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GABA_A Receptor Availability Is Not Altered In Adults With Autism Spectrum Disorder (ASD) Or In ASD Mouse Models

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Abstract

Preliminary studies have suggested that GABA_A receptors, and perhaps especially the GABA_A α 5 subtype, are deficient in autism spectrum disorder (ASD). However, prior studies were confounded by medication effects, and did not compare findings across species. Hence, we measured both total GABA_A and GABA_A α 5 receptor availability in two Positron Emission Tomography (PET) studies using the tracers [¹¹C]flumazenil (15 ASD adults, 15 controls) and [¹¹C]Ro15-4513 (12 ASD adults, 16 controls) respectively. All participants were medication-free. We also performed autoradiography, using the same tracers, in three mouse ASD models: *Cntnap2* k/o, *Shank3* k/o and 16p11.2 deletion. We found no significant differences in GABA_A or GABA_A α 5 receptor availability in any brain region in ASD. There were likewise no differences in any of the mouse models. However, humans with ASD did display altered performance on a GABA-sensitive perceptual task. GABA_A receptor availability is normal in adults with ASD, though GABA signaling may yet be functionally impaired.

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterised by impairments in social communication and interaction, alongside restricted and repetitive behaviours and interests.(1-3) The prevalence of ASD is estimated at 0.6-1.5%, with each case of the disorder being associated with projected lifetime care and loss of productivity costs of around \$1.5 million.(4, 5) Currently, there are no specific “disease-modifying” pharmacological treatments for ASD, and drug discovery has been hampered by poor understanding of the pathophysiology of the condition.

There is, however, increasing evidence for an excess of brain excitatory glutamate and/or a deficit in inhibitory gamma-aminobutyric acid (GABA) in ASD.(6-8) For instance, reduced expression of the GABA synthesis enzyme *glutamic acid decarboxylase* (GAD) has been observed in ASD brains post mortem.(9) In vivo, alterations in glutamate and glutamine have been reported in the cortex and basal ganglia of children, and in the basal ganglia of adults with ASD, as measured with proton magnetic resonance spectroscopy, [1H]MRS.(10-13) Reductions in GABA concentration have also been reported by several [1H]MRS studies of ASD children.(14, 15) However, a limitation of [1H]MRS is that it is unable to determine neurotransmitter receptor density.

Alterations in the expression of GABA receptors, of which GABA_A is the most prevalent type in the brain,(16-18) may be a key contributor to the excitatory/inhibitory imbalance in ASD. Reductions in the density of GABA_A receptors have been reported in post-mortem studies of ASD in the hippocampus(19) and cingulate cortex.(20, 21) Although these studies were valuable first steps, their sample sizes were small, and they included individuals taking medications such as anticonvulsants which might alter GABA_A expression, leaving it unclear whether their findings could be replicated by in vivo studies.

Two in vivo studies have reported reductions of GABA_A in ASD. Using [¹²³I]iomazenil Single Photon Emission Computed Tomography (SPECT), Mori et al. reported significantly decreased GABA_A availability in children with ASD.(22) However, this study included children with epilepsy and intellectual disability, and some participants were on anticonvulsants. Hence, it remained unclear whether it was ASD, or a confounding factor, which was associated with reduced GABA_A receptors. Therefore, we subsequently carried out a pilot study(23) using Positron Emission Tomography (PET) with the tracer [¹¹C]Ro15-4513. Our participants were medication-free adult males with ASD and no epilepsy or intellectual disability, and controls. We found significantly reduced availability of the GABA_A α5 subtype in ASD, with no alterations in the non-α5 subunits. However, this preliminary study was small, including 3 adults with ASD and 3 controls. Also, it did not clarify whether it is overall GABA_A, or GABA_A α5 specifically, that is altered in ASD. Hence we designed the present investigation to replicate and extend prior in vivo GABA_A studies in a new and larger sample; the data from the pilot were not included in the present analysis.

A further motivation for the present study was 'translational'. If the GABA_A receptor is abnormal in ASD, it might provide a drug target. However, to provide a rational screen for new treatments, drug development would require animal models that recapitulate any GABA_A alterations seen in humans. Altered GABA_A expression has indeed been observed in rodent models of ASD-associated syndromic disorders, including Fragile X syndrome(24) and Rett's syndrome.(25, 26). However, approximately 90% of ASD cases are nonsyndromic,(27) so we set out to examine whether GABA_A alterations are seen in animal models of nonsyndromic ASD.

A final motivation for this study was to investigate GABAergic function in ASD adults. This was based on our reasoning that the functional integrity of GABAergic circuits might be abnormal in ASD even if GABA_A receptors are unchanged. If so, this would suggest the presence of an abnormality elsewhere in the GABAergic system.

Hence, our aims in the present investigation were to: a) confirm whether GABA_A expression is altered in humans with nonsyndromic ASD; b) determine whether any putative changes are specific to the GABA_A $\alpha 5$ subtype; c) discover which, if any, of our chosen rodent models display the same pattern of GABA_A alterations seen in humans; and d) determine whether ASD is associated with functional impairment in GABAergic circuits using a 'proxy measure' of GABAergic function in humans.

We therefore conducted the first integrated, translational, investigation of GABA_A across both humans and mouse models of ASD. In humans, we carried out two PET studies in two experimental centres: in Stockholm using [¹¹C]flumazenil(28) and in London using [¹¹C]Ro15-4513.(29, 30) We chose [¹¹C]flumazenil as a non-selective tracer as it binds $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$,(31) covering the vast majority of GABA_A receptors in the brain(32) with approximately equal affinity. [¹¹C]Ro15-4513 was chosen for its 10-fold higher affinity for $\alpha 5$ over the other subtypes $\alpha 1-4$ and $\alpha 6$, at which it has approximately equal affinity.(31) The $\alpha 5$ subtype of the GABA_A receptor has been especially linked to ASD.(23, 33, 34) Both PET studies formed part of the Innovative Medicines Initiative (IMI) European Autism Interventions Multicenter Study (EU-AIMS) project.(35)

In mice, we selected three commonly used, etiologically diverse ASD models that represent a range of genetic risk factors for ASD in humans: *Cntnap2*(36) knockout mice, *Shank3*(37) knockout mice, and mice carrying a genetic deletion equivalent to human chromosome 16p11.2 deletion, which is one of the most common copy-number variants (CNVs) found in ASD.(38) These models were selected as well-established and well-characterized ASD models. Employing the same radiotracers used for PET, we performed autoradiography for quantification of GABA_A receptors in rodent brains. Autoradiography was used rather than small animal PET because it provides better spatial resolution, and because PET would have required the animals to be anaesthetized during scanning, which could impact on GABA.

Finally, to investigate the *functional* balance between excitatory/inhibitory neurotransmission using a visual psychophysics task, we used the paradoxical motion perception task (PMP), known to be a proxy measure of GABA functionality.(39, 40)

Results

PET studies

[¹¹C]flumazenil: No differences in GABA_A receptor availability

There was no evidence of group differences in estimated [¹¹C]flumazenil binding potential in the whole brain (independent samples t-test: $t(28)=1.304$, $p=0.203$, $d=0.476$), in brain grey matter ($t(28)=1.283$, $p=0.210$, $d=0.468$), or in any of the 13 specific brain regions we examined (all $p>0.156$). This suggests that there were no differences in GABA_A receptor availability in ASD. See **Figures 1, 2, and 3a**. There were no significant differences in [¹¹C]flumazenil tracer dosing between the two groups (see **Table S1**).

Sensitivity analyses including correction for partial volume effects, inclusion of male participants only, and exploratory voxel-wise analysis did not affect the results (see Supplementary Material.)

There were no correlations between grey matter GABA_A receptor availability and performance on any of a battery of ASD-related cognitive and social-cognitive tasks including the Embedded Figure Test,(41) Reading the Mind in the Eyes Test,(42) and the Faux Pas test.(43) See the Supplementary Methods & Results for details. There was no difference between the typically developing control and the ASD groups in terms of age or full-scale IQ. See **Table 1**.

[¹¹C]Ro15-4513: No differences in GABA_A non- $\alpha 5$ or $\alpha 5$ receptor availability

A primary analysis showed no group differences in total specific [¹¹C]Ro15-4513 binding, from 2TCM, in the whole brain (independent samples t-test: $t(25)=-1.392$, $p=0.176$, $d=0.574$), in brain grey matter ($t(25)=-1.516$, $p=0.142$, $d=0.614$), or in 11 of the 13 specific brain regions we examined (all $p>0.1$) In the cerebellum and the thalamic regions, nominally significant increases in binding were observed in the ASD group ($t(25)=-2.132$, $p=0.043$, $d=0.820$ and $t(25)=-2.513$, $p=0.019$, $d=0.974$ respectively), but these did not survive Bonferroni correction for multiple comparisons. There were no significant differences in [¹¹C]Ro15-4513 tracer dosing between the two groups (see **Table S1**).

In a secondary analysis of the [¹¹C]Ro15-4513 data, we used nonlinear spectral analysis (NLSA)(23) to distinguish non- $\alpha 5$ and $\alpha 5$ subtype availability. This confirmed that there were no differences in *either* non- $\alpha 5$ ($t(24)=-0.163$, $p=0.872$, $d=0.064$) or $\alpha 5$ ($t(23)=-0.805$, $p=0.429$, $d=0.322$) subtype receptor availabilities in adults with ASD in the whole brain. Across the brain areas, there were no significant regional differences in either non- $\alpha 5$ or $\alpha 5$ availability in any brain area, except for increased $\alpha 5$ availability in the insula in ASD ($t(18)=-2.345$, $p=0.029$, $d=1.063$) which did not survive correction for multiple comparisons. See **Figures 1, 3b, and 4**. In particular, we did not observe any differences in $\alpha 5$ availability

in the hippocampus or amygdala regions (all $p > 0.3$), i.e. in those areas in which significant differences were detected in our previous pilot study.(23) Finally, there was no difference between the typically developing control and the ASD groups in terms of age or full-scale IQ. See **Table 1**.

Quantitative autoradiography findings

GABA_A autoradiography was performed in three mouse models of ASD: *Cntnap2* knockout mice, *Shank3* knockout mice, and 16p11.2 deletion. Six brain regions were examined bilaterally: the frontal cortex, cingulate cortex, caudate/putamen, dorsal hippocampus, cerebellum, and amygdala. We observed no significant differences in the specific binding of either [³H]flumazenil or [³H]Ro15-4513 in any model or region compared to the control (wild type) animals (all $p > 0.05$). See **Figure 5**. For left and right amygdala results, see **Figure S1**.

Evidence of a functional GABAergic deficit (motion perception task)

The paradoxical motion perception task (PMP) was used as a proxy measure of GABA function in the visual cortex. We first verified that the paradoxical motion perception effect(39) was present in the healthy controls. The effect was significant: one-sample t-test: $t(17)=3.99$, $p < 0.001$, mean PMP ratio=1.844 (95% CI: 1.398-2.290 where 1 is no effect, demonstrating successful implementation of the task.

The magnitude of the contrast-induced motion perceptual impairment ratio was reduced in the ASD group compared to the controls. (Independent samples t-test with Welch-Satterthwaite correction for unequal variances: $t(28)=2.487$, $p=0.022$, $d=0.921$ two-tailed. Bootstrapped t-test: $p=0.028$ with 10,000 samples) See **Figure 6**. Individuals with an ASD were less impaired by the high contrast stimuli than the controls. This group difference was not accounted for by low-level differences in the ability to detect motion, because there were no group differences in perceptual threshold in either of the two control conditions (both $p > 0.4$). Furthermore, the paradoxical effect of contrast on motion perception was not correlated with age ($n=30$, $r=0.026$, $p=0.892$) or IQ ($n=29$, $r=0.243$, $p=0.203$).

Therefore, adults with ASD have a selective enhancement of motion perception under conditions of high contrast, a pattern of results considered indicative of a GABAergic signalling deficit.

Discussion

We conducted a multisite, translational molecular imaging study of GABA_A receptors in adults with ASD and rodent models. Our objective was to test *in vivo* the hypothesis that ASD is associated with reduced amounts of GABA_A receptors. We found no direct evidence in support of this hypothesis. Our results show that unmedicated adults with idiopathic ASD, and no intellectual disability, have normal availability of both total GABA_A receptors, and of the key GABA_A $\alpha 5$ receptor subtype. There were no correlations between GABA_A receptor availability and social cognitive task performance. Furthermore, strengthening our confidence in these findings, GABA_A availability was not altered in three common mouse models of ASD, namely *Cntnap2* knockout, *Shank3* knockout, and 16p11.2 deletion. Nonetheless, a visual perceptual proxy measure of cortical GABAergic signalling revealed evidence consistent with a *functional* GABA deficit in the ASD group in the [¹¹C]Ro15-4513 human study.

This is the first large *in vivo* study of GABA_A receptor availability in unmedicated adults with ASD. Our results contrast with reports of reduced GABA_A availability in children with ASD using SPECT.(22) The difference between those results and ours may indicate an age-specific alteration in GABA_A availability in ASD, but other methodological differences should also be considered. For instance, in the SPECT study the control group consisted of partial epilepsy patients, the majority of whom were medicated with anticonvulsants. In contrast, we recruited medication-free ASD and typically-developing control individuals in order to avoid any medical or medication confounds. The null findings in the present PET results also stands in contrast to our own pilot PET study(23) that reported reduced GABA_A $\alpha 5$ subtype availability in 3 ASD adults compared to 3 matched controls. It is possible that our prior finding was a Type I error.

While our results suggest that GABA_A receptor availability is not altered in ASD, other interpretations of our data should be considered. One possibility is that our studies lacked statistical power to detect any differences in GABA_A. Both of our PET studies were powered at 80% to detect an effect size typical of PET studies e.g. (44). A second possible explanation for our negative result is that the PET tracers we used were insensitive, yet we consider this unlikely as both [¹¹C]flumazenil and [¹¹C]Ro15-4513 have been used successfully to detect group differences in GABA_A receptor availability in other neuropsychiatric disorders,(44, 45) and in healthy controls differing in behavioral phenotype.(46)

While we found no detectable differences in GABA_A using [¹¹C]flumazenil and [¹¹C]Ro15-4513 PET in man, we did observe a significant alteration in performance on a visual perception task which has been proposed to index cortical GABAergic function.(39, 40) A number of other groups have reported similar findings in individuals with ASD.(47) This raises the possibility that the GABA system may be dysregulated in ASD, despite intact GABA_A receptor expression. For instance, it is possible that the synthesis and/or release of GABA is downregulated in ASD, leading to impaired GABAergic signaling.(48) Moreover

GABA_A receptors might be present in normal number – but be abnormally localized on postsynaptic neurons, affecting their functionality, as has been observed e.g. in epilepsy.(49) Future research could explore these possibilities.

Our rodent autoradiography results reflect our human findings. GABA_A was not altered in any of the three commonly used mouse genetic models of ASD we examined. This is the first study to examine GABA_A in any of these three models, but alterations in GABA_A might have been expected based on the functions of the affected genes. *Cntnap2* is a cell adhesion molecule belonging to neurexin family(50) and involved in dendritic arborization and spine development. While involved in excitatory synapse function, *Cntnap2* k/o also reduces inhibitory (GABAergic) transmission.(50) *Shank3* is another synaptic protein that plays a role in signalling to presynaptic buttons through the neuroligin-neurexin transsynaptic signalling complex,(51) and it has been linked to inhibitory signalling.(52, 53) Finally, 16p11.2 deletion is a chromosomal microdeletion associated with ASD in humans.(54) The gene(s) responsible for the phenotype are unclear, but synaptic genes may be key. (54) In contrast to our results, *increased* Ro15-4513 (but not muscimol) binding was recently reported in the left amygdala of valproate-exposed (VPA) rats.(55) Our study differed from that in a number of ways: we used mice not rats, we examined genetic rather than valproate-induced ASD models, and our animals were older, at 12 weeks of age vs. 7 weeks. Therefore our finding of no alterations in GABA_A tracer binding in any of the three models we examined does not rule out the possibility that other models might show such differences. We suggest, however, that our data at least establish that GABA_A alterations are not a shared phenotype across all ASD models.

Limitations of our research include the fact that due to prohibitions on exposing children to research PET, we were only able to recruit adults, so we are unable to exclude the possibility of abnormalities in GABA_A in children with ASD. Similarly, we were only able to recruit males in the [¹¹C]Ro15-4513 study, because of British regulations restricting the exposure of women of reproductive age to radiation for research. This limits the generalizability of our findings, although the majority of ASD cases are males.(3) Our patient population was also relatively high-functioning and free of medical comorbidities, with above average mean IQ scores and no epilepsy. While this represents a strength of the study in terms of avoidance of confounds, it also limits generalizability. It is possible that GABA_A receptor abnormalities are present in ASD individuals with epilepsy and/or intellectual disability. Testing this hypothesis using PET, however, could raise ethical issues over exposure to radiation in a vulnerable group who may be less able to give informed consent. Finally, we were able to selectively quantify GABA_A α5 subtype but could not perform the same procedure for other subtypes such as α2 and α3, owing to a lack of suitable tracers.

A further limitation of our study is that we included only a limited range (three) of mouse genetic models for autoradiography. GABA_A alterations in other models cannot be ruled out. It could furthermore be questioned whether the models we used are valid models for the phenotype of our human ASD participants. For instance, many humans with *Cntnap2*,

Shank3, and 16p11.2 mutations have epilepsy (see (56)), whereas we excluded people with epilepsy. However, we do not think that this is a key limitation, given that we report no difference in GABA_A in any of the models. Had we observed alterations in rodent GABA_A, it would have been difficult to exclude the possibility that they were correlates of epilepsy rather than of ASD *per se*, but the same concern does not apply to our null results. A final limitation of both PET and autoradiography is that they are only able to quantify the density of GABA_A receptors and not their functionality, for which purposes other methods such as electrophysiology would be required.

In conclusion, we used PET and autoradiography to investigate the inhibitory GABA system in adults with an ASD and mouse models of ASD. Our objective was to test a major prediction of the excitatory:inhibitory imbalance hypothesis, namely that GABA_A receptor availability is reduced in this disorder. We found normal availability of total GABA_A, receptors, and of the GABA_A non- α 5 and α 5 subtypes, in humans with ASD, and we recapitulated these findings in three diverse mouse models of ASD, although we did find behavioral differences consistent with a *functional* GABAergic deficit in humans.

Materials and Methods

Study Design

We carried out two human PET studies at two PET centers: Stockholm (Sweden) and London (UK). Each study was designed to compare GABA_A receptor availability in adults with ASD to adult controls. The two studies were intended to study a different aspect of GABA_A to provide convergent evidence. In Stockholm, we measured brain GABA_A receptor binding using PET with the non-selective tracer [¹¹C]flumazenil (also known as [¹¹C]Ro15-1788).⁽⁵⁷⁾ In London, we measured GABA_A non- α 5 and α 5 receptor availability using PET with the α 5-selective tracer [¹¹C]Ro15-4513. We included 15 (11 males) typically developing adult controls vs. 15 (11 males) ASD participants in the [¹¹C]flumazenil study in Stockholm, and a separate group of 16 (all males) typically developing controls vs. 12 (all males) ASD in the [¹¹C]Ro15-4513 study in London.

We also performed a quantitative autoradiography experiment to estimate GABA_A receptor density in brain slices from three mutant strains commonly used as ASD models, namely *Cntnap2* knockout, *Shank3* knockout, and 16p11.2df (deletion) (Jackson Laboratories, stock #013128). Control mice included wild type (WT) littermates. In all three models, there was an N=7-8 for the wild type mice and an N=8 for the knockouts. Randomization was not used as this was an observational study. PET and autoradiography image analysis and quantification was performed by individuals blind to group status.

Human PET: Participants and Recruitment

In Stockholm, the ASD participants were recruited via the Stockholm County healthcare services. In London the ASD participants were recruited from the Behavioural Genetics Clinic (BGC) at the Maudsley Hospital, a national referral service for the diagnosis of neurodevelopmental disorders. In each center, typically developing controls were recruited via local advertisements.

All participants were required to meet the following criteria: 1) Full Scale IQ of 70+ 2) Not currently taking any psychoactive medication, and no history of psychoactive medication use within at least the past six weeks. 3) No history of epilepsy, head injury or trauma with unconsciousness of more than five minutes, brain disease or infection, or serious medical illness by self-report. 4) No history of bipolar disorder, schizophrenia, drug or alcohol dependency or other DSM Axis I disorder 5) MRI safe 6) Physically in good health and suitable for PET according a brief physical examination. 7) For female participants, not pregnant. 8) Aged 18+.

In addition, participants in the ASD group were required to meet ICD-10 criteria for autism, and to have received a clinical diagnosis of ASD by an experienced psychiatrist involved in the study. Diagnoses were informed by the Autism Diagnostic Observation Schedule (ADOS) in all participants and where possible the Autism Diagnostic Interview – Revised (ADI-R) (10/12 ASD participants in the London sample.)

For details on past use of psychoactive medication, and a measure of functional impairment (employment status), see **Table S2**.

For the [¹¹C]flumazenil study in Stockholm, IQ was estimated from the Vocabulary and Matrix Reasoning subscales of the Wechsler Abbreviated Intelligence Scale IV (WAIS-IV), administered in Swedish.(58) For the [¹¹C]Ro15-4513 study in London, IQ was assessed using the English version of the Wechsler Abbreviated Scale of Intelligence (WASI) with four subscales: Vocabulary, Similarities, Block Design and Matrix Reasoning.

[¹¹C]flumazenil and [¹¹C]Ro15-4513 human PET data acquisition and processing

The following is a summary of the methods used in the two human PET studies. For more details, please see the Supplementary Material.

[¹¹C]flumazenil was prepared as previously described,(59) and injected in the antecubital vein as a 10 second bolus. [¹¹C]flumazenil PET was performed on a Siemens ECAT Exact HR 47 (CTI/Siemens, Knoxville, TN) run in 3D mode with Dual Energy Windows scatter correction. A 10 minute transmission scan was performed to correct for attenuation and scatter. PET data was collected in a dynamic scan lasting 63 minutes and binned into 26 time frames. PET images were reconstructed using filtered backprojection and corrected for head motion using a frame to first frames approach. T1 weighted MR images were coregistered to PET using SPM5. MRIs were segmented and regions of interest (ROIs) were delineated using Freesurfer.(60) Regional binding potential values (BP_{ND}) were calculated using the Simplified Reference Tissue Model (SRTM) with pons acting as reference region.

[¹¹C]Ro15-4513 was synthesised as previously described and injected in the dominant antecubital vein as a 15 second bolus. PET emission data was collected in 3D mode for 90 min. During the PET acquisition, arterial blood data were sampled via the radial artery using a combined automatic-manual approach. Dynamic PET data were corrected for inter-frame motion and aligned with the individual's structural T1 MR image. An atlas was coregistered on each subject's image space and a subset of regions including amygdala, hippocampus and nucleus accumbens, were considered. Two kinetic methods with metabolite-corrected plasma input functions were applied for the data analysis: (i) a two-tissue compartmental model (ii) a spectral-based quantification method, non-linear spectral analysis (NLSA) to separate GABA non- $\alpha 5$ and $\alpha 5$ -related components (V_1 and V_5 , respectively) from the total tracer uptake.(34)

Rodent autoradiography: overview and animal models

We performed quantitative autoradiography to estimate GABA_A receptor density in brain slices from *Cntnap2* knockout (*Cntnap2* *-/-*) (provided by Michael Saxe, Roche), *Shank3* knockout (*Shank3* *-/-*) (Michael Saxe, Roche) and 16p11.2df (deletion) (Jackson Laboratories, stock #013128). Control mice included wild type (WT) littermates. Mice were killed by decapitation, their brains rapidly removed and frozen in cooled isopentane. In order to approximate the patient demographics in the human ASD PET study as closely as possible, we used mature adult (12 weeks of age), male mice in all cases. In all three groups, there was an N=7-8 for the wild type mice and an N=8 for the knockouts.

Rodent autoradiography: protocol

Please see the Supplementary Material.

GABA visual task

In order to complement our PET measures of GABA_A receptor availability, we chose to investigate the *functional* balance between E:I neurotransmission using the paradoxical motion perception (PMP) task as a proxy measure. In this paradigm, high visual contrast normatively impairs perception of the direction of motion of sine wave grating stimuli, i.e. paradoxically, lower contrast stimuli are *easier* to perceive. This effect is believed(39) to reflect GABAergic lateral inhibition in the visual cortex.

GABA visual task: Method

During the PMP task, participants view brief presentations of a drifting sine wave gratings of varying size (small vs. large) and contrast (bright vs. faint). The task is to indicate the direction of motion. PMP stimuli were greyscale vertical sine-wave gratings masked with a Gaussian centred on the middle of the screen. Gratings had a spatial frequency of 1 cycle/degree of visual angle at the viewing distance of 50 cm. Each grating drifted at 2 degrees/second either left or right (randomly determined on each trial.) Stimulus size, defined as the spatial SD of the Gaussian mask, was 0.35 degrees for the small and 2.50 degrees for large stimuli. The bright stimuli used the maximum contrast of 127 provided by the Presentation software while the dim stimuli used a contrast of 5, which was chosen in piloting as the lowest contrast that was clearly perceptible to all participants.

There were 260 trials, 65 each of four trial types (small dim, small bright, large dim, large bright). Psychometric estimation was performed using a 2-up-1-down duration staircase. There were four staircases, one per stimulus type. Each staircase began at 250 milliseconds, with the step up (harder) at -14.1 milliseconds per 2 correct answers (i.e. the stimuli were made briefer), and step down (easier) was 18.2 milliseconds per 1 incorrect answer i.e. the stimuli were made longer. A failure to respond was counted as incorrect. Participants performed the task in a darkened room.

GABA visual task: Data Analysis

In line with previous work, contrast impairment on motion perception is defined as the ratio between the estimated threshold (in milliseconds) for large-bright compared to large-faint stimuli. Trial-by-trial results were analysed using a custom Microsoft Excel macro to detect reversals. We calculated perceptual thresholds for each stimulus condition by taking the mean of all of the reversals, as this is the best estimator of the psychometric threshold in a forced-choice staircase design.

Statistics

For all analyses, α was set at 0.05 and two-tailed tests were used. For the [¹¹C]flumazenil and [¹¹C]Ro15-4513 PET studies, group differences in estimated receptor availability (BP_{nd} and V_T respectively) were compared using an independent sample t-tests for each of the defined brain regions separately. Results are reported as uncorrected p-values, with remarks indicating whether significant results would survive Bonferroni correction over the number of brain regions within each study. For the PMP task, the magnitude of the contrast-induced motion perceptual impairment ratio was compared between groups using an independent samples t-test with Welch-Satterthwaite correction for unequal variances. For the rodent autoradiography, non-specific binding was subtracted from the total binding to give the specific binding values, which were compared between wild-type and mutant mice using unpaired t-tests corrected for multiple comparisons using Sidak correction.

Ethics

The [¹¹C]flumazenil study was approved by the Ethics and Radiation safety committees of the Karolinska Hospital and was performed in accordance with the Helsinki Declaration. The [¹¹C]Ro15-4513 PET experiment was approved by North London Research Ethics Committee 3, reference 10/H0709/90, and by the Administration of Radioactive Substances Advisory Committee (ARSAC) certificate 630 / 3764 / 28579. The PMP task was approved by Essex 2 National Research Ethics Committee (Reference 04-Q0102/26).

List of Supplementary Materials

Supplementary Methods & Results

Figure S1: Left and right amygdala autoradiography results

Table S1: PET tracer dosing in the two human studies

Table S2: Further information on functional outcome (employment status) and medication history in the ASD participants in the two PET studies

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Author Contributions: J.H. and M.A. performed statistical analysis and wrote the first draft of the paper. D.G.M. and J.B. designed the experiments. M.A.M., M.A., I.M., S.S. performed patient recruitment and carried out PET scanning, under the supervision of A.T., J.L., A.G., C.H., S.B., L.F., D.J.N., A.L.-H., and O.H. PET data analysis was performed by M.V., F.T. and M.A. J.H. and M.A.M. implemented and carried out the PMP task and analyzed these data. N.S., T.S., D.C. and K.H. carried out rodent autoradiography under the supervision of A.G. N.S. analyzed these data. D.G.M., O.H. and J.B. secured principle funding. All authors contributed to the final version of the manuscript.

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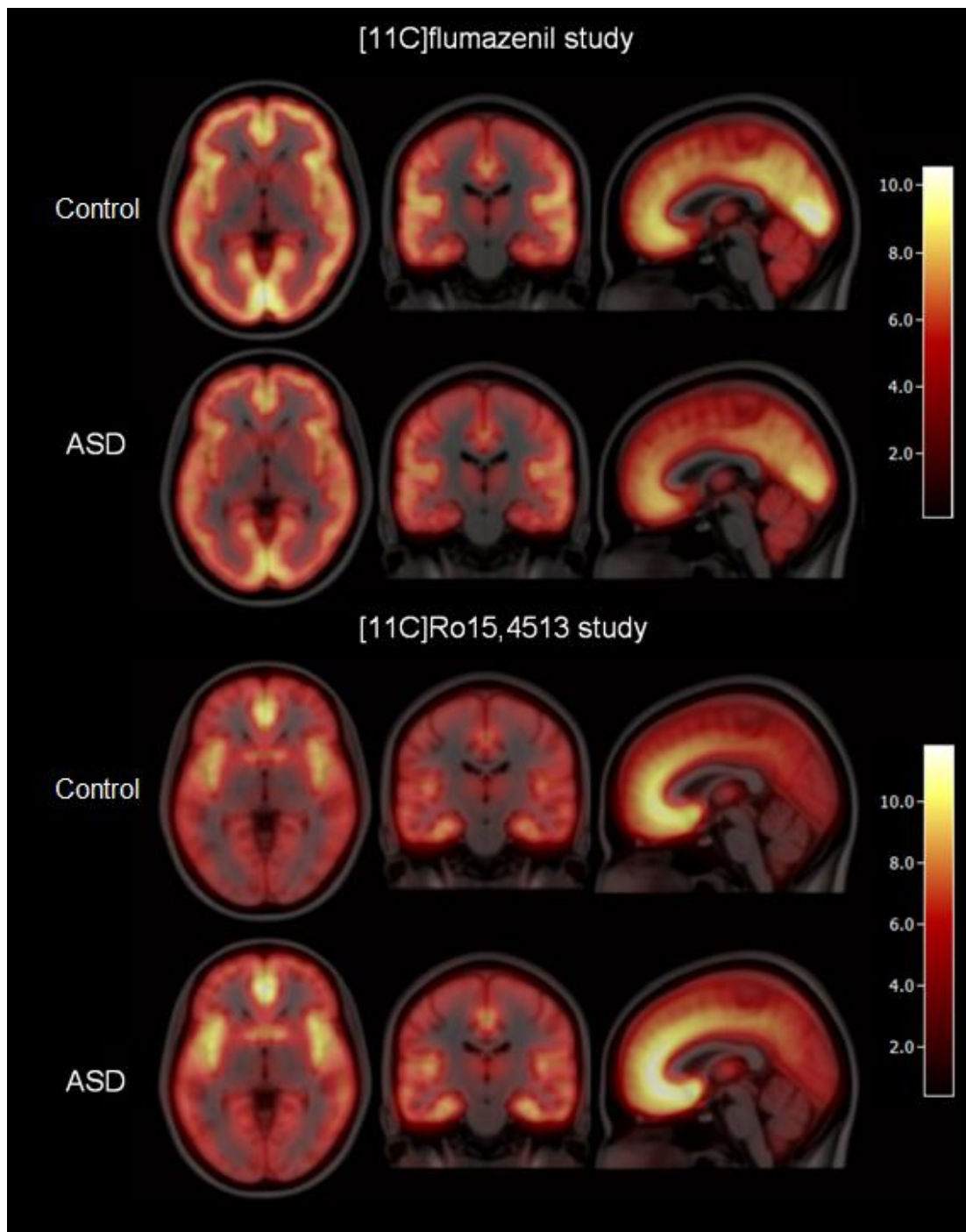
Figures

Figure 1. Estimated group mean $[^{11}\text{C}]$ flumazenil SRTM BP_{nd} and $[^{11}\text{C}]$ Ro15-4513 V_{T} in typically developing control adult and ASD adult samples. PET images were normalized into MNI-152 standard space, and are displayed overlaid on MNI-152 2mm resolution template anatomical MRI images. $[^{11}\text{C}]$ flumazenil $n=15$ control/15 ASD; $[^{11}\text{C}]$ Ro15-4513 $n=16$ control/12 ASD. V_{T} was calculated using the Logan method.

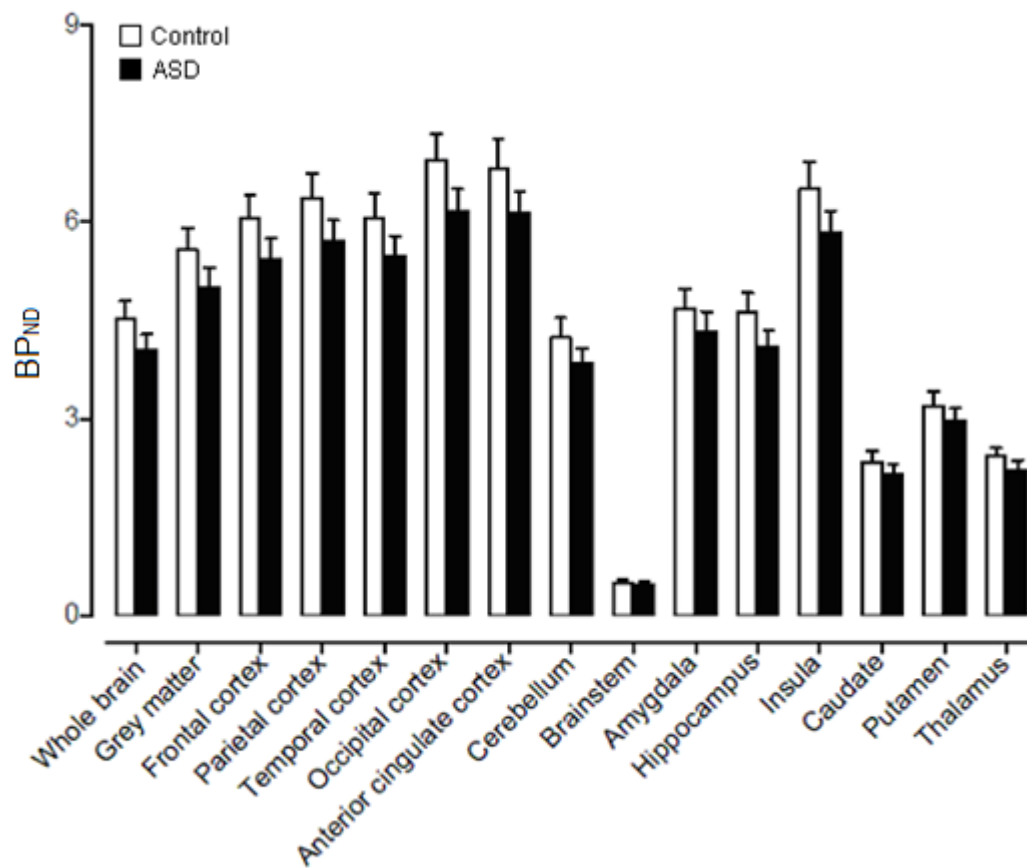


Figure 2. Estimated [¹¹C]flumazenil binding (mean estimated BP_{ND}) across the whole brain and in subdivisions (regions) of the brain in typically developing controls and ASD participants in the Stockholm sample. n=15 control/15 ASD.

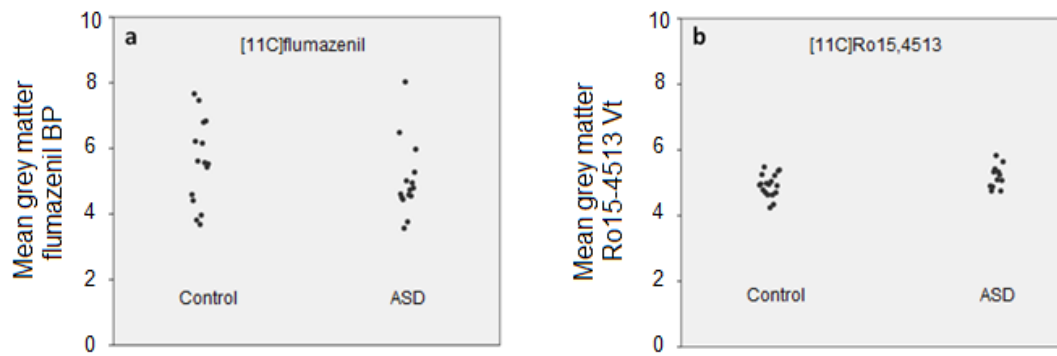


Figure 3. Scatterplots illustrating (a) estimated mean grey matter flumazenil BP_{ND} (b) estimated mean grey matter $[^{11}C]$ Ro15-4513 V_T for individual participants in the typically developing control and ASD adult groups. There were no evident outliers or subgroups with either tracer.

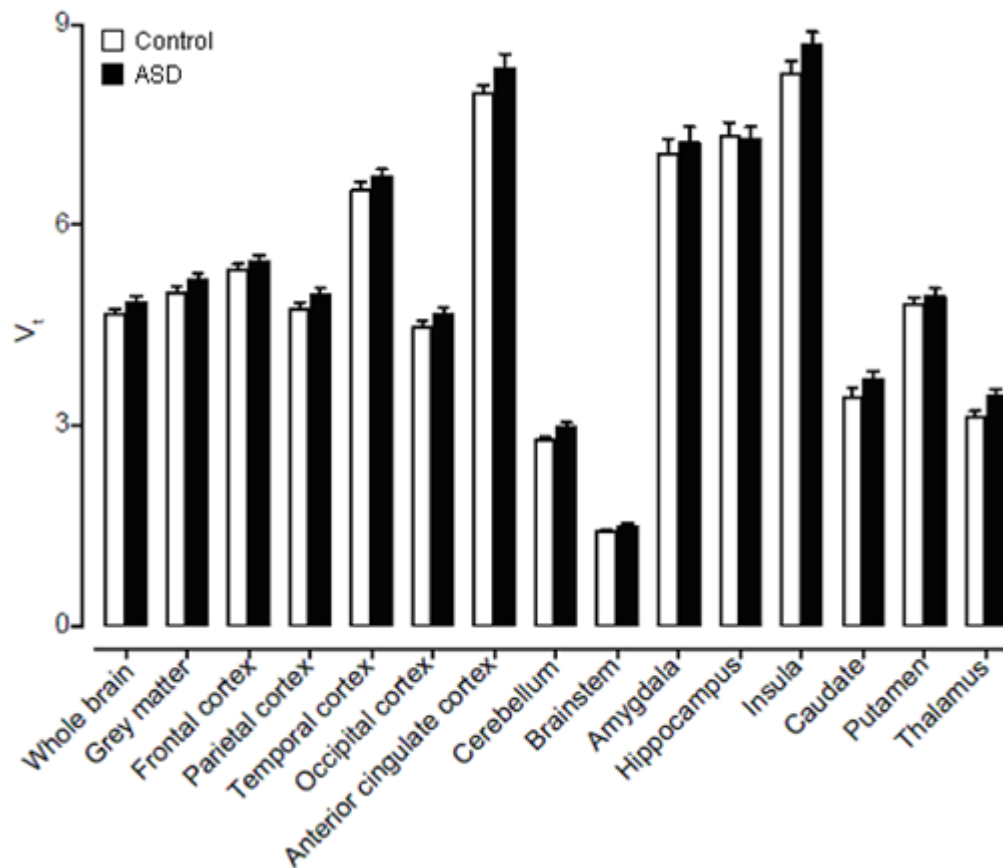
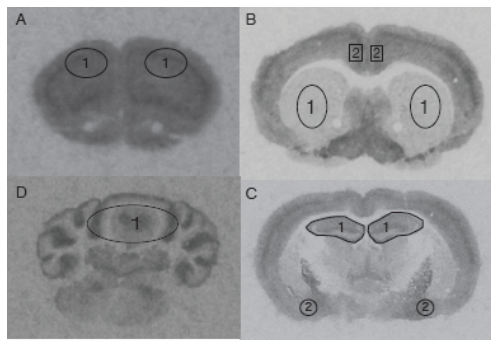


Figure 4. Estimated [¹¹C]Ro15-4513 specific binding, derived from two compartmental model (2TCM) of PET data, showing mean estimated V_T across the whole brain and in subdivisions (regions) of the brain in typically developing controls and ASD participants in the London sample. n=16 control/12 ASD



Representative images showing the brain levels used for analyses. Regions were defined based on Paxinos and Franklin's mouse brain atlas (2001). The numbers represent the specific regions that were analysed. Averages were calculated from both hemispheres.

A, 1 = frontal cortex

B, 1 = caudate putamen, 2 = cingulate cortex

C, 1 = dorsal hippocampus; 2 = amygdala

D, 1 = cerebellum

□ Wild-type

■ Transgenic

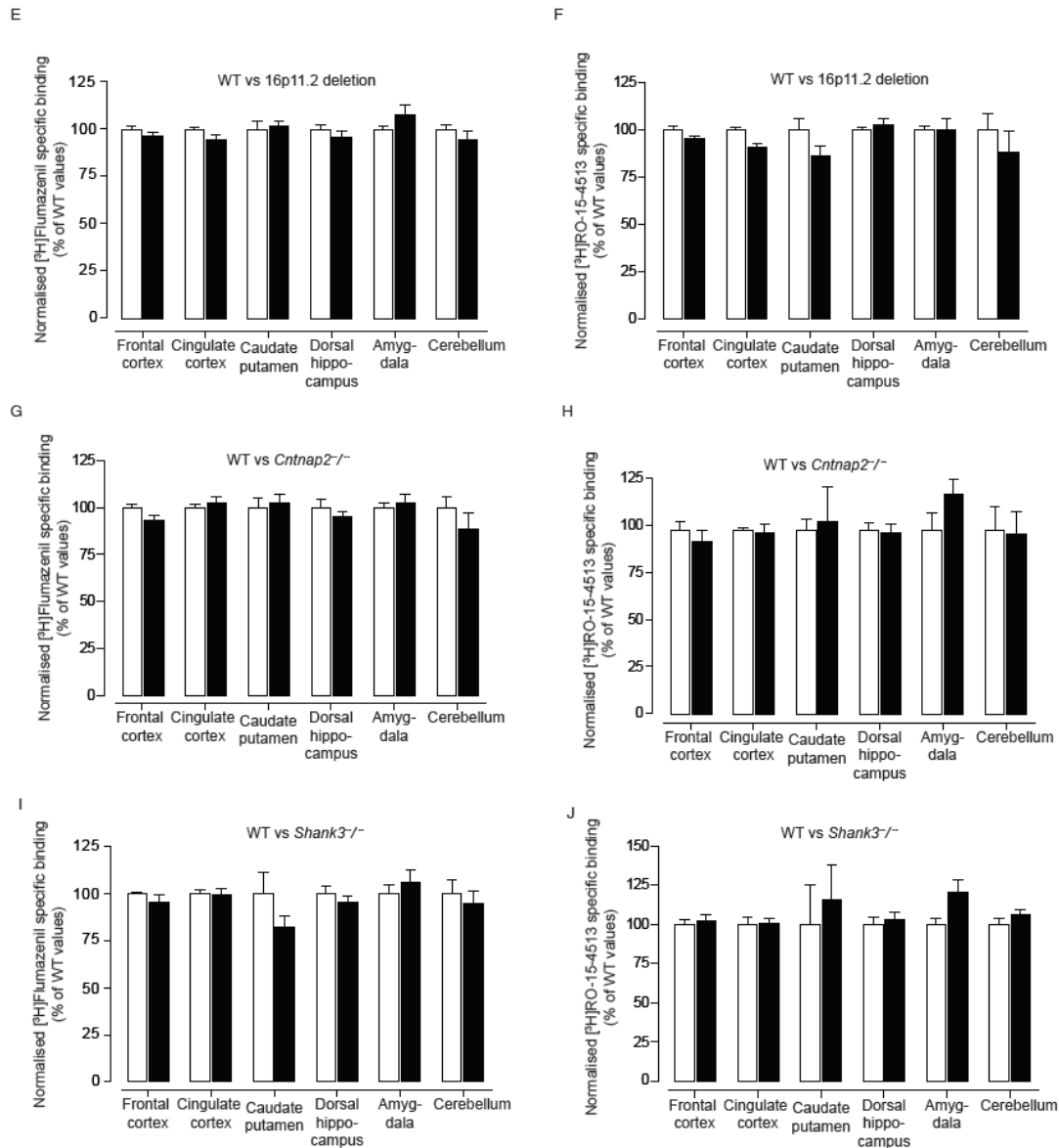


Figure 5. Mouse autoradiography results. A,B,C,D: examples of the brain levels used. E,G,I: [³H]flumazenil specific binding. F,H,J: [³H]Ro15-4513 specific binding. E,F: wild type (WT) vs. 16p11.2 deletion mice. G,H: WT vs. *Cntnap2*^{-/-} mice. I,J: WT vs. *Shank3*^{-/-} mice. Bars show group mean signal, normalized such that the mean signal in the WT group = 100%. Error bars show SEM.

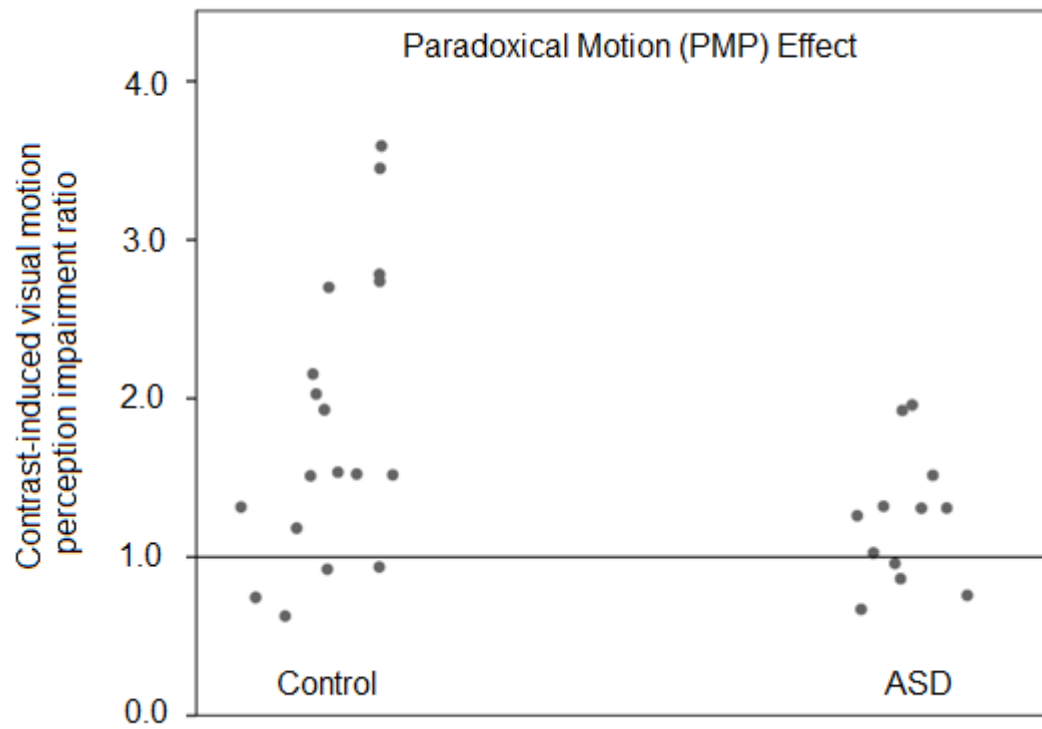


Figure 6. Contrast-induced motion perceptual impairment ratio in each participant, with higher values indicating more impairment. Impairment is considered a proxy for GABA-mediated contrast inhibition in the visual cortex.

Tables

Measure	Stockholm Flumazenil PET study			London Ro15-4513 PET study		
	Control	ASD	P-value (t-test)	Control	ASD	P-value (t-test)
Number Male / Female	11 / 4	15 / 0	NA	16 / 0	12 / 0	NA
Age	33.0 ± 9.4	33.0 ± 9.1	1.000	29.8 ± 9.1	30.7 ± 8.8	0.684
Full Scale IQ	113.1 ± 15.4	106.9 ± 17.9	0.318	122.7 ± 24.5	116.0 ± 13.9	0.256
ADI Social	NA	NA	NA	NA	15.5 ± 6.1	NA
ADI Communication	NA	NA	NA	NA	12.1 ± 7.0	NA
ADI Repetitive	NA	NA	NA	NA	4.5 ± 2.6	NA
ADOS Social	NA	7.5 ± 5.1	NA	NA	7.7 ± 2.1	NA
ADOS Communication	NA	6.9 ± 3.2	NA	NA	3.9 ± 1.8	NA

Table 1. PET participant demographics and ASD clinical scores from the two experimental studies. Values are shown as Mean ± Standard Deviation. P-values are the outcome of independent sample t-tests testing for a difference in group means, within each study. Clinical scores are not available for typically developing control participants. ADI scores were not available for the Stockholm cohort.