.1432	-1.438	0.15598
Glu/GABA ACC:GABA PGS	-0.2588	0.6429	-0.403	0.68875
Sex:Age	0.1820	0.3047	0.597	0.55272
Residual standard error	0.8045 (on 56 degrees of freedom)			
Multiple R squared	0.2397			
Adjusted R squared	0.1176			

Glu, Glutamate; GABA, γ-aminobutyric acid; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Table S13: Linear model outputs creatine referenced ^{1}H-MRS glutamate and glutamate PGS

ACC GLUTAMATE(cr) & GLUTAMATE PGS				
RBS ~ Glutamate ACC(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.37850	0.56091	-0.675	0.503
Glu ACC(cr)	-1.59631	1.63133	-0.979	0.332
Glu PGS	-0.21454	0.24939	-0.860	0.394
Glu PGS^2	-0.14332	0.19515	-0.734	0.466
Site2	0.35319	0.88410	0.399	0.691
Site3	0.40268	0.43395	0.928	0.358
Sex	-0.22719	0.25146	-0.903	0.371
Age	-0.06496	0.37514	-0.173	0.863
Glu ACC(cr):Glu PGS	-0.78465	1.45421	-0.540	0.592
Sex:Age	0.01556	0.29445	0.053	0.958
Residual standard error	0.7467 (on 51 degrees of freedom)			
Multiple R squared	0.04718			
Adjusted R squared	-0.121			
SRS ~ Glutamate ACC(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.94319	0.41300	-2.284	0.0246
Glu ACC(cr)	-1.06995	1.23806	-0.864	0.3897
Glu PGS	-0.06062	0.15740	-0.385	0.7010
Glu PGS^2	-0.18030	0.15843	-1.138	0.2580
Site2	0.26901	0.42258	0.637	0.5259
Site3	0.23265	0.31334	0.742	0.4596

Site3	**0.75960**	**0.37775**	**2.011**	**0.0483**
Sex	-0.30512	0.20230	-1.508	0.1361
Age	-0.04120	0.11202	-0.368	0.7142
Glu/GABA Thalamus:GABA PGS	**7.78175**	**3.12015**	**2.494**	**0.0151**
Sex:Age	0.17523	0.26557	0.660	0.5116
Residual standard error	0.7428 (on 68 degrees of freedom)			
Multiple R squared	0.3446			
Adjusted R squared	0.2579			

THALAMUS GLUTAMATE(cr) & GLUTAMATE PGS				
RBS ~ Glutamate Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.09407	0.44909	-0.209	0.835
Glu Thalamus(cr)	-0.79827	0.70866	-1.126	0.264
Glu PGS	-0.01741	0.20136	-0.086	0.931
Glu PGS²	-0.07630	0.19157	-0.398	0.692
Site2	0.18057	0.85807	0.210	0.834
Site3	0.34509	0.33897	1.018	0.313
Sex	-0.27365	0.24504	-1.117	0.268
Age	0.14277	0.36759	0.388	0.699
Glu Thalamus(cr):Glu PGS	0.77816	0.49316	1.578	0.120
Sex:Age	-0.09802	0.28481	-0.344	0.732
Residual standard error	0.8007 (on 61 degrees of freedom)			
Multiple R squared	0.1115			
Adjusted R squared	-0.01958			
SRS ~ Glutamate Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.77829	0.35688	-2.181	0.03141
Glu **Thalamus(cr)**	**-1.74989**	**0.59742**	**-2.929**	**0.00416**
Glu PGS	-0.07968	0.14348	-0.555	0.57984
Glu PGS²	-0.02080	0.15431	-0.135	0.89301
Site2	0.20394	0.41369	0.493	0.62306
Site3	0.28508	0.29832	0.956	0.34145

Table S13: Continued

Sex	0.14831	0.19547	0.759	0.4499
Age	-0.26155	0.30342	-0.862	0.3909
Glu ACC(cr):Glu PGS	2.20430	1.20619	1.827	0.0708
Sex:Age	0.17034	0.23616	0.721	0.4725
Residual standard error	0.8171 (on 94 degrees of freedom)			
Multiple R squared	0.07872			
Adjusted R squared	-0.009488			
SSP ~ Glutamate ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.05966	0.63131	0.094	0.9252
Glu ACC(cr)	-0.26099	1.88059	-0.139	0.8903
Glu PGS	0.19477	0.42179	0.462	0.6468
Glu PGS²	0.09297	0.33259	0.280	0.7813
Site2	-1.89572	0.95929	-1.976	0.0552
Site3	0.13236	0.49100	0.270	0.7889
Sex	0.23877	0.28655	0.833	0.4098
Age	0.11618	0.47091	0.247	0.8064
Glu ACC(cr):Glu PGS	-1.79385	1.66553	-1.077	0.2881
Sex:Age	-0.01203	0.35024	-0.034	0.9728
Residual standard error	0.8014 (on 39 degrees of freedom)			
Multiple R squared	0.2598			
Adjusted R squared	0.08902			
ADOS ~ Glutamate ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.37083	0.44208	0.839	0.4049
Glu ACC(cr)	-0.61882	1.39568	-0.443	0.6591
Glu PGS	0.29788	0.43634	0.683	0.4974
Glu PGS²	0.30203	0.34422	0.877	0.3838
Site2	0.64027	0.49404	1.296	0.1999
Site3	-0.23308	0.33236	-0.701	0.4858
Sex	**-0.60214**	**0.22800**	**-2.641**	**0.0105**
Age	-0.20609	0.36283	-0.568	0.5722
Glu ACC(cr):Glu PGS	1.73441	1.29657	1.338	0.1860
Sex:Age	0.02818	0.28670	0.098	0.9220
Residual standard error	0.7686 (on 60 degrees of freedom)			
Multiple R squared	0.274			
Adjusted R squared	0.1651			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Sex	0.08772	0.18839	0.466	0.64243
Age	-0.28321	0.29505	-0.960	0.33930
Glu Thalamus(cr):Glu PGS	0.44614	0.42487	1.050	0.29608
Sex:Age	0.18662	0.22913	0.814	0.41720
Residual standard error	0.8537 (on 106 degrees of freedom)			
Multiple R squared	0.1389			
Adjusted R squared	0.06576			

SSP ~ Glutamate Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.59060	0.53155	1.111	0.2723
Glu Thalamus(cr)	1.93404	1.06402	1.818	0.0756
Glu PGS	-0.05103	0.30489	-0.167	0.8678
Glu PGS²	-0.24889	0.27704	-0.898	0.3737
Site2	**-2.15766**	**0.93895**	**-2.298**	**0.0262**
Site3	-0.46998	0.44036	-1.067	0.2914
Sex	0.17960	0.27612	0.650	0.5187
Age	0.25403	0.45276	0.561	0.5775
Glu Thalamus(cr):Glu PGS	-0.45016	0.55483	-0.811	0.4213
Sex:Age	-0.06079	0.33821	-0.180	0.8581
Residual standard error	0.8445 (on 46 degrees of freedom)			
Multiple R squared	0.2504			
Adjusted R squared	0.1038			

ADOS ~ Glutamate Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.78937	0.36355	2.171	0.033073
Glu Thalamus(cr)	-0.19613	0.64591	-0.304	0.762237
Glu PGS	0.36939	0.23045	1.603	0.113164
Glu PGS²	0.35346	0.21142	1.672	0.098714
Site2	0.34829	0.41342	0.842	0.402215
Site3	-0.48190	0.30084	-1.602	0.113396
Sex	**-0.65538**	**0.18948**	**-3.459**	**0.000898**
Age	-0.09380	0.30280	-0.310	0.757597
Glu Thalamus(cr):Glu PGS	**1.03568**	**0.42072**	**2.462**	**0.016128**
Sex:Age	0.03253	0.24007	0.136	0.892570
Residual standard error	0.7392 (on 75 degrees of freedom)			
Multiple R squared	0.3897			
Adjusted R squared	0.3164			

Table S14: Linear model outputs creatine referenced ^{1}H-MRS GABA and glutamate PGS

ACC GABA(cr) & GLUTAMATE PGS				
RBS ~ GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.224017	0.562600	-0.398	0.692
GABA ACC(cr)	-2.853016	2.010090	-1.419	0.163
Glu PGS	0.358887	0.526676	0.681	0.499
Glu PGS²	0.056390	0.321770	0.175	0.862
Site2	-0.064946	0.884146	-0.073	0.942
Site3	0.241335	0.395740	0.610	0.545
Sex	-0.285854	0.268543	-1.064	0.293
Age	0.005929	0.382979	0.015	0.988
GABA ACC(cr):Glu PGS	2.903374	2.473108	1.174	0.247
Sex:Age	0.004651	0.310202	0.015	0.988
Residual standard error	0.775 (on 45 degrees of freedom)			
Multiple R squared	0.09077			
Adjusted R squared	-0.09108			
SRS ~ GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-1.2904	0.4272	-3.021	0.00334
GABA ACC(cr)	-1.1863	1.5052	-0.788	0.43284
Glu PGS	-0.2747	0.2078	-1.322	0.18982
Glu PGS²	-0.2203	0.1603	-1.375	0.17286
Site2	0.3650	0.4416	0.827	0.41074
Site3	0.2775	0.3397	0.817	0.41632
Sex	0.3249	0.1954	1.663	0.10012
Age	-0.2218	0.2931	-0.757	0.45146
GABA ACC(cr):Glu PGS	-0.7711	1.3847	-0.557	0.57909
Sex:Age	0.2020	0.2310	0.874	0.38434
Residual standard error	0.7905 (on 84 degrees of freedom)			
Multiple R squared	0.07709			
Adjusted R squared	-0.02179			
SSP ~ GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.4948	0.6297	2.374	0.02377
GABA ACC(cr)	3.3644	2.0650	1.629	0.11307
Glu PGS	0.8587	0.5379	1.596	0.12023
Glu PGS²	0.5411	0.3813	1.419	0.16558

THALAMUS GABA(cr) & GLUTAMATE PGS				
RBS ~ GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.317795	0.467677	0.680	0.500
GABA Thalamus(cr)	-0.115270	0.328920	-0.350	0.728
Glu PGS	-0.111892	0.223042	-0.502	0.618
Glu PGS²	-0.109762	0.250783	-0.438	0.664
Site2	-0.126807	1.020568	-0.124	0.902
Site3	-0.079528	0.336476	-0.236	0.814
Sex	-0.252051	0.320200	-0.787	0.435
Age	0.098442	0.470229	0.209	0.835
GABA Thalamus(cr):Glu PGS	-0.176824	0.428474	-0.413	0.682
Sex:Age	-0.009142	0.373715	-0.024	0.981
Residual standard error	0.9356 (on 47 degrees of freedom)			
Multiple R squared	0.05372			
Adjusted R squared	-0.1275			
SRS ~ GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.3020	0.3602	0.839	0.40405
GABA Thalamus(cr)	**0.4714**	**0.2328**	**2.024**	**0.04611**
Glu PGS	-0.1714	0.1868	-0.918	0.36124
Glu PGS²	-0.1691	0.2028	-0.834	0.40671
Site2	-0.8144	0.4167	-1.954	0.05398
Site3	**-0.8191**	**0.2625**	**-3.121**	**0.00247**
Sex	0.1182	0.2117	0.559	0.57798
Age	-0.5449	0.3311	-1.645	0.10361
GABA Thalamus(cr):Glu PGS	-0.2067	0.2679	-0.772	0.44244
Sex:Age	0.4355	0.2583	1.686	0.09554
Residual standard error	0.8698 (on 84 degrees of freedom)			
Multiple R squared	0.1415			
Adjusted R squared	0.04952			
SSP ~ GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.08656	0.41675	0.208	0.8367
GABA Thalamus(cr)	0.05171	0.30543	0.169	0.8665
Glu PGS	0.19384	0.25174	0.770	0.4465
Glu PGS²	0.07795	0.25775	0.302	0.7641

Table S14: Continued

Site2	**-2.2622**	**0.7913**	**-2.859**	**0.00742**
Site3	-0.8780	0.4599	-1.909	0.06526
Sex	0.1202	0.2518	0.477	0.63649
Age	0.3207	0.3920	0.818	0.41931
GABA ACC(cr):Glu PGS	2.0868	2.6072	0.800	0.42938
Sex:Age	-0.2445	0.3050	-0.802	0.42862
Residual standard error	0.6582 (on 32 degrees of freedom)			
Multiple R squared	0.3732			
Adjusted R squared	0.1969			
ADOS ~ GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.076676	0.412154	-0.186	0.853
GABA ACC(cr)	-1.690662	1.694015	-0.998	0.323
Glu PGS	-0.578992	0.539880	-1.072	0.288
Glu PGS²	-0.144200	0.363196	-0.397	0.693
Site2	0.669041	0.449816	1.487	0.143
Site3	-0.273399	0.298662	-0.915	0.364
Sex	-0.430556	0.222099	-1.939	0.058
Age	0.006721	0.347932	0.019	0.985
GABA ACC(cr):Glu PGS	-2.417390	2.583862	-0.936	0.354
Sex:Age	-0.048997	0.273519	-0.179	0.859
Residual standard error	0.7137 (on 52 degrees of freedom)			
Multiple R squared	0.2283			
Adjusted R squared	0.09475			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Table S15: Linear model outputs creatine referenced ^{1}H-MRS glutamate/GABA ratio and glutamate PGS

ACC GLUTAMATE/GABA(cr) RATIO & GLUTAMATE PGS				
RBS ~ Glutamate/GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.362685	0.524121	0.692	0.492
Glu/GABA(cr) ACC	2.730957	1.648849	1.656	0.104
GluPGS	-0.117570	0.442853	-0.265	0.792
GluPGS²	-0.060028	0.411685	-0.146	0.885

Site2	-1.38973	0.84067	-1.653	0.1073
Site3	0.55782	0.31213	1.787	0.0826
Sex	-0.07718	0.27696	-0.279	0.7821
Age	0.54834	0.44278	1.238	0.2238
GABA Thalamus(cr):Glu PGS	-0.04614	0.38388	-0.120	0.9050
Sex:Age	-0.30084	0.33485	-0.898	0.3751
Residual standard error	0.7531 (on 35 degrees of freedom)			
Multiple R squared	0.2828			
Adjusted R squared	0.09841			
ADOS ~ GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.01657	0.33938	2.995	0.004002
GABA Thalamus(cr)	0.22677	0.21884	1.036	0.304321
Glu PGS	0.16238	0.24514	0.662	0.510309
Glu PGS^2	0.21240	0.23660	0.898	0.372980
Site2	-0.24085	0.45612	-0.528	0.599457
Site3	**-0.89753**	**0.22079**	**-4.065**	**0.000144**
Sex	**-0.51467**	**0.21276**	**-2.419**	**0.018662**
Age	-0.17519	0.34759	-0.504	0.616126
GABA Thalamus(cr):Glu PGS	0.04532	0.25742	0.176	0.860858
Sex:Age	0.11520	0.27324	0.422	0.674844
Residual standard error	0.7574 (on 59 degrees of freedom)			
Multiple R squared	0.3222			
Adjusted R squared	0.2188			

THALAMUS GLUTAMATE/GABA(cr) RATIO & GLUTAMATE PGS				
RBS ~ Glutamate/GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.14338	0.76975	-0.186	0.853
Glu/GABA(cr) Thalamus	-3.82837	6.42770	-0.596	0.554
GluPGS	4.35605	7.41985	0.587	0.560
GluPGS^2	-0.09172	0.22302	-0.411	0.682

Table S15: Continued

Site2	-24.274627	15.480602	-1.568	0.124
Site3	0.012403	0.393356	0.032	0.975
Sex	-0.190874	0.259717	-0.735	0.466
Age	0.032100	0.377554	0.085	0.933
Glu/GABA(cr) ACC:Glu PGS	-0.940686	0.808073	-1.164	0.250
Sex:Age	-0.009745	0.299047	-0.033	0.974
Residual standard error	0.7622 (on 47 degrees of freedom)			
Multiple R squared	0.08189			
Adjusted R squared	-0.09392			
SRS ~ Glutamate/GABA ACC(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.4824	0.3928	-1.228	0.223
Glu/GABA(cr) ACC	0.2340	0.2610	0.897	0.372
GluPGS	-0.1600	0.1602	-0.999	0.321
GluPGS^2	-0.1248	0.1745	-0.715	0.476
Site2	-0.5158	0.4586	-1.125	0.264
Site3	-0.2475	0.3334	-0.742	0.460
Sex	0.2454	0.1972	1.244	0.217
Age	-0.1467	0.3065	-0.479	0.633
Glu/GABA(cr) ACC:Glu PGS	-0.0300	0.4104	-0.073	0.942
Sex:Age	0.1621	0.2360	0.687	0.494
Residual standard error	0.8052 (on 87 degrees of freedom)			
Multiple R squared	0.1118			
Adjusted R squared	0.01987			
SSP ~ Glutamate/GABA ACC(cr) * Glutamate PGS + Glutamate PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.78202	0.64404	-1.214	0.233
Glu/GABA(cr) ACC	-4.50219	2.77093	-1.625	0.113
GluPGS	0.13955	0.71149	0.196	0.846
GluPGS^2	0.06633	0.46463	0.143	0.887
Site2	54.31873	54.89255	0.990	0.329
Site3	0.51644	0.46147	1.119	0.271
Sex	0.23057	0.28783	0.801	0.429
Age	-0.07201	0.46345	-0.155	0.877
Glu/GABA(cr) ACC:Glu PGS	-0.82218	4.37532	-0.188	0.852
Sex:Age	0.08105	0.34887	0.232	0.818
Residual standard error	0.7956 (on 35 degrees of freedom)			
Multiple R squared	0.3118			
Adjusted R squared	0.1349			

Site2	-0.05568	0.92775	-0.060	0.952
Site3	-0.05225	0.25338	-0.206	0.837
Sex	-0.19173	0.29695	-0.646	0.521
Age	0.10002	0.42520	0.235	0.815
Glu/GABA(cr) Thalamus:Glu PGS	49.78655	82.80662	0.601	0.550
Sex:Age	-0.01182	0.34073	-0.035	0.972
Residual standard error	0.8717 (on 55 degrees of freedom)			
Multiple R squared	0.04531			
Adjusted R squared	-0.1109			

SRS ~ Glutamate/GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.3447	0.3230	-1.067	0.28859
Glu/GABA(cr) Thalamus	-2.6203	1.3987	-1.873	0.06406
GluPGS	**1.9144**	**0.9594**	**1.995**	**0.04883**
GluPGS²	-0.1304	0.1547	-0.843	0.40135
Site2	-0.3292	0.4031	-0.817	0.41615
Site3	**-0.6055**	**0.2123**	**-2.853**	**0.00531**
Sex	0.2043	0.1976	1.034	0.30367
Age	-0.4069	0.3069	-1.326	0.18800
Glu/GABA(cr) Thalamus:Glu PGS	**23.4559**	**10.7495**	**2.182**	**0.03155**
Sex:Age	0.3396	0.2420	1.403	0.16380
Residual standard error	0.848 (on 96 degrees of freedom)			
Multiple R squared	0.1387			
Adjusted R squared	0.05792			

SSP ~ Glutamate/GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.91548	0.78764	1.162	0.252
Glu/GABA(cr) Thalamus	6.93802	6.59623	1.052	0.299
GluPGS	-7.12096	7.59188	-0.938	0.354
GluPGS²	-0.01324	0.27351	-0.048	0.962
Site2	-1.56277	0.86438	-1.808	0.078
Site3	0.32029	0.26074	1.228	0.226
Sex	-0.14888	0.29854	-0.499	0.621
Age	0.67099	0.45392	1.478	0.147
Glu/GABA(cr) Thalamus:Glu PGS	-81.71833	85.04074	-0.961	0.342
Sex:Age	-0.39627	0.34678	-1.143	0.260
Residual standard error	0.7995 (on 41 degrees of freedom)			
Multiple R squared	0.2304			
Adjusted R squared	0.06142			

Table S15: Continued

ADOS ~ Glutamate/GABA ACC(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.7444	0.4184	1.779	0.0807
Glu/GABA(cr) ACC	0.3212	0.7056	0.455	0.6507
GluPGS	0.2721	0.4647	0.585	0.5606
GluPGS²	0.4338	0.3735	1.162	0.2503
Site2	0.1439	0.5502	0.262	0.7946
Site3	**-0.6875**	**0.3277**	**-2.098**	**0.0405**
Sex	-0.4744	0.2520	-1.883	0.0650
Age	-0.2819	0.3901	-0.723	0.4728
Glu/GABA(cr) ACC:Glu PGS	-0.5182	1.1722	-0.442	0.6601
Sex:Age	0.1104	0.3043	0.363	0.7181
Residual standard error	0.7948 (on 56 degrees of freedom)			
Multiple R squared	0.2579			
Adjusted R squared	0.1387			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Table S16: Linear model outputs creatine referenced ^{1}H-MRS glutamate and GABA PGS

ACC GLUTAMATE(cr) & GABA PGS				
RBS ~ Glutamate ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.4088	0.5406	-0.756	0.453
Glu ACC(cr)	-1.0073	1.4986	-0.672	0.505
GABA PGS	-0.7567	1.2172	-0.622	0.537
GABA PGS²	-0.6809	1.2159	-0.560	0.578
Site2	0.4020	0.8658	0.464	0.644
Site3	0.3764	0.4077	0.923	0.360
Sex	-0.1441	0.2565	-0.562	0.577
Age	-0.2026	0.3905	-0.519	0.606
Glu ACC(cr): GABA PGS	0.1255	1.2532	0.100	0.921
Sex:Age	0.1299	0.3111	0.418	0.678
Residual standard error	0.7456 (on 51 degrees of freedom)			
Multiple R squared	0.04978			
Adjusted R squared	-0.1179			

ADOS ~ Glutamate/GABA Thalamus(cr) * Glutamate PGS + Glutamate PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.72524	0.30660	2.365	0.020868
Glu/GABA(cr) Thalamus	-0.40344	0.86175	-0.468	0.641167
GluPGS	0.15562	0.27427	0.567	0.572306
GluPGS²	0.27197	0.22290	1.220	0.226627
Site2	0.08396	0.41562	0.202	0.840518
Site3	**-0.76044**	**0.20276**	**-3.751**	**0.000367**
Sex	**-0.46573**	**0.20942**	**-2.224**	**0.029477**
Age	-0.06662	0.34158	-0.195	0.845942
Glu/GABA(cr) Thalamus:Glu PGS	-0.71734	1.44076	-0.498	0.620170
Sex:Age	0.03844	0.27572	0.139	0.889530
Residual standard error	0.7743 (on 68 degrees of freedom)			
Multiple R squared	0.2879			
Adjusted R squared	0.1937			

THALAMUS GLUTAMATE(cr) & GABA PGS				
RBS ~ Glutamate Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.09877	0.42003	-0.235	0.8149
Glu Thalamus(cr)	-0.59123	0.66997	-0.882	0.3810
GABA PGS	0.24168	1.06335	0.227	0.8210
GABA PGS²	0.28340	1.05771	0.268	0.7897
Site2	0.26782	0.80578	0.332	0.7407
Site3	0.30756	0.31085	0.989	0.3264
Sex	-0.25235	0.23354	-1.081	0.2841
Age	0.10804	0.35006	0.309	0.7587
Glu Thalamus(cr):GABA PGS	**0.78395**	**0.31230**	**2.510**	**0.0147**
Sex:Age	-0.10772	0.27072	-0.398	0.6921
Residual standard error	0.7485 (on 61 degrees of freedom)			
Multiple R squared	0.2236			
Adjusted R squared	0.1091			

Table S16: Continued

SRS ~ Glutamate ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-1.02638	0.42098	-2.438	0.0166
Glu ACC(cr)	-1.58050	1.23242	-1.282	0.2028
GABA PGS	-0.08211	1.09108	-0.075	0.9402
GABA PGS²	-0.15033	1.09633	-0.137	0.8912
Site2	0.22805	0.42559	0.536	0.5933
Site3	0.16683	0.31366	0.532	0.5961
Sex	0.22280	0.19854	1.122	0.2646
Age	-0.22416	0.32176	-0.697	0.4877
Glu ACC(cr): GABA PGS	0.62760	1.17454	0.534	0.5944
Sex:Age	0.15867	0.25291	0.627	0.5319
Residual standard error	0.8353 (on 94 degrees of freedom)			
Multiple R squared	0.03725			
Adjusted R squared	-0.05492			
SSP ~ Glutamate ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.2231	0.6148	-0.363	0.7186
Glu ACC(cr)	-0.8533	1.7653	-0.483	0.6315
GABA PGS	2.8205	1.4245	1.980	0.0548
GABA PGS²	2.8217	1.4358	1.965	0.0565
Site2	-1.7321	0.9461	-1.831	0.0748
Site3	0.4965	0.4749	1.046	0.3022
Sex	0.1347	0.2948	0.457	0.6502
Age	0.1357	0.4919	0.276	0.7842
Glu ACC(cr): GABA PGS	-0.7615	1.4543	-0.524	0.6035
Sex:Age	-0.1092	0.3729	-0.293	0.7712
Residual standard error	0.8073 (on 39 degrees of freedom)			
Multiple R squared	0.2488			
Adjusted R squared	0.07544			
ADOS ~ Glutamate ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.54149	0.44982	1.204	0.2334
Glu ACC(cr)	-0.55596	1.33226	-0.417	0.6779
GABA PGS	-1.69609	1.36585	-1.242	0.2192
GABA PGS²	-1.54133	1.33697	-1.153	0.2535
Site2	0.38445	0.49353	0.779	0.4390

SRS ~ Glutamate Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.7667	0.3557	-2.156	0.0334
Glu Thalamus(cr)	-1.5923	0.6062	-2.627	0.0099
GABA PGS	-0.2106	0.9953	-0.212	0.8328
GABA PGS²	-0.1685	0.9939	-0.170	0.8657
Site2	0.1803	0.4065	0.443	0.6583
Site3	0.2091	0.2971	0.704	0.4830
Sex	0.1345	0.1860	0.723	0.4712
Age	-0.3335	0.2992	-1.115	0.2675
Glu Thalamus(cr):GABA PGS	0.4525	0.3074	1.472	0.1440
Sex:Age	0.2226	0.2308	0.964	0.3370
Residual standard error	0.8475 (on 106 degrees of freedom)			
Multiple R squared	0.1513			
Adjusted R squared	0.07924			
SSP ~ Glutamate Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.77625	0.51104	1.519	0.1356
Glu Thalamus(cr)	**2.41725**	**1.07434**	**2.250**	**0.0293**
GABA PGS	**2.96901**	**1.36519**	**2.175**	**0.0348**
GABA PGS²	**2.80962**	**1.37038**	**2.050**	**0.0461**
Site2	-2.43109	0.91905	-2.645	0.0111
Site3	-0.56356	0.43407	-1.298	0.2006
Sex	0.05606	0.26941	0.208	0.8361
Age	0.45494	0.46629	0.976	0.3343
Glu Thalamus(cr):GABA PGS	-0.55597	0.57479	-0.967	0.3385
Sex:Age	-0.26594	0.34460	-0.772	0.4442
Residual standard error	0.8152 (on 46 degrees of freedom)			
Multiple R squared	0.3016			
Adjusted R squared	0.1649			
ADOS ~ Glutamate Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.5978	0.3778	1.582	0.1178
Glu Thalamus(cr)	-0.6595	0.6425	-1.026	0.3080
GABA PGS	-1.8376	1.0533	-1.745	0.0852
GABA PGS²	-1.7817	1.0377	-1.717	0.0901
Site2	0.1221	0.4284	0.285	0.7764

Table S16: Continued

Site3	-0.50829	0.32720	-1.553	0.1256
Sex	**-0.54495**	**0.22854**	**-2.384**	**0.0203**
Age	-0.26610	0.38076	-0.699	0.4873
Glu ACC(cr): GABA PGS	-0.47370	1.24176	-0.381	0.7042
Sex:Age	0.07941	0.30100	0.264	0.7928
Residual standard error	0.8052 (on 60 degrees of freedom)			
Multiple R squared	0.2246			
Adjusted R squared	0.1083			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Table S17: Linear model outputs creatine referenced ^{1}H-MRS GABA and GABA PGS

ACC GABA(cr) & GABA PGS				
RBS ~ GABA ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.13852	0.57307	-0.242	0.810
GABA ACC(cr)	-2.21546	1.91522	-1.157	0.253
GABA PGS	-1.02005	1.31691	-0.775	0.443
GABA PGS²	-1.20181	1.33482	-0.900	0.373
Site2	0.32666	0.91172	0.358	0.722
Site3	0.13834	0.39571	0.350	0.728
Sex	-0.27753	0.28527	-0.973	0.336
Age	-0.05212	0.40998	-0.127	0.899
GABA ACC(cr): GABA PGS	2.71073	1.95484	1.387	0.172
Sex:Age	0.01513	0.33763	0.045	0.964
Residual standard error	0.7705 (on 45 degrees of freedom)			
Multiple R squared	0.1012			
Adjusted R squared	-0.07852			
SRS ~ GABA ACC(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-1.2850	0.4348	-2.955	0.00405
GABA ACC(cr)	-1.1462	1.4959	-0.766	0.44567
GABA PGS	-0.4017	1.0877	-0.369	0.71284
GABA PGS²	-0.3247	1.0911	-0.298	0.76674
Site2	0.3125	0.4487	0.697	0.48796
Site3	0.2475	0.3447	0.718	0.47474

Site3	-0.5361	0.3092	-1.734	0.0871
Sex	**-0.4936**	**0.2002**	**-2.465**	**0.0160**
Age	-0.2628	0.3208	-0.819	0.4152
Glu Thalamus(cr):GABA PGS	0.1308	0.2813	0.465	0.6433
Sex:Age	0.1819	0.2525	0.720	0.4736
Residual standard error	0.7758 (on 75 degrees of freedom)			
Multiple R squared	0.3277			
Adjusted R squared	0.2471			

THALAMUS GABA(cr) & GABA PGS				
RBS ~ GABA Thalamus(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.36379	0.44391	0.820	0.417
GABA Thalamus(cr)	0.04877	0.30544	0.160	0.874
GABA PGS	2.00828	1.41232	1.422	0.162
GABA PGS^2	2.29000	1.39701	1.639	0.108
Site2	-0.01179	0.94982	-0.012	0.990
Site3	-0.08263	0.30508	-0.271	0.788
Sex	-0.27729	0.31604	-0.877	0.385
Age	-0.04963	0.45365	-0.109	0.913
GABA Thalamus(cr):GABA PGS	-0.01231	0.35650	-0.035	0.973
Sex:Age	0.04450	0.36240	0.123	0.903
Residual standard error	0.8633 (on 47 degrees of freedom)			
Multiple R squared	0.1943			
Adjusted R squared	0.04006			
SRS ~ GABA Thalamus(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.2807	0.3581	0.784	0.43530
GABA Thalamus(cr)	0.4459	0.2280	1.956	0.05383
GABA PGS	1.3625	1.1719	1.163	0.24828
GABA PGS^2	1.4539	1.1630	1.250	0.21472
Site2	-0.7643	0.4146	-1.843	0.06881
Site3	**-0.8109**	**0.2596**	**-3.124**	**0.00245**

Table S17: Continued

Sex	0.3564	0.1966	1.813	0.07347
Age	-0.2333	0.3082	-0.757	0.45120
GABA ACC(cr): GABA PGS	-1.0069	1.4744	-0.683	0.49654
Sex:Age	0.2176	0.2441	0.891	0.37523
Residual standard error	0.7976 (on 84 degrees of freedom)			
Multiple R squared	0.06054			
Adjusted R squared	-0.04011			
SSP ~ GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.61980	0.66402	2.439	0.0204
GABA ACC(cr)	2.46323	2.19669	1.121	0.2705
GABA PGS	1.23732	1.25466	0.986	0.3314
GABA PGS^2	0.93848	1.29046	0.727	0.4724
Site2	**-2.13122**	**0.82099**	**-2.596**	**0.0141**
Site3	-0.89971	0.48653	-1.849	0.0737
Sex	-0.08258	0.28115	-0.294	0.7709
Age	0.57425	0.42630	1.347	0.1874
GABA ACC(cr): GABA PGS	2.70185	2.21834	1.218	0.2321
Sex:Age	-0.44884	0.33208	-1.352	0.1860
Residual standard error	0.6573 (on 32 degrees of freedom)			
Multiple R squared	0.375			
Adjusted R squared	0.1992			
ADOS ~ GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.02681	0.40051	-0.067	0.9469
GABA ACC(cr)	-2.34316	1.76643	-1.326	0.1905
GABA PGS	**-2.94274**	**1.36953**	**-2.149**	**0.0363**
GABA PGS^2	**-2.87843**	**1.33640**	**-2.154**	**0.0359**
Site2	0.45921	0.44263	1.037	0.3043
Site3	-0.36691	0.29401	-1.248	0.2176
Sex	-0.37774	0.21227	-1.779	0.0810
Age	-0.27671	0.33961	-0.815	0.4189
GABA ACC(cr): GABA PGS	0.66551	1.70654	0.390	0.6981
Sex:Age	0.14073	0.26558	0.530	0.5984
Residual standard error	0.7069 (on 52 degrees of freedom)			
Multiple R squared	0.2428			
Adjusted R squared	0.1118			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, significant results ($p<0.05$) are **marked in bold**.

Sex	0.1238	0.2149	0.576	0.56619
Age	-0.4371	0.3386	-1.291	0.20028
GABA Thalamus(cr):GABA PGS	-0.2308	0.2802	-0.824	0.41235
Sex:Age	0.3441	0.2653	1.297	0.19813
Residual standard error	0.865 (on 84 degrees of freedom)			
Multiple R squared	0.151			
Adjusted R squared	0.06001			
SSP ~ GABA Thalamus(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.15835	0.41566	0.381	0.7055
GABA Thalamus(cr)	0.04432	0.29626	0.150	0.8819
GABA PGS	1.57488	1.62800	0.967	0.3400
GABA PGS^2	1.31946	1.67991	0.785	0.4375
Site2	-1.61628	0.80121	-2.017	0.0514
Site3	0.54296	0.29046	1.869	0.0700
Sex	-0.19449	0.27551	-0.706	0.4849
Age	0.84325	0.43465	1.940	0.0605
GABA Thalamus(cr):GABA PGS	0.22843	0.33857	0.675	0.5043
Sex:Age	-0.54292	0.33014	-1.644	0.1090
Residual standard error	0.7118 (on 35 degrees of freedom)			
Multiple R squared	0.3594			
Adjusted R squared	0.1946			
ADOS ~ GABA Thalamus(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.000537	0.338552	2.955	0.004483
GABA Thalamus(cr)	0.264098	0.227481	1.161	0.250331
GABA PGS	-0.779533	1.228295	-0.635	0.528113
GABA PGS^2	-0.710971	1.210523	-0.587	0.559226
Site2	-0.353242	0.468762	-0.754	0.454109
Site3	**-0.914000**	**0.228818**	**-3.994**	**0.000182**
Sex	**-0.475388**	**0.226514**	**-2.099**	**0.040132**
Age	-0.315075	0.352677	-0.893	0.375282
GABA Thalamus(cr):GABA PGS	0.008064	0.229448	0.035	0.972084
Sex:Age	0.236483	0.277150	0.853	0.396964
Residual standard error	0.7604 (on 59 degrees of freedom)			
Multiple R squared	0.3167			
Adjusted R squared	0.2124			

Table S18: Linear model outputs creatine referenced ^{1}H-MRS glutamate/GABA ratio and GABA PGS

ACC GLUTAMATE/GABA(cr) RATIO & GABA PGS				
RBS ~ Glutamate/GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.38352	0.48288	0.794	0.43105
Glu/GABA(cr) ACC	**3.62940**	**1.23535**	**2.938**	**0.00511**
GABA PGS	-1.22347	1.12763	-1.085	0.28346
GABA PGS^2	-0.56309	1.10481	-0.510	0.61266
Site2	**40.39689**	**15.55927**	**2.596**	**0.01254**
Site3	0.02029	0.34519	0.059	0.95338
Sex	-0.15676	0.24617	-0.637	0.52734
Age	-0.04039	0.36799	-0.110	0.91308
Glu/GABA(cr) ACC:GABA PGS	-5.41621	1.63167	-3.319	0.00175
Sex:Age	0.03273	0.29285	0.112	0.91150
Residual standard error	0.7005 (on 47 degrees of freedom)			
Multiple R squared	0.2244			
Adjusted R squared	0.07591			
SRS ~ Glutamate/GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.2595	0.4068	-0.638	0.5252
Glu/GABA(cr) ACC	2.4638	1.2639	1.949	0.0545
GABA PGS	-0.7037	1.0360	-0.679	0.4988
GABA PGS^2	-0.5082	1.0314	-0.493	0.6234
Site2	-0.5341	0.4480	-1.192	0.2364
Site3	-0.3203	0.3239	-0.989	0.3254
Sex	0.3075	0.1948	1.578	0.1181
Age	-0.1118	0.3118	-0.358	0.7209
Glu/GABA(cr) ACC:GABA PGS	-1.6913	0.9495	-1.781	0.0784
Sex:Age	0.1497	0.2414	0.620	0.5370
Residual standard error	0.7943 (on 87 degrees of freedom)			
Multiple R squared	0.1356			
Adjusted R squared	0.0462			
SSP ~ Glutamate/GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-0.98220	0.61866	-1.588	0.1214
Glu/GABA(cr) ACC	-4.43341	2.23425	-1.984	0.0551
GABA PGS	1.59532	1.59216	1.002	0.3232
GABA PGS^2	2.41421	1.31170	1.841	0.0742

THALAMUS GLUTAMATE/GABA(cr) RATIO & GABA PGS				
RBS ~ Glutamate/GABA Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	5.83946	3.86408	1.511	0.136
Glu/GABA(cr) Thalamus	64.48703	43.86759	1.470	0.147
GABA PGS	7.59027	4.56958	1.661	0.102
GABA PGS²	1.35538	1.18611	1.143	0.258
Site2	0.27848	0.88560	0.314	0.754
Site3	0.07607	0.24554	0.310	0.758
Sex	-0.19419	0.28302	-0.686	0.496
Age	0.01848	0.41974	0.044	0.965
Glu/GABA(cr) Thalamus:GABA PGS	71.95760	48.90423	1.471	0.147
Sex:Age	0.04716	0.34107	0.138	0.891
Residual standard error	0.8253 (on 55 degrees of freedom)			
Multiple R squared	0.1441			
Adjusted R squared	0.00409			
SRS ~ Glutamate/GABA Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.9009	0.5587	1.612	0.11017
Glu/GABA(cr) Thalamus	**11.7094**	**5.5958**	**2.093**	**0.03903**
GABA PGS	1.3700	1.1210	1.222	0.22467
GABA PGS²	0.2076	0.9972	0.208	0.83553
Site2	-0.3006	0.4035	-0.745	0.45815
Site3	**-0.5837**	**0.2152**	**-2.712**	**0.00792**
Sex	0.2154	0.1983	1.086	0.28003
Age	-0.4292	0.3162	-1.357	0.17789
Glu/GABA(cr) Thalamus:GABA PGS	**14.0061**	**6.2422**	**2.244**	**0.02714**
Sex:Age	0.3623	0.2488	1.456	0.14857
Residual standard error	0.8485 (on 96 degrees of freedom)			
Multiple R squared	0.1377			
Adjusted R squared	0.05684			
SSP ~ Glutamate/GABA Thalamus(cr) * GABA PGS + GABA PGS² + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	-3.8224	3.7773	-1.012	0.3175
Glu/GABA(cr) Thalamus	-46.1832	42.6246	-1.083	0.2849
GABA PGS	-2.2408	4.6559	-0.481	0.6329
GABA PGS²	2.2153	1.2952	1.710	0.0948

Table S18: Continued

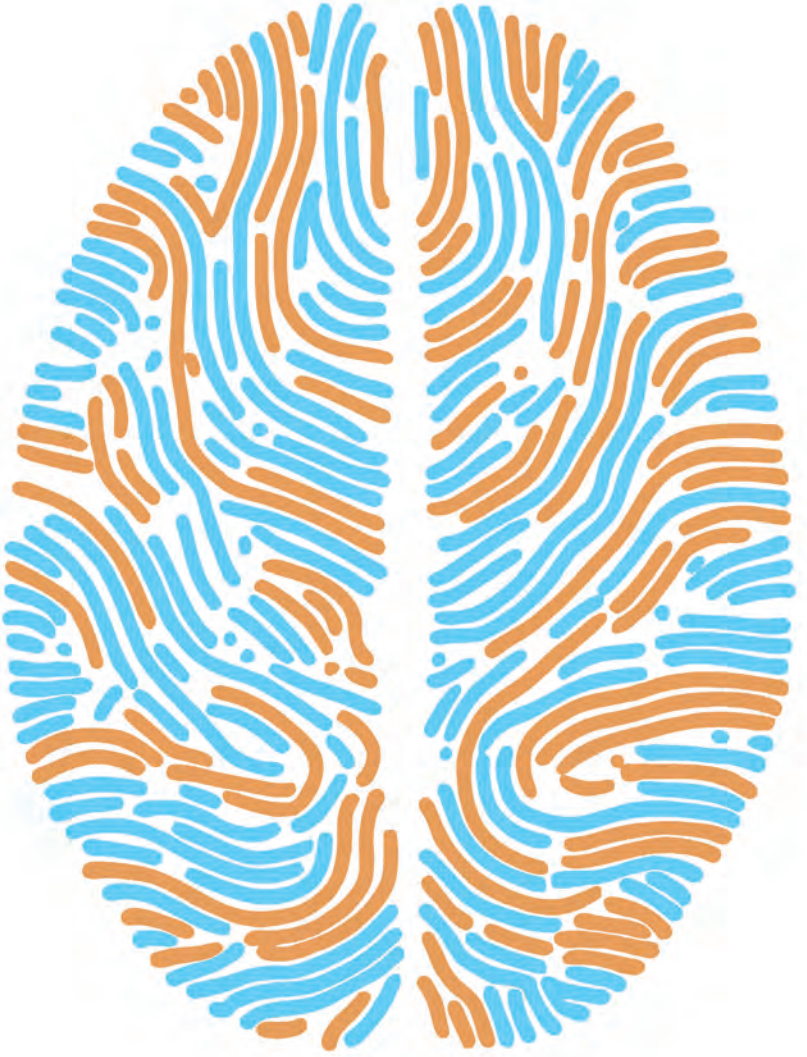
Site2	148.43174	153.90075	0.964	0.3414
Site3	0.72613	0.43489	1.670	0.1039
Sex	0.18210	0.29704	0.613	0.5438
Age	0.06905	0.47595	0.145	0.8855
Glu/GABA(cr) ACC:GABA PGS	-6.69848	9.55842	-0.701	0.4881
Sex:Age	-0.05640	0.36263	-0.156	0.8773
Residual standard error	0.7672 (on 35 degrees of freedom)			
Multiple R squared	0.3602			
Adjusted R squared	0.1956			
ADOS ~ Glutamate/GABA ACC(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site				
Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	0.9506	0.4473	2.125	0.0380
Glu/GABA(cr) ACC	1.5604	1.5963	0.978	0.3325
GABA PGS	-1.8364	1.4074	-1.305	0.1973
GABA PGS^2	-1.6018	1.3767	-1.163	0.2496
Site2	-0.0483	0.5395	-0.090	0.9290
Site3	**-0.8409**	**0.3164**	**-2.658**	**0.0102**
Sex	-0.4431	0.2463	-1.799	0.0774
Age	-0.3517	0.3910	-0.899	0.3723
Glu/GABA(cr) ACC:GABA PGS	-1.1521	1.1967	-0.963	0.3398
Sex:Age	0.1635	0.3027	0.540	0.5911
Residual standard error	0.799 (on 56 degrees of freedom)			
Multiple R squared	0.2501			
Adjusted R squared	0.1296			

Glu, Glutamate; GABA, γ-aminobutyric acid; (cr), creatine referenced ^{1}H-MRS; ACC, anterior cingulate cortex; PGS, Polygenic score; Glu/GABA, ratio of glutamate/GABA. ":" indicates the model estimation for interaction effects between variables. Covariates are labeled in gray, results discussed in manuscript are labeled in blue, significant results ($p<0.05$) are **marked in bold**.

Site2	**-1.7479**	**0.8140**	**-2.147**	**0.0377**
Site3	0.3632	0.2522	1.440	0.1575
Sex	-0.2355	0.2713	-0.868	0.3905
Age	**1.0079**	**0.4429**	**2.276**	**0.0282**
Glu/GABA(cr) Thalamus:GABA PGS	-52.5662	47.5101	-1.106	0.2750
Sex:Age	-0.6765	0.3402	-1.988	0.0535
Residual standard error	0.7463 (on 41 degrees of freedom)			
Multiple R squared	0.3294			
Adjusted R squared	0.1822			

ADOS ~ Glutamate/GABA Thalamus(cr) * GABA PGS + GABA PGS^2 + Age * Sex + Site

Coefficients:	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.4877	0.4222	3.524	0.000766
Glu/GABA(cr) Thalamus	**9.2548**	**3.7549**	**2.465**	**0.016243**
GABA PGS	-0.7887	1.1066	-0.713	0.478432
GABA PGS^2	-1.7492	1.0485	-1.668	0.099858
Site2	-0.1280	0.4033	-0.317	0.751871
Site3	**-0.9005**	**0.1967**	**-4.577**	**2.06e-05**
Sex	-0.3015	0.2028	-1.487	0.141756
Age	-0.1984	0.3348	-0.593	0.555388
Glu/GABA(cr) Thalamus:GABA PGS	**11.8642**	**4.8173**	**2.463**	**0.016321**
Sex:Age	0.1533	0.2665	0.575	0.566968
Residual standard error	0.7436 (on 68 degrees of freedom)			
Multiple R squared	0.3432			
Adjusted R squared	0.2563			



Chapter 6

Discussion

Summary

The aim of this thesis was to disentangle part of the complex relationships between brain and behavior underlying autism using a dimensional and multimodal approach. To do this, we took advantage of large multicenter cohorts with autistic and neurotypical participants who were deeply phenotyped, genotyped and whose brains were scanned using various neuroimaging modalities. The results showed that there are differing alterations of excitation and inhibition that link to various behavioral traits of autism, functional activity during inhibitory control, and brain structure differences throughout development.

In **Chapter 1** I introduced autism and the current state of understanding its biological etiology, focusing on the excitatory/inhibitory (E/I) imbalance theory. I showed that the heterogeneity within autism, combined with the so far mainly inconsistent and categorical approaches that have been used for investigating E/I imbalance in autism, has led to a lack of deeper understanding of underlying mechanisms. I introduced the nuanced nature of E/I imbalance and argued for the need of using dimensional and multimodal approaches to capture variations in E/I imbalances to unravel the brain differences that may underlie different autism characteristics.

In **Chapter 2** I investigated longitudinal changes in glutamate concentrations in ACC and striatum, and explored their associations with repetitive behaviors and brain activity during inhibitory control in the TACTICS cohort (1). This chapter included participants with OCD as well but given the focus of this thesis I will here highlight the results regarding the autism group. I found a larger decrease of ACC glutamate in autistic compared to neurotypical participants, while increased repetitive behaviors were also associated with decreased ACC glutamate. Additionally, increased compulsive behaviors were associated with increased functional activity in striatum during failed inhibitory control. These results show that through development, E/I mechanisms, here captured by glutamate concentrations and functional brain activity, affect autistic adolescents and traits associated with autism in distinct ways.

In **Chapter 3** I introduced the LEAP cohort (2) and glutamate and GABA gene-sets, consisting of genes encoding for proteins involved in glutamate and GABA neurotransmitter communication pathways in the brain. Aggregated genetic variation of glutamate genes was associated with all Autism Diagnostic Interview (ADI-R, (3)) and Autism Diagnostic Observation Schedule-2 (ADOS-2, (4)) subscales,

while the GABA genetic variation had a trend significant association with sensory processing. This established a clear distinction between E/I mechanisms underlying sensory processing differences in autism in contrast with social and restricted and repetitive behaviors captured by the ADI-R and ADOS-2. I applied gene-expression analysis utilizing gene expression data from the Allen Human Brain Atlas (AHBA (5)) to correlate cortical thickness differences between autistic and neurotypical participants with gene expression of the glutamate and GABA genes. I found significant correlations between both gene-sets in adolescents and adults, but in opposite directions. The gene-expression findings were replicated in the independent ABIDE cohort (6), although the correlation in the adult group was in the opposite direction compared to the LEAP cohort. This indicates differences in cortical thickness alterations in the autistic and/or neurotypical groups across these datasets, while still showing strong effects of glutamate and GABA gene expression for these differences. These results suggest that glutamate and GABA genes have underlying effects on cortical thickness differences in autism, but that the effects of these may differ throughout development.

In **Chapter 4** I built on the findings in Chapter 2 and 3 using the LEAP and TACTICS datasets from both chapters, here applying Bayesian Constraint based Causal Discovery (BCCD) to investigate causal relationships between functional activity during inhibitory control, polygenic scores for autism in the glutamate and GABA gene-sets and behavioral measures of autism traits. Additionally, here I was able to capture the links between glutamate genes to behavioral traits measured by the ADI-R in the LEAP cohort, this time using both a different analysis method and a different genetic measure compared to chapter 3. We attempted to replicate this gene to behavior result using a third independent dataset, the Simon Simplex Collection (SSC (7)). However here we did not replicate these findings, which is discussed in more detail below.

In **Chapter 5** I combined genetic and *in vivo* estimates of glutamate and GABA in the brain by combining glutamate and GABA gene markers and *in vivo* ^{1}H-MRS measures of glutamate and GABA concentrations simultaneously. The purpose was to understand both how genetic and ^{1}H-MRS markers are associated with each other, and how they together affect behavioral characteristics of autism. Aggregated genetic variation of glutamate genes was associated with GABA concentrations in the thalamus, and vice versa, genetic variation of GABA genes was associated with glutamate concentrations in the same region. This shows that links between neurotransmitter gene-sets and their measured ^{1}H-MRS concentrations are not direct, and that glutamate and GABA genetic mechanisms interact with metabolite

concentrations. There were also interactions between thalamic glutamate/GABA ratios and GABA PGS, which were associated with both social-communicative behaviors (Social Responsiveness Scale-Revised, SRS-R (8)) and with core clinical autism characteristics (ADOS-2). Thalamus glutamate/GABA ratios also showed interaction effects with glutamate PGS and were associated with SRS-R scores. Additionally, there were interactions between ACC glutamate concentrations and glutamate PGS on social-communicative (SRS-2), sensory processing (Short Sensory Profile, SSP (9)) and core clinical autism characteristics (ADOS-2). These findings suggest that genetic and metabolic aspects of glutamatergic and GABAergic processes in the brain interact to affect behavioral autism characteristics.

Collectively, ACC glutamate concentration differences in autism are associated with repetitive behaviors (Chapter 2) and with variation in brain structure (Chapter 3). Moreover, polygenic scores for glutamate appear to drive these differences in ACC glutamate concentrations (Chapter 4), which links genetic information to metabolite concentrations in autism for the first time. There are also complex interplays between genetic and ^{1}H-MRS markers of excitation and inhibition that are linked to behavioral autism characteristics (Chapter 5). An overview of the main findings that span across data modalities can be seen in Figure 1. What emerges from these results is that sensory processing differences appears to have differing underlying mechanisms compared to social and repetitive behaviors (Chapters 3, 4 and 5). While these discoveries are not enough to clearly distinguish specific alterations in E/I mechanisms that underlie specific behavioral characteristic of autism, they are a valuable first step to investigate these associations in more detail. These findings also show the importance of including *both* glutamate and GABA measures in investigations of E/I mechanisms in a multimodal fashion, as much of the behavior relies on the interplay between them. It is also important to take age and developmental trajectories into account, as these seem to interact and have strong effects on findings, which in turn will help explain heterogeneities and developmental differences across autistic individuals. Ultimately, the findings of this thesis argue for the urgent need of continuing to use dimensional and multimodal approaches to really disentangle the biological etiologies of the heterogeneities of autism.

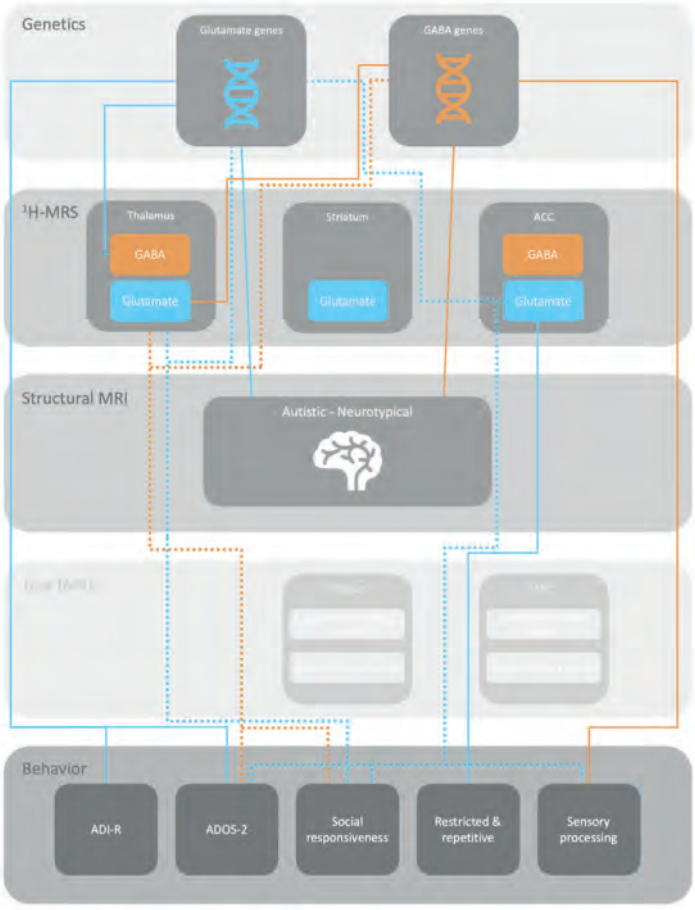


Figure 1. Overview of results

Significant associations across modalities, blue lines are associations with glutamate measures, orange lines are associated with GABA measures, interaction effects are outlined in dotted lines for emphasis. From top left to right, the results show associations between glutamate genes with behaviors captured by ADI-R and ADOS-2 (Chapter 3). Glutamate genes are also associated with ^{1}H-MRS measured GABA concentrations in thalamus (Chapter 5). There are also interaction effects between glutamate genes and glutamate/GABA ratios in the thalamus with social responsiveness behaviors (Chapter 5). Glutamate genes also interact with ACC glutamate concentrations on sensory processing and restricted & repetitive behaviors, and ADOS-2 (Chapter 5). GABA genes are associated with ^{1}H-MRS glutamate concentrations in the thalamus (Chapter 5), and with sensory processing behaviors (Chapter 3). GABA genes also interact with glutamate/GABA ratios in the thalamus affecting ADOS-2 and social responsiveness behaviors (Chapter 5). Both glutamate and GABA genes affect structural MRI captured cortical thickness differences between autistic and neurotypical participants (Chapter 3). Decreased ACC glutamate is associated with increased repetitive behaviors (Chapter 2). Behavioral measures were, from the left; ADI-R, autism diagnostic interview-revised; ADOS-2, autism diagnostic observation schedule-2; social responsiveness, SRS-2; restricted & repetitive, RBS-R; sensory processing, SSP. Note that the results in this thesis, as illustrated in this figure, do not cover every single finding within this thesis, as only results spanning across data modalities are included here.

So, what kind of imbalance?

As stated in Chapter 1, the E/I imbalance theory and previous work aiming to disentangle the imbalances underlying autism have shown convincing evidence for both overexcitation and overinhibition in autism (10,11). Here the aim was to disentangle whether different measures of these imbalances underlie different autism traits. This is indeed what the findings support, which is illustrated in the overview in Figure 1.

Concluding that there are varying E/I imbalances underlying distinct autism traits may beg the question of what the utility of the E/I framework really is as it can be used to explain over/under excitation, over/under inhibition, and/or differences between individuals and brain regions, to a degree where it starts to touch on the problem of demarcation (12). The way the E/I imbalance theory is often discussed in studies is not to try to test its validity or specificity but is rather arbitrarily slapped on as an explanatory framework regardless of whether results were expected or not, and regardless of directionality (10,11,13–15). I therefore believe that the E/I imbalance theory should be regarded not so much as a theory, but rather as a specific framework from which further, better testable hypotheses, can be formulated.

The findings within this thesis show that different aspects of glutamatergic and GABAergic mechanisms link to brain and behavior traits of autism in distinct ways. Further, these findings are better understood through the lens of a dimensional approach to the E/I imbalance theory (13). Understanding that there are homeostatic mechanisms of E/I systems, and that initial imbalances may have compensatory effects across the brain, is important for interpreting these results. Additionally, knowing that genes that are associated with autism and involved in excitatory and inhibitory mechanisms in the brain, also have differing effects throughout development (16) further explains the findings in this thesis, particularly in Chapters 2 and 4.

I would also argue that the E/I framework reinforces the importance of looking at multiple metabolites at once, as focusing on just one is not conducive to truly increase mechanistic insight or identifying useful biomarkers. One metabolite or neurotransmitter will not be enough to explain E/I imbalance heterogeneity in autism. For example, the glutamate genes to ADI-R associations (Chapters 3 and 4) do not indicate that GABA is not involved in behavioral characteristics captured by the ADI-R. Rather what this finding shows is that there is a shift in alterations of

glutamate mechanisms that relate to autism specifically, which is strongly associated with these behaviors. In Chapter 5 I also identified several interactions between ratios of glutamate/GABA and both glutamate and GABA PGS with behavioral measures of autism traits, cementing that investigating these metabolites together and using and multimodal data, really is necessary for disentangling E/I imbalances in autism.

Strengths and limitations

The datasets used here are unique both in terms of the large number of participants and the broad set of combined neuroimaging, genetic and behavioral data collected. This large amount of data has allowed for applying multiple novel analysis methods throughout this thesis and combining and investigating several data modalities in ways that have not been done previously, especially in autism cohorts. Both the LEAP and TACTICS cohorts are multicenter datasets, which while beneficial in many ways, also come with its limitations. One major limitation is site effects, where different researchers, recruitment strategies, MR scanners, and sometimes also discrepancies in execution of protocols lead to data loss and variations in data across sites. This has been addressed in the most appropriate ways across the analyses performed throughout this thesis, such as including site as covariates in analyses where possible and using standardized sequences.

It should also be noted that both glutamate and GABA ^{1}H-MRS measures has technical limitations, as the signals are noisy and reflect combined signals. The estimated glutamate signal is not fully separated from the glutamine signal, and the estimated glutamate concentrations likely therefore partially contain some glutamine. The GABA signal also contains some co-edited macromolecules. Additionally, glutamate and GABA are functions are not fully independent, much like the interplay between excitatory and inhibitory functions in the brain as a whole (14,17). These limitations implies that rather than looking at excitation and/or inhibition in isolated measures, we are looking at metabolite systems.

Thanks to the openly available datasets used here (SSC, AHBA and ABIDE), it was possible to perform some replication analyses. Nonetheless, considering especially the LEAP cohort, there is currently no comparable dataset available that has similar a number of participants with genetic, neuroimaging (especially ^{1}H-MRS) and deeply phenotyped data. This means the datasets in this thesis are not directly comparable, which also becomes clear in the not so straightforward replication attempts.

Replication, validation, and generalization across cohorts

In any field of research, it is important that results are reliable, replicable, and can be generalized across populations. This becomes particularly important when conducting research on conditions such as autism, as the long-term goal is to improve understanding of the condition to improve quality of life. Additionally, participating in studies may be a more stressful experience for autistic participants, and extra care should therefore be taken to make their contributions as valuable as possible. That is why replication analyses were made where data were available, particularly in Chapters 3 and 4. While some results were partially replicated, the findings also highlighted that not only is replication not always straight forward, but datasets are not always comparable.

Focusing first on Chapter 3, where we used the ABIDE dataset to replicate gene expression (from the AHBA) with cortical thickness (CT) differences between autistic and neurotypical participants. While the gene expression to CT correlations were indeed replicated from LEAP to ABIDE, the direction of the correlations in adults was different, which indicates that cortical thickness differences between autistic and neurotypical participants vary across the LEAP and ABIDE datasets. Inconsistencies between these cohorts in analyses using structural imaging data have been found elsewhere (18), where a deeper dive into the ABIDE data, used as part of the ENIGMA consortium (19), showed large differences across sites between diagnostic groups. This can partially be attributed to the nature of the cohort, as ABIDE is a legacy cohort where data was collated retrospectively only after independent data collection at the different sites. In contrast, in LEAP and TACTICS data was collected according to streamlined pre-defined protocols across sites.

In Chapter 4, the Simon Simplex Collection (SSC) cohort was used to replicate the glutamate polygenic scores (PGS) to ADI-R causal relationships found in the LEAP cohort. These findings were not replicated, which could be due to several factors. Although SSC is also a pre-defined multicenter cohort, the inclusion criteria across the LEAP and SSC cohorts were different, as SSC used diagnostic cut-off thresholds of the ADI-R and ADOS-2 scores as inclusion criteria, while these were also measured but not used as inclusion criteria in the LEAP cohort. Additionally, the gene-set PGS differed across LEAP and SSC in post-hoc tests, indicating that the cohorts were genetically different. However, the PGS could potentially be less reliable in the SSC cohort as the GWAS used as reference is based on a European dataset (20). Of note is also that the LEAP data was exclusively collected at European research centers.

The SSC data was collected in the USA, while ABIDE consists of a combination of majority USA sites, but with some European sites as well. It could be that there are subtle clinical or cultural differences between European and North American datasets, either due to data collection and access to participants, differences in access to care across countries and continents, or other factors affecting either clinical expressions of autism and/or application of diagnostic instruments and procedures.

One could argue that different cohorts can never actually be fully comparable, as there will always be variation e.g. in recruitment of participants or differences between measures and that is therefore not a matter of replication but rather of validation, or generalization. I believe that there will indeed always be differences across datasets and that this is not intrinsically negative, but I also insist that if we can never claim to have performed replication analyses due to variations between cohorts, such study designs lack ecological validity. It could also be argued that variation induced in a legacy cohort such as ABIDE is beneficial rather than undesirable, as results that persist and generalize in less homogenous datasets could be considered more reliable. However, it is important to keep in mind that large datasets, while increasing power for analyses, do not necessarily solve all problems. Variation across data collection sites or cohorts may not reflect clinical variation. While it is important to include heterogeneous expressions of autism across individuals in data collection, differences between and within autism cohorts do not necessarily represent differences between or within autistic individuals. Issues with cohorts not being comparable, and variations introduced by large site effects, may have stronger negative effects on findings than the potential benefit of increased power (18,21).

Although LEAP, ABIDE and SSC are the largest autism datasets of their kinds, they do not fully overlap in measures available, making complete replications difficult. Using similar measures available across them and performing partial replication analyses as done here, is useful as it provides some replication to potentially increase confidence in results, but non-replications across these datasets do not necessarily invalidate results. This highlights that there is a need for systematic investigations into what really are the similarities and differences across them, and caution needs to be taken both to interpret non-replications and replications. If findings may be considered reliable e.g. in the LEAP cohort even if not fully replicated in another dataset, it is not entirely clear what this means for generalization of results until we really know *why* results were not replicated. I believe this will be one of the big challenges moving forward as more large datasets become available, not just within autism research, but in large openly available neuroimaging cohorts in general.

Clinical implications

How we understand and define autism since its original definition in the early 1900's has shifted greatly thanks to the increased knowledge and redefinition of the condition, including increased sensitivity to more subtle phenotypic expressions in those with typical or higher IQ, or camouflaging and suppression of symptoms. These factors have been affected by a greater understanding of what is happening in the brain and how this relates to clinical characteristics. However, diagnostic procedures and treatment evaluations are still exclusively based on descriptive and behavioral outcomes. It is time to move beyond entirely behavior-based assessments and incorporate knowledge of underlying mechanisms in the brain to better predict what support and treatment options may be most beneficial for whom. To make such a shift, we need to have a better understanding of what these underlying mechanisms in the brain are, and a crucial step for doing so is to disentangle the heterogeneous expressions of autism (10,13,22).

The work in this thesis has demonstrated that autism is mediated by several alterations in the brain and established that these mechanisms can be best understood when looking at several of these E/I markers together. While the results presented here do not provide one to one mappings between biomarkers and certain clinical traits, they provide us with greater understanding to now inform more specific research questions. Sensory processing differences in autism seem to be driven by different alterations of excitation and inhibition in the brain compared to restricted-repetitive and social domains. Is this mediated in specific parts of the brain, and does it differ for different sensory domains (e.g. auditory processing, or sense of touch)? Understanding these relationships will help us find more fine-tuned biomarkers, allowing us to better identify diagnostic markers, support options, and facilitate subtyping, which can subsequently be followed by better targeted therapeutic options and improved quality of life.

Moving forward

Multimodal dimensional analysis allows us to look at alterations across domains to identify how these may influence each other, and ultimately influence clinical characteristics of autism. Future work should leverage this in experimental approaches, for example using medications that impact glutamate and/or GABA. While pharmacological studies have been performed previously, they typically use categorical approaches and several did not find significant group level effects,

even though some participants may have had positive responses to medications (23,24). The findings in this thesis demonstrate that variations in E/I imbalances likely affect different behavioral domains, which needs to be considered in future pharmacological trials.

In this thesis the focus was on core clinical traits; restricted and repetitive behaviors, social interactions, and sensory processing differences. Autism also manifests in various ways, often with co-occurring conditions such as anxiety, burnout, depression or gastro-intestinal problems. These should be addressed as well to provide an even more nuanced understanding of the underlying mechanisms of autism. Further, the behavioral measures used here were based on interviews and questionnaires, and using other measures of e.g. sensory processing or cognition has the potential to provide more objective markers of behavioral autism characteristics. More objective phenotyping across varying sensory domains are needed to disentangle interindividual variations in sensory processing differences. Examples of such measures are tasks involving sensory detection thresholds of e.g. auditory or tactile stimulation, which provides measures independent from self- or parent-reports. Such measures are also included in the third wave of the LEAP data collection, and will be incorporated into future analyses.

There are several novel neuroimaging methods that approximate excitation and inhibition in various ways, capturing distinct, but informative, aspects of neuronal functioning and communication. For example, E/I ratios have been estimated both using fMRI (25) and EEG (26) measures, which are based on various assumptions of brain function and structure affecting excitatory and inhibitory mechanisms in the brain. Other measures that aim to capture, or modulate, excitation and inhibition are Positron Emission Tomography (PET) imaging and brain stimulation approaches, where the latter has been suggested to have potential therapeutic benefits for autism traits (27). Excitation and inhibition are fundamental properties of brain functioning and exists on multiple levels; intracellularly, between local populations of neurons in brain regions, and across brain regions in networks. The measures we have available today, including the ones just mentioned and the *in vivo* ^{1}H-MRS and genetic methods used within this thesis, all capture different aspects of excitatory and inhibitory mechanisms, in differing levels of spatial and temporal resolution, and have the potential to unravel distinct, or converging, alterations of E/I in autism and how they relate to different clinical characteristics.

As excitation and inhibition are properties of many layers of functioning in the brain, and is affected by several mechanisms, we do not yet have a cohesive

definition or measurement of excitation and inhibition, let alone their (im)balance and the ratios between them. We need to systematically evaluate how these different approximations of E/I mechanisms relate to each other to understand how they can be useful for both understanding biological mechanisms and for identifying biomarkers. This also includes defining what level of E/I functioning and development you are investigating, as e.g. animal models, *in vitro*, or pharmacological studies are measuring or altering individual mechanisms on a cellular level, while measures like EEG or ^{1}H-MRS capture large scale networks and resting state levels of excitation and inhibition (28). By doing so, we can also formulate specific testable hypotheses for distinct alterations that may be affected in autism.

The results in this thesis emphasize differences in the brain across development, however, we were mainly restricted to cross-sectional age ranges of the participants included in our cohorts and more longitudinal analyses are necessary to investigate individual developmental trajectories. Longitudinal data collection does come with challenges, including changes and updates in equipment between waves of measurement, different researchers performing the data collection, and participant drop out, which all affect data collection and quality. It is however not impossible, and in the LEAP cohort the third wave of data collection has just been completed, spanning over 5 years since the first wave of data collection with three data points available.

Additionally, all participants in the autism groups were already diagnosed prior to participating and to find early diagnostic and biological markers even younger participants should be included, potentially before diagnosis is typically given. There are studies currently being undertaken with this in mind. For example, within AIMS-2-TRIALS there is ongoing data collection of the Preschool Imaging Project (PIP: https://www.aims-2-trials.eu/pip/), where autistic and typically developing children (as well as those with ADHD and developmental delay) from three years of age participate. Data collection matches the measures available in the LEAP cohort and is acquired longitudinally. PIP also includes children that express autism characteristics without yet having received a formal diagnosis. There are also initiatives to capture autism predictors in infants in initiatives such as Eurosibs (29) and The British Autism Study of Infant Siblings (BASIS; www.basisnetwork.org), and those that test transdiagnostic differences in neurodivergence in childhood (CANDY, https://www.candy-project.eu/).

There is a need for a shift in the field of autism research, not only moving away from case-control analyses to focus more dimensional or subgroup analysis, but also

initiatives to systematically assess how various neuroimaging measures interact or capture distinct aspects of alterations of E/I and brain functioning, to truly understand what is underlying the heterogeneous expressions of autism traits. There are many novel and promising datasets and analysis methods becoming available, which will be informative in the coming years to build on the findings in this thesis.

Ethical and practical considerations

Research on autism, or any neurodevelopmental condition or disorder, should always operate from the goal to improve quality of life of the group being studied. Not that all studies must have immediate practical or clinical implications, but research questions and long-term goals should be defined to do so. This may sound obvious, but research on autistic individuals has historically also been harmful (30–32). Today, there are discussions and tensions surrounding the topic of autism research, where stakeholders with lived experience question how and whether all autism research is beneficial or ethical (30–32). These are valid concerns, and continuing these discussions are important to bridge mutual understandings between researchers and stakeholders (32).

Genetic research

Genetic data is considered identifiable data, as everyone's genome is unique. This necessitates mindfulness when using genetic data in research, especially as it is now possible to use genetic screening to identify e.g. likelihoods for certain conditions or disorders. There is a growing concern that genetic research could lead to identifying or singling out autistic individuals or those who have a high genetic likelihood for autism, without their consent (33). In this thesis, all access to genetic data and its analyses have been performed on data from participants who gave informed consent to its collection and analysis approaches. The analyses throughout this thesis which included genetics can in no way be used to develop genetic markers for autism or autism traits. Genetic variations, polygenic scores, and postmortem gene-expression data used here are measures selected exclusively to understand glutamatergic and GABAergic mechanisms in the brain. These methods are based on common genetic variance which is highly unlikely to ever be used as diagnostic or predictive measures on an individual level.

Within the realm of genomics, there are several approaches that were not used here. For example, investigations of rare genes and copy number variants (CNVs)

with stronger effects, as well as epigenetics, are also known to affect to autism characteristics and has great potential in increasing mechanistic understanding of autism (34,35).

Pharmacological development

There is a clear connection between investigating glutamate and GABA functions in the brain in autism and the development and testing of (new) pharmacological interventions. For this reason, it is important to be clear that the use of pharmacological alternatives to the difficulties expressed by autistic people should always be optional, as is the case with medications for other neurodevelopmental conditions such as ADHD. Previous pharmacological studies on autism have had mixed results, likely due to the lack of stratification and precision medicine approaches typically used in clinical trials. This has led to several promising pharmacological approaches not surviving clinical trials, despite potentially having positive effects for some individuals. Recent work aiming to stratify responders and non-responders to e.g. bumetanide (15,36,37), will continue to be of great importance for developing better targeted therapeutic options. This undertaking will be much more effective by understanding what mechanisms may underlie which experiences autistic individuals may want support with. For example, if we can identify disturbances in specific circuits that relate to certain traits or behavioral domains, we can select pharmacological trial designs more likely to be effective for specific traits or individuals. An example of this is the GOAT trial, part of the TACTICS consortium, which investigated the effects of memantine and focused on compulsive and impulsive behaviors across OCD and autism, targeting glutamate dense fronto-striatal circuits due to its involvement in these behavioral domains (38,39).

It is also important to acknowledge that pharmacological options are not necessarily the only, or most effective, alternative for all autistic individuals. It is one of many routes that should be better investigated and understood as support options for autistic individuals are improved.

Data acquisition with diagnostic groups

Keeping in mind that the end goal of research on diagnostic groups is to contribute to improving of quality of life, there are also important considerations regarding data acquisition involving autistic individuals. Firstly, the experience of participating in research can be overwhelming for anyone and should be made as accommodating as possible. This includes e.g. giving participants enough time

to process and prepare for tasks, both before visiting the research center and during testing.

It is also important that the participation in studies is made as valuable as possible. This may initially sound contradictory to making the participant's experience as pleasant and accommodating as possible. However, having a stressed or nervous participant often lead to more movement during e.g. MRI or EEG, which leads to lower quality and loss of data, not completing all measurements, and makes participants less likely to return for longitudinal studies. Other factors that improve data quality, especially in multicenter studies, include continuously checking the collected data across sites to make sure that protocols are executed properly[1], that there are no problems with equipment that would otherwise not be detected until data processing, and making sure that all researchers involved are well trained on the data acquisition techniques and have an understanding of how to best accommodate participants. These are important steps to avoid differences across diagnostic groups, sites, testers, and equipment. Discovering at the end of a study that e.g. settings in an MR sequence were incorrect at one site, rendering collected data unusable, is a waste of both funding and participants' time and efforts, which becomes problematic when some participants may find the experience of participating in research particularly stressful. This becomes especially important when aiming to address the bias of studies on autism more often recruiting participants with lower support needs, e.g. with verbal abilities or without intellectual disability.

The need for considerations further extends to data management after data collection, as this is an important step where data is checked and quality controlled and potential errors may lead to even further, unnecessary, data loss. I believe that it is unethical to lose data whenever avoidable, especially from participants in diagnostic groups, due to errors that could be prevented had these factors been considered from conception of the study to end of data acquisition, processing and analysis. It is, after all, for them that we do this research, and their efforts and contributions should be treated as carefully as we possibly can.

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[1] I have been impressed to see just how many creative ways one can deviate from standard operating procedures.

Final conclusions

To conclude, in this thesis I have identified several associations across genetic, various MR-based and behavioral modalities to increase understanding of how E/I imbalances may be expressed in various ways in autism. This work also highlights the urgent need for further multimodal approaches and datasets that includes several measures of excitation and inhibition, genetic measures, and behavioral measures that goes beyond questionnaires and interviews. Furthermore, it is important to investigate glutamate and GABA together, as excitatory and inhibitory proxies, as they are strongly related and interact to affect other brain and behavior measures. As new approaches to capturing E/I dynamics are developed, and more autism datasets become available, there are promising new ventures ahead which I believe have the potential to finally increase our understanding of the various expressions and support needs of autistic individuals. To do so, there needs to be a more intentional approach to how we use the E/I framework to help define research questions and interpret results, rather than using it as a catch-all explanatory theory for all neuroimaging findings pertaining to autism.

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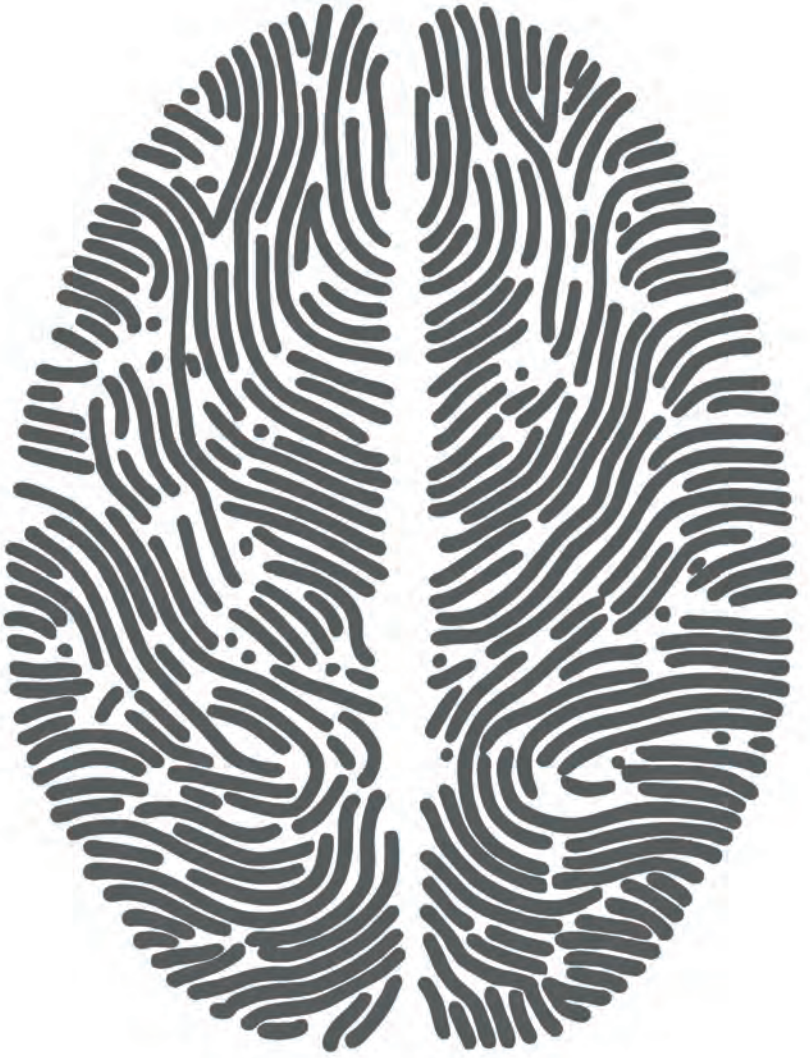


Appendices

Data management

The research in this thesis is based on existing datasets from the LEAP (https://www.aims-2-trials.eu/leap-front-page/) and TACTICS (https://cordis.europa.eu/project/id/278948/reporting) studies.

Ethics and privacy

This thesis is based on the results of research involving existing data from studies with human participants, which were conducted in accordance with relevant national and international legislation and regulations, guidelines, codes of conduct and Radboud UMC policy. The privacy of the participants was warranted by the use of pseudonymized data. For the purpose of the research in this thesis, only the pseudonymized data was shared, the keyfile was not shared with the data.

Data collection and storage

The data in chapters 2, 3, 4 and 5 have been analyzed and processed in project folders at the DCCN: 3022035.04 (LEAP) and 3015043.01 (TACTICS).

Data sharing according to the FAIR principles

Data collected in LEAP are stored and curated at the central EU-AIMS database at the Pasteur Institute in Paris. LEAP data is currently only accessible to consortium members who get an analysis proposal approved, and it will be available for use to the wider research public through open-access publication via a secure database that will become available in the near future (https://elixir-luxembourg.org/). TACTICS data will not be available for the wider research public, as per consortium guidelines. All studies are or will be published open access.

Nederlandse Samenvatting

Autisme (autisme spectrum stoornis) is een van de meest voorkomende ontwikkelingsaandoeningen. Er is echter veel ontbrekende kennis van de onderliggende mechanismen in de hersenen en over hoe autisme zich ontwikkelt en de verschillende manieren waarop het tot uiting kan komen. In dit proefschrift komen een aantal onderzoeken aan bod die zich richten op de populaire *excitation/ inhibition (E/I)* disbalanstheorie van autisme. Het doel van deze onderzoeken was om meer inzicht te krijgen in de heterogeniteit van autisme door te kijken naar genetische aspecten met betrekking tot glutamaat en GABA functies (respectievelijk betrokken bij *excitation* en *inhibition*), maten vanuit magnetische beeldvorming (MRI) en gedragskenmerken. De bevindingen bieden steun voor een centrale rol van de neurotransmitters glutamaat en GABA, zowel fysiologisch als gedragsmatig, in de onderliggende mechanismen van autisme. Verschillen in glutamaat in de voorste cingulate hersenschors (afgekort ACC) zijn geassocieerd met repetitief gedrag (Hoofdstuk 2), en met variaties in de hersenstructuur (Hoofdstuk 3). Bovendien blijken polygene scores van glutamaat gecorreleerd met deze verschillen in ACC concentraties van glutamaat (Hoofdstuk 5). Hoofdstuk vijf laat hiermee voor het eerst een relatie tussen genetische informatie en concentraties van glutamaat in de hersenen zien. Deze interactie is bovendien gerelateerd aan verschillende gedragskenmerken van autisme, waaronder verschillen op sociaal en sensorisch vlak. Ik vond hiernaast ook interacties tussen glutamaat en GABA op genetisch en hersenniveau die op verschillende wijze betrokken waren bij kenmerken van autisme (Hoofdstuk 5).

De bevindingen uit dit proefschrift onderschrijven onvoldoende *welke* veranderingen in E/I mechanismen tot kenmerken van autisme leiden, maar fungeren des te meer als een waardevolle eerste stap om dit verder te onderzoeken. Mijn onderzoek als geheel pleit voor (1) het gelijktijdig onderzoeken van het glutamaat en GABA neurotransmitter systeem in de hersenen, (2) het gebruik van verschillende methoden als genetica en beeldvormend onderzoek, en (3) het onderzoeken van het effect van leeftijd en ontwikkelingsfactoren. De interactie tussen deze verschillende aspecten vormt de ingang voor meer inzicht en hopelijk een uiteindelijke verklaring voor de heterogeniteit van autisme en de (individuele) verschillen gedurende de ontwikkeling.

Acknowledgments

In the 7 years I have lived in the Netherlands, there have been a lot of ups and downs. Through everything, I have met and gotten to know incredible people that I am honored and grateful to have in my life, including those who are no longer here. Without them, this thesis would not be what it is today, and as a matter of fact neither would I.

First of all, thank you **Jan**, for believing in me enough to let me embark on this journey, pushing me where I needed it, and encouraging me when I needed that too. And **Jill**, the pigeon to my eagle, whom my academic work is fundamentally based on (I still live in the glow of your heritage, in several ways). But above that, I am very grateful for the great discussions we have, both about the science and everything else. Thank you **Nick**, especially for agreeing to become my supervisor when I was already halfway through my PhD, and doing so in a fully remote capacity, which I understand is not an obvious yes. I am very grateful for the input(s) and new perspectives you have given me.

My colleagues at the Donders have made work a lot more fun, even during the long nights in the MR basement waiting for the children to (try to) fall asleep, discussing meetings, venting and supporting each other, making coffee breaks take much longer (and productive) than planned. Thank you for making this experience as fun as it has been. This also extends to the people in remote labs and groups across Europe that I have gotten the privilege to work with.

Thank you **Amy** and **Marije** for being my Dutch moms (and kids on occasion), and bringing me out of my comfort zone both literally and figuratively. **Tineke** for the sushi, constant love and support, and insightful perspectives. **Vivian** for all the fun things we have done (and tried to do). And **Lucas** for reminding me to get some fresh air, and for the male perspective I sometimes needed.

Thank you to my friends that have stayed in my life even though I left Sweden over 7 years ago, especially **Lovisa**, **Amanda**, **Lotten** and **Tove**. Thank you for all the video calls, for letting me stay at yours whenever I go back home home, and for reminding me every now and then that it is actually quite wild that this weird girl from Värmland somehow ended up doing cognitive neuroscience.

I also want to say thank you to my paranymphs for agreeing to it (although at time of writing I do not know what you are conspiring); **Amy**, **Lucas**, **Nils**, **Nat** and **Jill**

(although for this your title here is *party*nymph). Extra thank you Nat for, seemingly, being ok with me coming with supervisor-type questions when I needed help, despite not being my supervisor.

To **Beau**, even though things have not always been easy, I would not have been able to do this without your support.

Mormor, who did not get to see me finish this PhD, but always reminded me I was working really hard and doing very well, while also often asking when I would finally come back to Sweden.

When I was about to graduate high school, I went to **pappa** and asked what I was supposed to do with my life. He asked me what I had done in school that was the most interesting, and I replied it was my presentation about the brain. This eventually led to him telling me that neuroscience is in fact a thing you can do, and ever since it has been my goal to do exactly what I am doing now. I cannot think of anyone who has had a bigger impact on me getting to where I am now, or becoming the person that I am, than you.

Len, the whirlwind that came into my life at a point where I was really not a peak-performance-fighter-jet. The unconditional, unwavering support and motivation (and distraction when needed) made the last push of this PhD so much better than it would have been otherwise. I am incredibly lucky and eternally grateful, for everything.

And most importantly, **Sören** and **Koda**, even though they will never read this themselves, because they are dogs. The best thing that happened to me when I came to the Netherlands was that I got the privilege to be their caregiver. I cannot explain in words, but I have done my best to show that their unconditional love, I also have for them.

Curriculum Vitae

Viola Hollestein was born 5 December 1994, in Bærum Norway, but grew up in Karlstad Sweden (she actually doesn't even speak Norwegian, or remember those first 6 months of her life). She completed her Bachelor's degree (BSc) with a major in Cognitive Neuroscience at Skövde University in Sweden, where her thesis integrated previously opposing theories of neural correlates of synesthesia. She completed her Master's degree (MSc) cum laude with a major in Cognitive Neuroscience and a minor in Philosophy of mind, at Radboud University, the Netherlands. This is where her interest in neurodevelopmental conditions, and especially autism, began. During her internship she analyzed TACTICS data, while she also became a certified MR user at the Donders Institute, scanning children and adults with various conditions for several studies. This was also when she was introduced to multicenter multimodal data analysis, including the introduction to ^{1}H-MRS, which eventually became the foundation for the conception of her PhD.

Viola began her PhD in April of 2020 (which of course, was not the easiest time to embark on a PhD), supervised by Prof. Jan Buitelaar and Dr. Jill Naaijen, who halfway through were joined by Dr. Nick Puts. Throughout her PhD she was involved in the data collection of the third wave of LEAP, as well as the PIP and Multiplex projects, working in the data collection team at the Donders Institute as well as with the international teams across these projects, while performing the data analyses presented throughout this thesis.

Following the completion of her PhD, Viola will continue to work with neuroimaging and genetics data in the AIMS-2-TRIALS consortium, then at King's College London under supervision of Prof. Declan Murphy and Prof. Christine Ecker.

Portfolio

Invited talks

2024 Invited speaker, Autism Research Institute (ARI) think tank. Title "*Using glutamate and GABA genes to understand mechanisms underlying brain and behavior differences in autism*"

2023 Invited speaker, INSAR panel. Title *"Linking glutamate and GABA gene-sets, cortical thickness, and behavioral characteristics of autism"*

2023 Invited speaker at AIMS-2-TRIALS webinar. Title *"Understanding excitation and inhibition in autism: Linking genetics, brain and behavior"*

2023 Invited speaker at Donders Session event "*Neurodiversity: how to define it and why it is important for researchers*"

2022 Invited speaker at Dondrite event. Title "*Neurodiversity: how to define it and why it is relevant to researchers*"

2022 Invited speaker at CINP panel. Title "*A virtual histology and genetics approach investigating excitatory/inhibitory imbalance in autism"*

Coordinated symposia

2023 OHBM, Organiser, Educational course: Acquisition and analysis of in vivo markers of 'Excitation and Inhibition' in humans. Presentation title "*Linking glutamate and GABA genetic variation and gene expression to behavior and brain"*

Accepted conference talks

2023 Presentation at GABA MRS Symposium. Title *"Linking MRS glutamate with fMRI, behavioral and genetic measures using causal discovery analysis"*

Poster presentations

2024 Poster presentation at OHBM: "*The causal roles of glutamate and GABA genes on brain and behavior in autism"*

2023 Poster presentation at AIMS-2-TRIALS General Assembly. Title *"Measuring MRS in preschool children and adults – progress and quality control from PIP and LEAP"*

2022 Poster presentation AIMS-2-TIRALS General Assembly. Title "*The role of glutamate and GABA gene-sets in behavioral autism characteristics and cortical brain structure*"

2022 Poster presentation at ECNP workshop. Title *"Glutamate and GABA gene-sets, cortical thickness, and clinical characteristics of autism: probes of causality"*

2020 Poster presentation at OHBM. Title "*A virtual histology and genetics approach investigating excitatory/inhibitory imbalance in autism*"

Training and courses

2024 Donders Career Event
2023 Donders Discussions
2023 Course: Analytic storytelling
2023 Course: Design and Illustration
2022 Course: Writing scientific articles
2021 Course: Effective writing strategies
2020 Training: Advanced (f)MRI toolkit, Donders Institute
2020 Training: MRS editing school
2020 eBROK certification (re-certified 2023)

Donders Graduate School for Cognitive Neuroscience

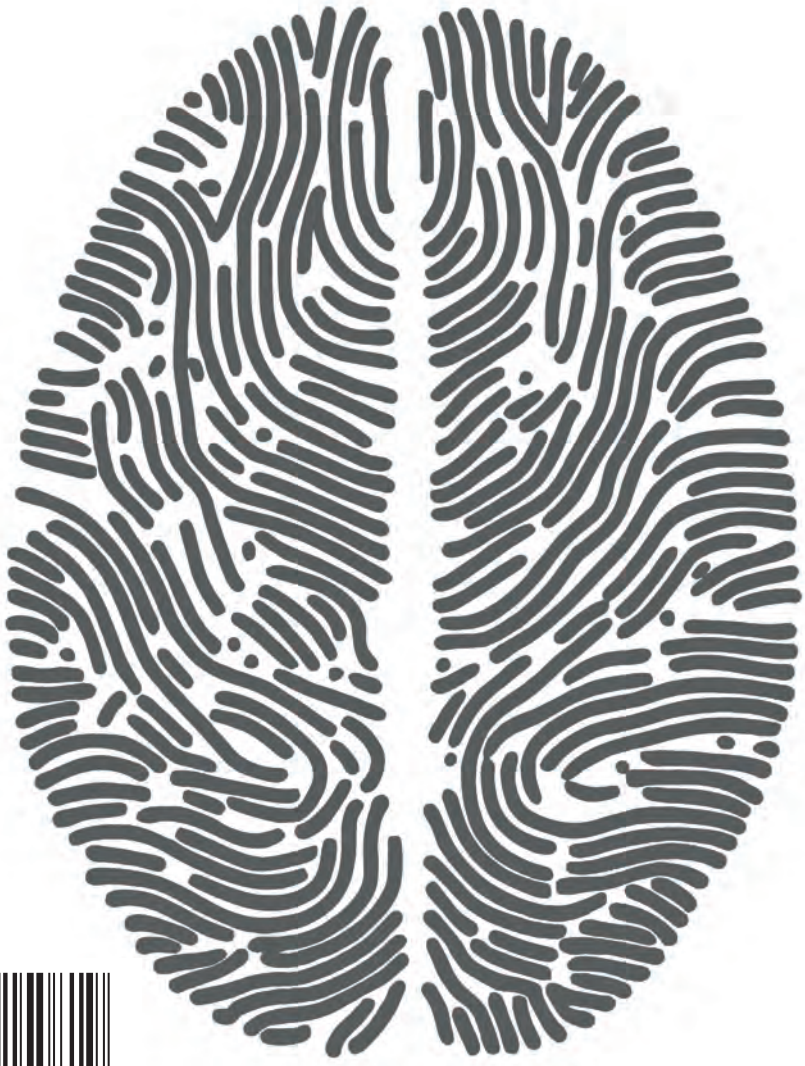
For a successful research Institute, it is vital to train the next generation of scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School in 2009. The mission of the Donders Graduate School is to guide our graduates to become skilled academics who are equipped for a wide range of professions. To achieve this, we do our utmost to ensure that our PhD candidates receive support and supervision of the highest quality.

Since 2009, the Donders Graduate School has grown into a vibrant community of highly talented national and international PhD candidates, with over 500 PhD candidates enrolled. Their backgrounds cover a wide range of disciplines, from physics to psychology, medicine to psycholinguistics, and biology to artificial intelligence. Similarly, their interdisciplinary research covers genetic, molecular, and cellular processes at one end and computational, system-level neuroscience with cognitive and behavioural analysis at the other end. We ask all PhD candidates within the Donders Graduate School to publish their PhD thesis in de Donders Thesis Series. This series currently includes over 600 PhD theses from our PhD graduates and thereby provides a comprehensive overview of the diverse types of research performed at the Donders Institute. A complete overview of the Donders Thesis Series can be found on our website: https://www.ru.nl/donders/donders-series

The Donders Graduate School tracks the careers of our PhD graduates carefully. In general, the PhD graduates end up at high-quality positions in different sectors, for a complete overview see https://www.ru.nl/donders/destination-our-former-phd. A large proportion of our PhD alumni continue in academia (>50%). Most of them first work as a postdoc before growing into more senior research positions. They work at top institutes worldwide, such as University of Oxford, University of Cambridge, Stanford University, Princeton University, UCL London, MPI Leipzig, Karolinska Institute, UC Berkeley, EPFL Lausanne, and many others. In addition, a large group of PhD graduates continue in clinical positions, sometimes combining it with academic research. Clinical positions can be divided into medical doctors, for instance, in genetics, geriatrics, psychiatry, or neurology, and in psychologists, for instance as healthcare psychologist, clinical neuropsychologist, or clinical psychologist. Furthermore, there are PhD graduates who continue to work as researchers outside academia, for instance at non-profit or government organizations, or in pharmaceutical companies. There are also PhD graduates who work in education, such as teachers in high school, or as lecturers in higher

education. Others continue in a wide range of positions, such as policy advisors, project managers, consultants, data scientists, web- or software developers, business owners, regulatory affairs specialists, engineers, managers, or IT architects. As such, the career paths of Donders PhD graduates span a broad range of sectors and professions, but the common factor is that they almost all have become successful professionals.

For more information on the Donders Graduate School, as well as past and upcoming defences please visit: http://www.ru.nl/donders/graduate-school/phd/



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