

Serotonin and the Neural Processing of Facial Emotions in Adults With Autism

An fMRI Study Using Acute Tryptophan Depletion

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Context: People with autism spectrum disorders (ASDs) have lifelong deficits in social behavior and differences in behavioral as well as neural responses to facial expressions of emotion. The biological basis to this is incompletely understood, but it may include differences in the role of neurotransmitters such as serotonin, which modulate facial emotion processing in health. While some individuals with ASD have significant differences in the serotonin system, to our knowledge, no one has investigated its role during facial emotion processing in adults with ASD and control subjects using acute tryptophan depletion (ATD) and functional magnetic resonance imaging.

Objective: To compare the effects of ATD on brain responses to primary facial expressions of emotion in men with ASD and healthy control subjects.

Design: Double-blind, placebo-controlled, crossover trial of ATD and functional magnetic resonance imaging to measure brain activity during incidental processing of disgust, fearful, happy, and sad facial expressions.

Setting: Institute of Psychiatry, King's College London, and South London and Maudsley National Health Service Foundation Trust, England.

Participants: Fourteen men of normal intelligence with autism and 14 control subjects who did not significantly differ in sex, age, or overall intelligence.

Main Outcome Measures: Blood oxygenation level-dependent response to facial expressions of emotion.

Results: Brain activation was differentially modulated by ATD depending on diagnostic group and emotion type within regions of the social brain network. For example, processing of disgust faces was associated with interactions in medial frontal and lingual gyri, whereas processing of happy faces was associated with interactions in middle frontal gyrus and putamen.

Conclusions: Modulation of the processing of facial expressions of emotion by serotonin significantly differs in people with ASD compared with control subjects. The differences vary with emotion type and occur in social brain regions that have been shown to be associated with group differences in serotonin synthesis/receptor or transporter density.

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AUTISM SPECTRUM DISORDER (ASD) is a highly genetic neurodevelopmental disorder affecting between 63¹ and 116 per 10 000 people.²

A core diagnostic feature of ASD is impaired social communication.³ Adaptive social behavior is key to human survival and highly dependent on the ability to identify and respond to emotional information from the environment, especially faces.⁴ There is substantial evidence that people with ASD have deficits in recognizing and responding to facial expressions of emotion.⁵ However, the biological basis of these deficits is not fully understood.

Behavioral studies of facial emotion processing in people with ASD report a range of impairments that may contribute to clinically observed deficits in social communication and behavior. For instance, faces are less salient for individuals with ASD,^{6,7} and some (but not all) studies⁸⁻¹³ have reported that individuals with ASD have impairments in recognition of primary emotions as compared with control subjects. Neuroimaging studies of facial emotion processing suggest that, compared with healthy control subjects, people with ASD have functional differences in a network of face-processing regions, including hypoactivation of the extrastri-

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Table 1. Demographic Characteristics of Study Participants

Characteristics	Mean (SD) [Range]	
	Control (n = 14)	ASD (n = 14)
Age, y	31 (11) [16-50]	31 (13) [16-57]
WAIS-III full-scale IQ	123 (20) [77-147]	115 (13) [88-135]
WAIS-III verbal IQ	124 (21) [83-147]	112 (12) [84-131]
WAIS-III performance IQ	120 (23) [76-153]	118 (14) [95-139]
AuQ	12 (5) [3-21]	31 (9) [15-42] ^a
AgQ	78 (18) [46-109]	110 (22) [73-149] ^a
OCI-R	10 (10) [1-39]	25 (15) [5-61] ^a
BDI placebo day	3 (4) [0-11]	10 (7) [0-11] ^a
BDI ATD day	2 (2) [0-6]	9 (9) [0-34] ^a
BAI placebo day	3 (3) [0-9]	11 (13) [0-53] ^a
BAI ATD day	2 (2) [0-7]	10 (14) [0-56] ^a

Characteristics	Mean (SD) [Range]	
	Control	ASD (n = 12)
ADI-R social domain (cutoff for ASD, 10)	NA	17 (11) [10-23]
ADI-R communication domain (cutoff for ASD, 8)	NA	11 (4) [8-22]
ADI-R stereotypy domain (cutoff for ASD, 3)	NA	5 (2) [3-8]

Abbreviations: ADI-R, Autism Diagnostic Inventory-Revised; AgQ, Aggression Quotient; ASD, autism spectrum disorder; ATD, acute tryptophan depletion; AuQ, Autism-Spectrum Quotient; BAI, Becks Anxiety Inventory; BDI, Becks Depression Inventory; IQ, intelligence quotient; OCI-R, Obsessive-Compulsion Inventory-Revised; WAIS, Wechsler Adult Intelligence Scale.

^a $P < .05$ *t* test—significant difference between groups.

ate and limbic areas.¹⁴⁻²³ The biological basis for these behavioral and imaging differences is unknown but may include modulatory effects of serotonin. For example, in healthy populations, there is increasing evidence that serotonin modulates social communication²⁴ and the functioning of brain systems crucial to facial emotion processing.^{25,26} Furthermore, there is preliminary evidence of abnormalities of the serotonergic system in ASD²⁷; for example, a significant proportion of people with ASD may have hyperserotonemia.²⁸⁻³³ And significant associations between ASD and genetic polymorphisms for serotonin synthesis,³⁴ transporters,³⁵ and receptors³⁶ have been reported. Brain levels of serotonin are dependent on the blood concentration of its chemical precursor tryptophan, which is in direct competition with other large neutral amino acids to cross the blood-brain barrier,^{37,38} and it has been reported that people with ASD, compared with control subjects, have a significantly lower ratio of tryptophan to other large neutral amino acids³⁹ and that reducing the availability of dietary tryptophan leads to worsening autistic symptoms in adults with ASD.^{40,41} Additionally, there are preliminary neuroimaging reports that individuals with ASD have significant differences from control subjects in serotonin synthesis^{42,43} as well as reductions in serotonin_{2A} receptor and serotonin transporter binding in brain regions, including the cingulate cortices, which are involved in social communication.^{44,45} In summary, prior research suggests that the serotonin system is altered in some individuals with ASD and

that this may be related to impairments in social functioning. However, the modulatory effect of serotonin on the brain activity of people with ASD during social cognition tasks—for example, when processing facial emotions—is unknown.

In healthy individuals, pharmacologic functional magnetic resonance imaging (fMRI) studies of facial emotion processing using acute tryptophan depletion (ATD), a non-invasive technique for reducing serotonin levels in the brain,^{46,47} reported that serotonin²⁶ modulates function of face-processing regions during incidental processing of facial expressions of emotion but this varies with emotion type.^{25,48-51} For example, our prior study of facial emotion processing using fMRI and ATD in healthy individuals found that ATD is associated with increased activation in face processing regions of cingulate cortex and inferior frontal gyrus compared with non-ATD (placebo) in fearful and happy faces, but decreased activity in these brain regions to expressions of disgust.

Brain regions involved in the processing of facial expressions of emotion (1) show differences in activation in people with ASD compared with healthy control subjects, (2) demonstrate modulation of activation by manipulation of brain serotonin levels in healthy control subjects, and (3) are associated with serotonin abnormalities in people with ASD such as synthesis differences and decreased receptors and transporters. These regions encompass the frontal lobe (eg, inferior frontal gyrus), temporal lobe (eg, fusiform gyrus), extrastriate cortex (eg, lingual gyrus), and limbic regions (eg, cingulate gyrus and insula). However, to our knowledge, no one has investigated the modulatory effect of serotonin on neural activity when individuals with ASD process facial emotions. Hence, we compared the role of serotonin in emotion processing in adults with autism and control subjects using ATD and fMRI during incidental processing of 4 primary emotional expressions depicting fear, sadness, happiness, and disgust. We tested the main hypothesis that there are significant group differences in the effects of ATD on blood oxygenation level-dependent (BOLD) response to facial expressions of emotion in relevant brain regions, including inferior frontal, fusiform, lingual and cingulated gyri, and insula.

METHODS

SUBJECTS

Fourteen right-handed men with ASD (mean [SD] age, 31 [13] years; range, 16-57 years; mean [SD] Full-Scale Intelligence Quotient, 115 [13]) were recruited through a clinical research program at the Maudsley Hospital/Institute of Psychiatry (London) (**Table 1**). An ASD diagnosis was based on application of International Classification of Diseases and Related Health Problems, 10th revision, research criteria³ and confirmed using the Autism Diagnostic Interview-Revised⁵² to ensure that all participants with ASD met the criteria for childhood autism. We were unable to obtain an Autism Diagnostic Interview for 2 of the subjects. In these 2 cases, the diagnosis was confirmed using the Autism Diagnostic Observational Schedule.⁵³ All cases of ASD with an Autism Diagnostic Interview-Revised reached algorithm cutoffs in the 3 domains of impaired reciprocal social interaction, communication and repetitive behaviors, and stereotyped patterns. For

the Autism Diagnostic Observational Schedule, subjects had to reach the total algorithm cutoff score, although failure to reach cutoff in 1 of the domains by 1 point was permitted.

We also included 14 healthy, right-handed men as control subjects, recruited from local advertisements, who did not differ significantly in age (mean [SD] age, 31 [11] years; range, 16-50 years) or overall intelligence (mean [SD] Full-Scale Intelligence Quotient, 123 [20]) as measured by the Wechsler Adult Intelligence Scale-III from our ASD sample.⁵⁴

All subjects underwent routine clinical and genetic screening blood tests to ensure good medical health and a structured clinical examination to exclude psychiatric illness (eg, schizophrenia and major depression), head injury, genetic disorder associated with autism (eg, fragile X syndrome or tuberous sclerosis), and neurologic or medical disorders that might affect brain function (eg, epilepsy or hypertension). None of the participants were abusing alcohol or taking any illicit drugs; and none were taking antipsychotic medication, mood stabilizers, or benzodiazepines. After receiving a description of the study, all subjects gave written, informed consent. This study was approved by the ethical committee of the South London and Maudsley National Health Service Foundation Trust and the Institute of Psychiatry, King's College London.

DEPLETION/PLACEBO PROCEDURE

Subjects were tested on 2 separate occasions separated by 1 week (range, 0.6-3 weeks). After fasting from midnight, at around 8:30 AM (approximately 5 hours prior to scanning), subjects drank a 100-g amino acid mixture that contained 15 large neutral amino acids but lacked aspartic and glutamic acid to avoid possible toxicity⁵⁷ and either contained 2.3 g of tryptophan for the placebo condition or no tryptophan for the ATD condition. In a double-blind, counterbalanced, crossover design, subjects were assigned to order of drink consumption (placebo or ATD on first visit) (For amino acid recipe, see eAppendix; <http://www.archgenpsychiatry.com>).

SERIAL MONITORING OF AFFECTIVE STATE AND BLOOD CHEMISTRY

Prior to the start of the study, participants completed baseline autistic traits-related self-assessment measurements using the Autism-Spectrum Quotient⁵⁵ and the Obsessive-Compulsive Inventory-Revised.⁵⁶ In addition, they completed the Aggression Questionnaire⁵⁷ to screen out high aggression, anger, and hostility—given that the ATD procedure may increase levels of aggression in susceptible individuals.^{58,59} Before consumption of an amino acid drink at each session, participants also completed the Beck Depression Inventory⁶⁰ and the Beck Anxiety Inventory.⁶¹ Between-group differences for the Autism-Spectrum Quotient, Obsessive-Compulsive Inventory-Revised, Aggression Questionnaire, Beck Depression Inventory, and Beck Anxiety Inventory were examined using the independent-samples *t* test.

Blood sampling to measure tryptophan levels were collected before drinking the amino acid mixture (baseline) and then prior to scanning (typically 4.5 hours after ingestion). This timeframe is considered to be optimal for capitalizing on the effects of ATD on behavioral measures, blood plasma levels,^{58,62} and brain serotonin synthesis.^{63,64} Total plasma tryptophan levels were determined from each blood sample using previously described methods.⁶⁵ Self-report visual analogue scales were administered before (baseline) and 4.5 hours after (follow-up) ATD or placebo drink ingestion to measure mood, aggression, and physical symptoms thought to be associated with ATD including nausea, dizziness, irritability, and anxiety (eAppen-

dix). Differences between points on the tryptophan plasma levels and visual analogue scale measures were examined with a mixed between-within subjects analysis of variance. Statistical tests were run in SPSS PASW17 (<http://www-01.ibm.com/software/analytics/spss/products/statistics/>).

fMRI EMOTIONAL FACE PARADIGM

Five hours after ingestion of the amino acid drink, each subject participated in 4 6-minute sessions employing event-related fMRI.⁶⁶ In each experiment, subjects were presented with facial expressions of 1 of 4 primary emotions (happy, sad, disgust, or fear) and neutral expressions from a standardized series of prototypical facial expressions posed by 10 different volunteers in a randomized order.⁶⁷ This incidental facial emotion processing task required subjects to decide on the sex of each face and indicate their decision by pressing 1 of 2 buttons accordingly with the right thumb. In prescan testing, all subjects were able to identify the sex of the faces at an accuracy of 100% (eAppendix and eFigure 1).

fMRI ACQUISITION

Magnetic resonance images were acquired using a GE Signa 1.5 T Horizon LX system (General Electric) at the Maudsley Hospital, London, England. A quadrature birdcage headcoil was used for radiofrequency transmission and reception. A gradient echo echoplanar imaging data set was acquired that provided whole-brain coverage and was later used to register the fMRI data sets acquired from each individual in standard stereotactic space. Each functional imaging run consisted of 180 T2-weighted images acquired at each of 16 near-axial noncontiguous 7-mm thick planes parallel to the anterior commissure-posterior commissure line (echo time, 40 ms; time to repeat, 2 s, in-plane resolution, 3 mm; interslice gap, 0.7 mm; matrix size, 64 × 64 pixels).

fMRI IMAGE ANALYSIS XBAM

Data were analyzed with the XBAM version 4 software developed at the Institute of Psychiatry, London, using a nonparametric approach (<http://www.brainmap.co.uk>).

A brief overview of the method follows; however, a more detailed version can be found in the eAppendix.

Within each run, every point was realigned to the mean of all the images in the run to remove subject-induced motion artifacts,⁶⁸ then smoothed using a Gaussian filter (full-width at half maximum, 7.2 mm) to improve the signal to noise characteristics of the images. Using a wavelet-based resampling method for functional MRI data, a time series analysis was conducted on each individual subject to compute their sum of squares ratio reflecting the BOLD effect.⁶⁹ These individual maps were registered into standard Talairach space using rigid body and affine transformations.⁷⁰ Group brain activations maps were computed for each experimental condition, with hypothesis testing performed by voxel or cluster-level analyses giving excellent type 1 error control.⁷¹ Using data-driven, permutation-based methods with minimal distributional assumptions, we performed time-series analyses for group maps and intergroup permutation for within/between-group analysis of variance (ANOVA) to compute the distribution of the sum of squares ratio under the null hypothesis.⁷² Thresholding to the required level of significance was then performed, first at a voxel-wise *P* value of .05, then grouped into 3-dimensional clusters followed by determination of a cluster-level null distribution with a *P* value producing <.99 false positive over the whole brain.

Table 2. Plasma Tryptophan Levels and Reaction Times

Drink	Period	Variable	Mean (SD)	
			Control	ASD
Placebo ^a	Pre-drink	Plasma tryptophan ^b	71 (10)	78 (13)
	Post-drink ^c	Plasma tryptophan ^b	140 (54)	190 (77) ^d
		Sad reaction time, s	1.2 (0.2)	1.1 (0.2) ^d
ATD	Pre-drink	Plasma tryptophan ^b	75 (13)	76 (5)
	Post-drink ^c	Plasma tryptophan ^b	20 (12)	23 (9) ^d
		Sad reaction time, s	1.0 (0.2)	1.2 (0.3) ^d

Abbreviations: ASD, autism spectrum disorder; ATD, acute tryptophan depletion.

^aPlacebo drink was a balance amino acid drink.

^bMeasured in millimoles per liter.

^cMeasured 4.5 hours after drink.

^dAnalysis of variance interaction drink time: $P < .005$.

ANOVA Testing

We generated sum of squares ratio maps of the BOLD response to facial expressions of emotion by subtracting the contrast of the neutral expression (vs fixation cross) from the prototypic (100%) facial emotion expression (vs fixation cross) for each emotion type. These data were then subjected to ANOVA testing.

Interactions Between Tryptophan Status (Placebo vs ATD) and Group Membership (Control vs ASD) for Individual Emotion Type

A 2 group (control vs ASD) \times 2 tryptophan condition (placebo vs ATD) factorial repeated-measures ANOVA analysis was conducted. In other words, group membership (control vs ASD) was used as the between-condition variable for each emotion type and tryptophan status (placebo vs ATD) as the within-condition variable. Group \times tryptophan condition interactions refer to brain regions in which the effect on the BOLD response to the facial emotion expression is different in each group, depending on tryptophan condition (placebo or ATD).

Main Effect of Group (Control vs ASD) and Tryptophan Status (Placebo vs ATD) on BOLD Response

If no interaction was found in the 2 group \times 2 tryptophan condition factorial repeated-measures ANOVA, then the main effect of group was derived.

From each emotion type interaction or main effect of group, XBAM extracted each subject's mean BOLD signal responses (sum of squares) found within the significant clusters and these values were exported to and graphed using SPSS.

Post-hoc Tests of Effect of Tryptophan Status (Placebo vs ATD) on BOLD Response to Prototypic Expressions Within Each Group (Control vs ASD)

Post-hoc repeated-measure 1-way ANOVAs for each prototypic emotional expression, contrasting the neural responses of the placebo to those of the ATD conditions, were undertaken to determine the modulatory effects of tryptophan status on BOLD response to emotion expressions associated with the interaction effects. These ANOVAs were restricted to regions of interests (ROIs) that demonstrated significant interactions between group (control vs ASD) and tryptophan status (placebo vs ATD) for each emotion type.

fMRI Task Response Measures

Sex response accuracy and response time to sex decision were acquired along with the fMRI data. A mixed between-within subjects ANOVA was run in SPSS to examine differences of sex accuracy and/or response time.

RESULTS

BASELINE MEASURES

Subject demographics can be found in Table 1. Control subjects scored within the normal range on all measures taken at baseline (Aggression Questionnaire, Autism-Spectrum Quotient, and Obsessive-Compulsive Inventory-Revised) and pre-drink (Beck Depression Inventory and Beck Anxiety Inventory). Subjects with ASD scored significantly higher on all 5 measures compared with control subjects (for all t tests, $P < .04$) (Table 1).

TRYPTOPHAN BLOOD LEVELS

There were no significant differences in total plasma tryptophan concentration across groups on either day at baseline (mean [SD], 75 [11] $\mu\text{mol/L}$; to convert to milligrams per deciliter, divide by 48.967). As expected, in each group, there was a significant interaction between tryptophan status (placebo vs ATD) and time (baseline vs 4.5 hours) (control subjects: Wilks lambda=0.04; $F_{3,11}=80$; $P < .001$ and ASD: Wilks lambda=0.03; $F_{3,11}=140$; $P < .001$). After 4.5 hours, consumption of the placebo drink significantly increased the total plasma tryptophan concentrations in both control subjects and those with ASD. Following the ATD drink, the total plasma tryptophan concentrations were significantly reduced in both control subjects and those with ASD (**Table 2**).

AFFECTIVE MEASURES AND fMRI TASK RESPONSES

Despite between-group differences in baseline measures of aggression, depression, and anxiety, there were no significant differences in reported adverse effects (ie, nausea or vomiting) or visual analogue scale measurements caused by the consumption of the amino acid drink (eTable 1).

fMRI MOVEMENT AND TASK RESPONSES

There were no group by drink differences in the x , y , or z movement parameters and none of the subjects exceeded maximum displacement of more than 1 mm. For the fMRI task, there were no significant differences for percentage of correct sex decision responses or response times for the disgust, fear, and happy stimuli. However, for the sad task response time, there was a significant interaction between tryptophan status and group (Wilks lambda=0.6; $F_{2,27}=18$; $P < .005$; partial eta squared=0.41) such that control subjects were faster on the ATD day and those with ASD were slowed by ATD (Table 2).

Table 3. Anatomical Location of ANOVAs: Interactions (Tryptophan Status and Group) and Main Effect of Group

Emotion	x, y, z	Size, voxels	P Value	Region	BA
Disgust	-7, 30, -18	923	.007	L medial frontal gyrus	11
	-4, -11, 53	482	.009	L medial frontal gyrus	6
	-7, -82, -2	388	.03	L lingual gyrus	18
Happy	-4, 48, 37	649	.02	L medial frontal gyrus	9
Sad	54, 19, 42	892	.009	R middle frontal gyrus	8
	18, 22, -7	265	.04	R putamen	
Main Effect of Group (Control vs ASD)					
Fear	-29, -67, -4	1365	<.001	L lingual gyrus	18

Abbreviations: ANOVA, analysis of variance; ASD, autism spectrum disorder; ATD, acute tryptophan depletion; BA, Brodman area; L, left; R, right.

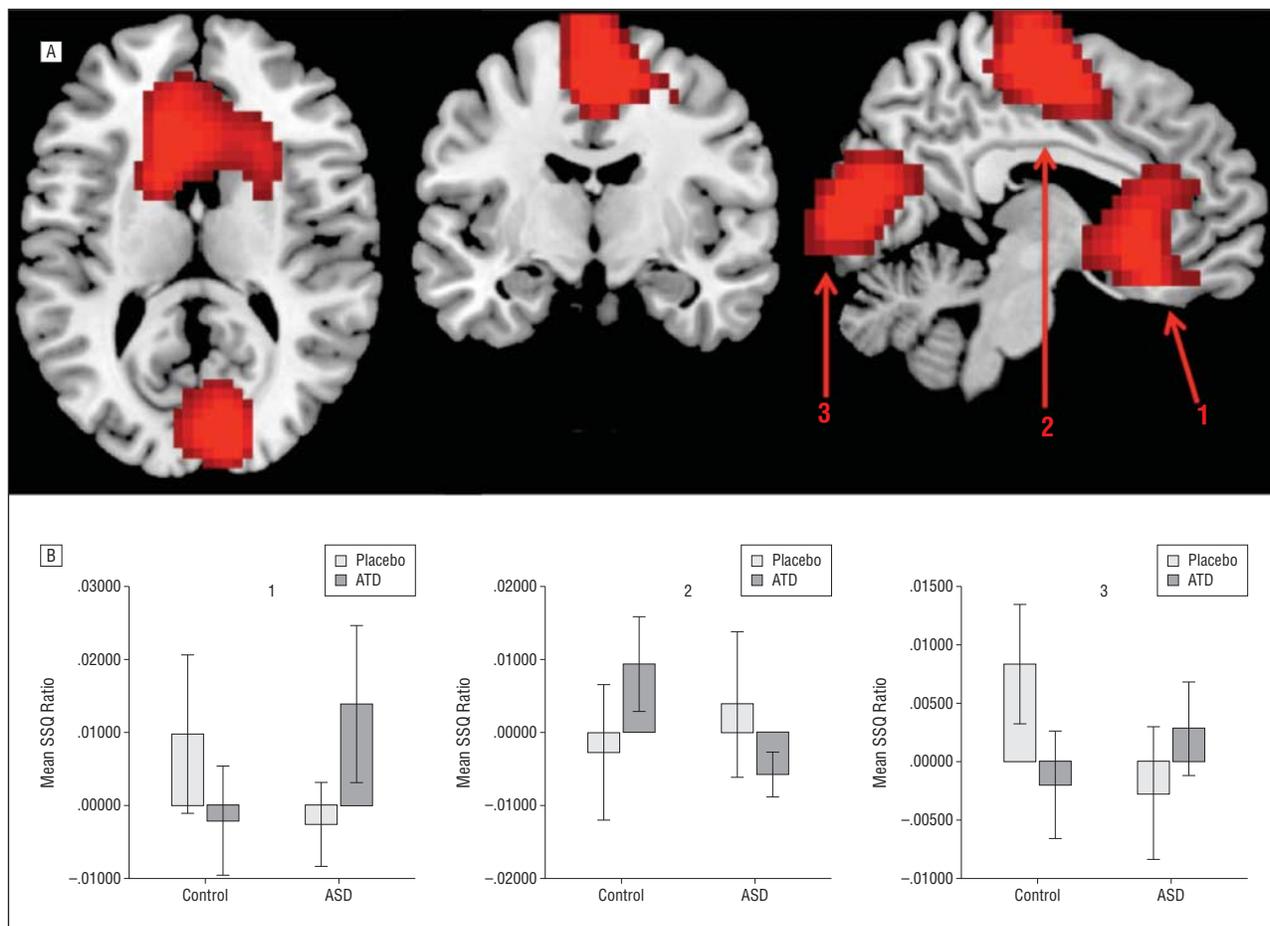


Figure 1. Disgust facial expression experiment. A, Maps of interaction of serotonin status (placebo vs acute tryptophan depletion [ATD]) by group (control vs autism spectrum disorder [ASD]) from the disgust experiment. B, Plot of mean blood oxygenation level–dependent signal (sum of squares [SSQ]) extracted from each disgust interaction cluster: (1) Left medial frontal gyrus Brodman area 11; (2) Left medial frontal gyrus Brodman area 6; and (3) Left lingual gyrus. Error bars=95% confidence intervals. Right side of figure is left side of brain.

fMRI RESULTS

Group Brain Activation Maps

Regardless of group and emotion type, the contrasts of prototypic (100% intensity) facial expressions vs fixation cross under the placebo condition revealed significant activation in established components of face-processing networks, including fusiform and extrastriate cortices; insula; and superior temporal, cingulate, and medial frontal gyri (eTable 1).

ANOVA Mapping

Significant interaction effects between tryptophan status (placebo vs ATD) and group membership (control vs ASD) were observed in the disgust, happy, and sad expression experiments, and a significant main effect of group was observed in the fear experiment. Each effect cluster was identified by the Talairach coordinates of the centroid of the cluster but were also described by structures within and extending to the boundary of the entire cluster.

Table 4. Anatomical Location of ANOVAs: Post-Hoc Repeated-Measure 1-Way ANOVA^a

Emotion	Control				ASD					
	x, y, z	Size, voxels	P Value	Region	BA	x, y, z	Size, voxels	P Value	Region	BA
Disgust	4, 33, 4	622	.006	R anterior cingulate	24	22, 7, 31	409	.02	R cingulate gyrus	32
	7, 4, 48	361	.004	R medial frontal gyrus	6	-4, -15, 48	122	.05	L medial frontal gyrus	6
	-4, -82, 4	102	.003	L lingual gyrus	18	-7, -70, 15	293	.01	L cuneus	18
Happy	4, 63, 9	105	.04	R medial frontal gyrus	10	4, 67, -7	40	.05	R medial frontal gyrus	10
	4, 22, 20	266	.04	R anterior cingulate	32	-7, 48, 31	198	.007	L medial frontal gyrus	9
Sad	-4, -22, 59	346	.04	L medial frontal gyrus	6	29, 19, 48	378	.03	R middle frontal gyrus	8
	43, -7, 37	163	.05	R precentral gyrus	6					
	22, 22, -2	56	.05	R putamen		14, 22, -7	190	.04	R putamen	

Abbreviations: ANOVA, analysis of variance; ASD, autism spectrum disorder; ATD, acute tryptophan depletion; BA, Brodman area; L, left; R, right.
^aANOVAs: bold font, placebo>ATD; regular font, placebo<ATD.

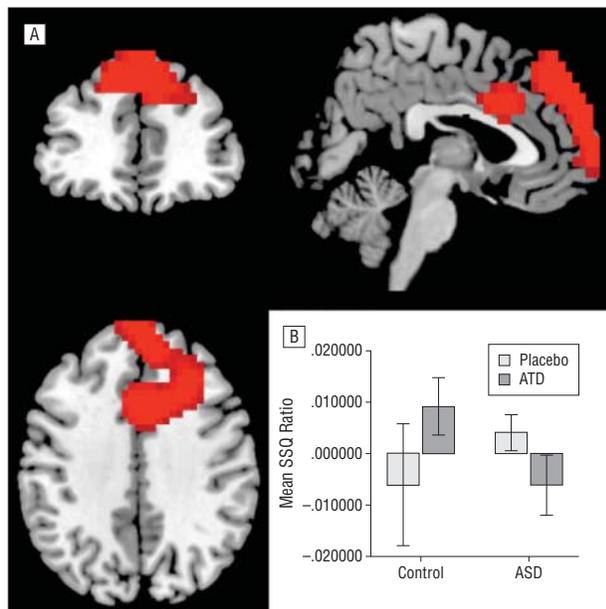


Figure 2. Happy facial expression experiment. A, Map of interaction of serotonin status (placebo vs acute tryptophan depletion [ATD]) and group (control vs autism spectrum disorder [ASD]) from the happy experiment. B, Plot of mean blood oxygenation level-dependent signal (sum of squares [SSQ]) extracted from the happy interaction cluster: left medial frontal gyrus Brodman area 9. Error bars=95% confidence intervals. Right side of figure is left side of the brain.

Disgust. In the disgust experiment, the interaction produced 1 cluster, centered in the left medial frontal gyrus (Brodman area [BA] 11), which encompassed left orbital frontal and rectus gyri (BA 6) as well as right medial frontal gyrus (BA 11), right anterior cingulate (BA 24, 32), and caudate nucleus. Another cluster was centered in the superior part of the medial frontal lobe (BA 6), which extended to bilateral medial frontal gyrus (BA 6) and right cingulate gyrus (BA 24). Also, a cluster in the lingual gyrus (BA 18) extended from the left cerebellum, and from the lingual gyrus (BA 17, 18) to the bilateral cuneus (BA 17, 19) and right precuneus (BA 31) (**Table 3** and **Figure 1**).

In the post-hoc ROI analyses for the disgust expressions experiment, ATD in the control group significantly increased BOLD signal response in the cingulate

gyrus ROI, but it decreased BOLD response in the medial frontal gyrus and lingual gyrus/cuneus. In contrast, ATD in the ASD group decreased BOLD signal in the cingulate gyrus ROI and increased BOLD in the medial frontal and lingual gyrus/cuneus ROI (**Table 4**).

Happy. The happy interaction's only cluster was centered in the left medial frontal gyrus (BA 9) that included left superior (BA 9) and medial frontal (BA 6) gyri, right superior and medial frontal gyri (BA 8, 10), and the anterior cingulate (BA 32) (**Table 3** and **Figure 2**). Within the left medial frontal gyrus ROI, ATD in the control group increased the BOLD response, while in the ASD group, this led to a decrease in activation (**Table 4**).

Sad. For the sad interaction, 1 cluster was centered in the right middle frontal gyrus (BA 8) encompassing medial (BA 6, 25), inferior (BA 47), middle (BA 6, 8, 9), and superior (BA 8) frontal gyri; right superior temporal gyrus (BA 38); right cingulate gyrus (BA 24, 31); and paracentral (BA 5), pre- (BA 4) and post- (BA 6) central gyri. Additionally a cluster demonstrating an interaction was found in the right putamen (**Table 3** and **Figure 3**). Within the middle/medial frontal ROI, ATD in the control group increased the BOLD signal, while for the ASD group, the signal was decreased. The opposite occurred within the putamen ROI; ATD in the control group decreased and for the ASD group increased the BOLD signal response (**Table 4**).

Fear. There were no significant interactions between tryptophan status and group membership for the fearful expressions experiment. However, we observed a significant main effect of group membership in which people with ASD showed greater activation relative to control subjects for both tryptophan conditions in a cluster centered in the left lingual gyrus (BA 18) that included left parahippocampal gyrus (BA 30); cuneus (BA 17) and middle occipital gyrus (BA 18); right fusiform gyrus (BA 20); inferior (BA 37), middle (BA 20, 21, 22), and superior (BA 41) temporal gyri; inferior and middle occipital gyri (BA 19); and cuneus (BA 18) (**Table 4** and **Figure 4**).

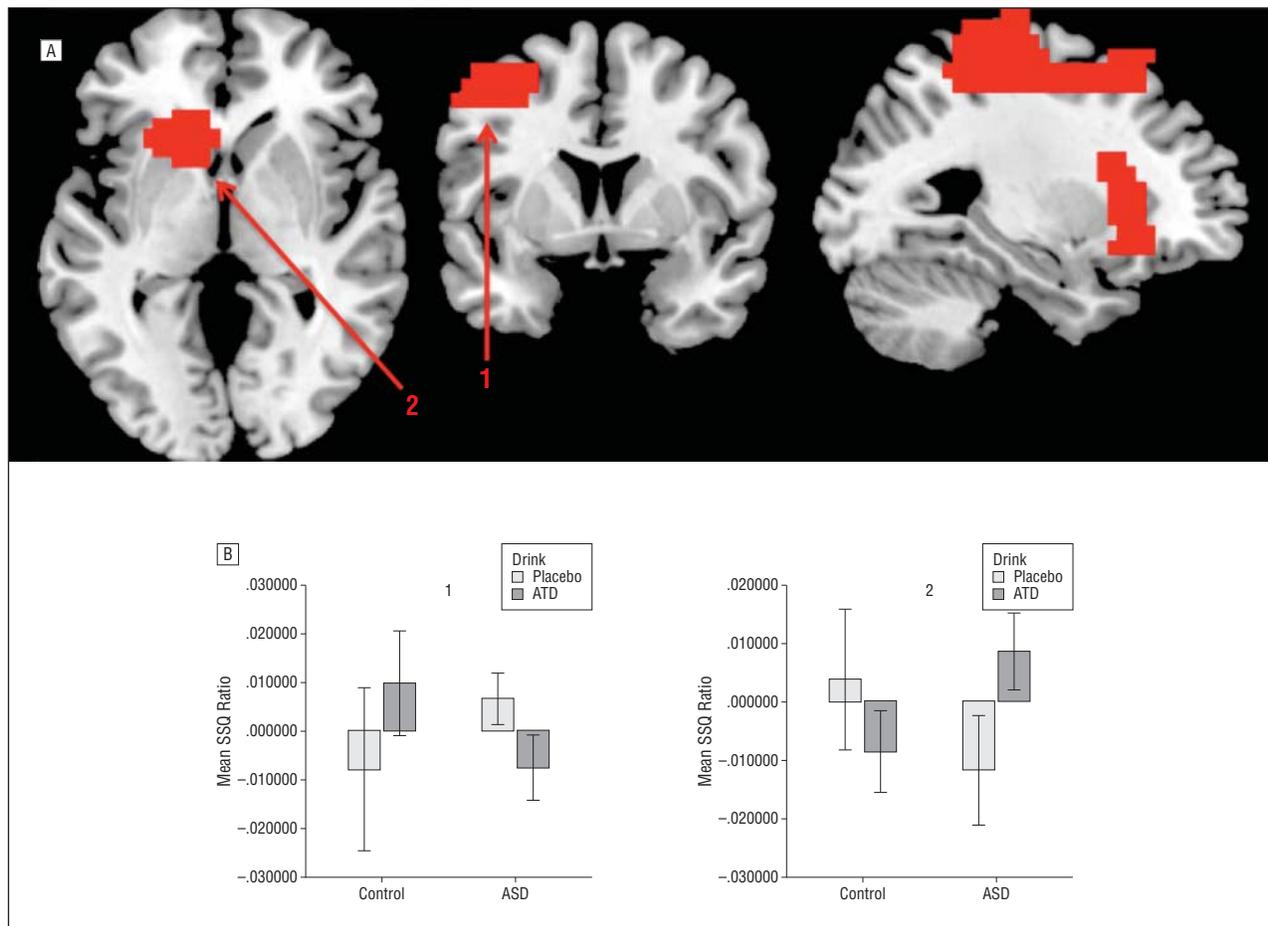


Figure 3. Sad facial expression experiment. A, Maps of interaction of serotonin status (placebo vs acute tryptophan depletion [ATD]) and group (control vs autism spectrum disorder [ASD]) from the sad experiment. B, Plot of mean blood oxygenation level–dependent signal (sum of squares [SSQ]) extracted from each sad interaction cluster: (1) Right middle frontal gyrus Brodman area 8 and (2) Right putamen. Error bars=95% confidence intervals. Right side of figure is left side of the brain.

COMMENT

To our knowledge, this is the first event-related fMRI study in people with ASD and control subjects to examine the effect of ATD on neural responses to 4 emotional expressions—fear, happiness, sadness, and disgust.

As expected, ATD led to a significant reducing of blood tryptophan concentration in both groups, but this change did not differ between groups. Also, those with ASD and the healthy control subjects performed equally well in the sex recognition tasks across tryptophan conditions (placebo vs ATD). The high-performance accuracy of both groups under placebo and ATD conditions and results of the basic contrasts of BOLD response to emotional and neutral expressions in the placebo condition is consistent with prior evidence that a sex discrimination task is relatively undemanding but still elicits attention to face stimuli such that brain regions traditionally involved in emotion processing are engaged.^{66,73} For example, our contrasts of the prototypic emotional expression to the fixation cross in the placebo condition in both groups revealed an increased BOLD response in established components of the face-processing networks, including the cerebellum, fusiform-extrastriate cortices, brainstem, superior temporal cortex, amygdala, insula, thalamus,

basal ganglia, and medial frontal and anterior cingulate gyri (eTable 1).^{14,25,66,74}

By contrast, the modulatory effects of ATD on the functional anatomy of facial emotion processing varied with group membership (control vs ASD), emotion type (disgust, fear, happy, and sad) and brain region. Regions affected included face-processing areas of medial, middle and inferior frontal gyri, lingual gyrus, fusiform gyrus, putamen, and cingulate gyrus.

For example, in the disgust expressions experiment, between-group differences in the modulatory effects of ATD were demonstrated in both the medial frontal cingulate and lingual gyri. Post-hoc testing revealed that in the right cingulate gyrus, the BOLD signal was significantly reduced in control subjects but increased in people with ASD following ATD. However, an opposite pattern of differential effects was found in the lingual gyrus cluster. Modulatory effects of ATD on activity in components of facial emotion processing networks were also demonstrated in the happy, sad, and fearful expression experiments (Table 3), showing widespread effects of ATD, which nevertheless vary with emotion type. As an additional test of whether brain regions showing interactions between group and tryptophan status were involved in facial emotion processing, we overlapped the

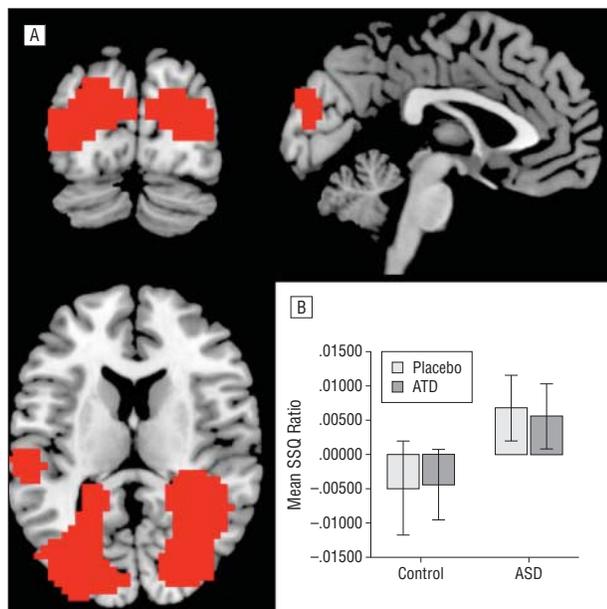


Figure 4. Fear facial expression experiment. A, Maps of main effect group (control vs autism spectrum disorder [ASD]) analysis of variance from the fear experiment. B, Plot of mean blood oxygenation level-dependent signal (sum of squares [SSQ]) extracted from the fear main effect of group cluster: left lingual gyrus Brodman area 18. Error bars=95% confidence intervals. Right side of figure is left side of the brain.

interaction maps with the brain activation maps derived from the emotion-neutral contrasts in healthy control subjects for each emotion type. These overlap maps confirmed that regions showing interaction effects are active during emotion processing, although interaction effects also extend beyond these regions (eFigures 2-4).

The between-group differences in modulatory effects of ATD we report may be partially explained by differences in brain serotonin metabolism and/or receptor systems. For example, prior research has shown that alteration of the serotonin system with dietary tryptophan depletion causes decreases of frontal gyrus serotonin₂ receptor binding in healthy control subjects.⁷⁵ Furthermore, we and others have previously reported that people with ASD have significant differences in serotonin synthesis^{42,43} and significantly reduced serotonin_{2A} receptor binding in the frontal as well as in the cingulate gyri when compared with control subjects.⁴⁵ Reduction of serotonin transporter binding in people with ASD compared with control subjects has also been reported in the frontal and cingulate gyri as well as in the precuneus.⁴⁴ Hence, the differential effects of ATD on brain activity during emotion processing that we report in these regions may reflect between-group differences in serotonin metabolism, innervation, and function.

Thus, our results suggest that perturbation of serotonin function during facial emotion processing differentially affects brain function in people with ASD and healthy control subjects, respectively. Such marked differential effects of ATD suggest that serotonin dysfunction may be implicated in primary abnormalities of brain activity during facial emotion processing in ASD as reported by us and others (ie, independently of ATD).¹⁴ It should be noted that in addition to its role as a neurotransmitter,

serotonin also acts as a trophic, or differentiation factor, in the development of the human brain.^{76,77} Given that ASD is a neurodevelopmental disorder, the regionally specific differences in brain activity seen during facial emotion processing (both with and without ATD) may also be influenced by abnormal development of relevant brain regions owing to disrupted trophic effects of serotonin. For example, those regions that we found demonstrating significant between-group differences in BOLD response to facial expressions of emotion following ATD (including cingulate gyrus, medial and middle frontal gyri, lingual gyrus and cuneus, and putamen) may be negatively influenced by declines in serotonin as well as other developmental indices of brain macrostructural and microstructural deficits. For example, a magnetic resonance spectroscopy study reported significant differences in the neuronal metabolism and integrity of medial frontal and cingulate gyri in men with ASD and these differences correlated with social communication deficits.⁷⁸ Furthermore, differences in brain gray and white matter anatomy have been reported in medial frontal and occipital lobes, putamen, and anterior cingulate gyrus.^{14,79-82} Hence, further studies are required to determine the extent to which differences in the neurobiology of facial emotion processing in ASD are primarily determined by acute alterations in serotonin neurotransmission or by differences in brain maturation, which may be secondary to altered trophic effects of serotonin.

While significant group differences in affective measures may have impacted our results, they do not fully explain them. Thus, in agreement with prior studies,⁸³⁻⁸⁵ our subjects with ASD had significantly higher scores on baseline affective measures compared with the control group; however, ATD did not significantly affect any of the visual analogue scales' affective measures scores (eTable 2). Furthermore, there were no significant correlations between baseline depression, anxiety, or obsessive-compulsive scores and BOLD response for either group using a Pearson product moment correlation coefficient. Hence, these significant group differences in affective measures are unlikely to fully explain our results. While we did not measure the behavioral effects of ATD on explicit emotion recognition, we did use an incidental (sex discrimination) rather than explicit (emotion recognition) task because emotion appraisal in routine social interaction often occurs automatically (without conscious deliberation)⁷⁴; therefore, we believe our paradigm to be more appropriate for investigating the effects of ATD on the brain systems that are routinely engaged in social interaction. Nevertheless, future studies should include measures of emotion recognition and arousal (eg, by measuring galvanic skin response) to help determine the potential behavioral relevance of altered serotonin function. Furthermore, given that activity in social brain regions during facial emotion processing changes with age,⁸² future studies should examine differential effects of ATD on modulation of age-related changes in brain activity during facial emotion processing in people with ASD and healthy control subjects.

A further limitation of our study is that it is not clear whether these widespread effects of ATD on facial emotion processing that we report reflect independent effects

of ATD on specific brain regions, reflecting the distributed nature of serotonergic innervation in the brain,⁸⁶⁻⁸⁹ or alternatively, whether the modulatory effects of ATD may be indirectly mediated through primary effects on key modulatory structures such as the anterior cingulate cortex. These questions could be addressed in future studies by using techniques modeling effective connections (ie, the effect 1 neural system exerts on another)⁹⁰ in relation to ATD effects on components of facial emotion processing systems in both people with ASD and healthy individuals. Moreover, as previously noted,⁸⁹ the density of serotonin transporter and receptor subtypes varies across different parts of the social brain, and it is possible that the functions of these different receptor subtypes are not modulated in a uniform way by ATD. Hence, further studies are also required on how modulation of specific serotonin receptor types affects different aspects of brain function during emotion processing tasks, perhaps by integrating molecular positron-emission tomographic imaging and pharmacofMRI to investigate whether regionally specific group differences in serotonin synthesis/receptor or transporter density relate to differences in brain function. Finally, we were unable to generalize our findings to other ASD groups (eg, children with ASD). The ethical considerations involved in the pharmacomanipulation of these groups are complex, and a necessary first step is studies that demonstrate differences in adults who can give informed consent.

The effects of acute depletion of serotonin on brain function during facial emotion processing differ significantly in people with ASD compared with control subjects and these differences vary with emotion type. The variations occur in brain regions that are involved in facial emotion processing and are also reported to have brain serotonin abnormalities such as synthesis differences and decreased numbers of receptors and transporters. Our results suggest that serotonin dysfunction in ASD may be implicated in altered brain activity during facial emotion processing relative to healthy control individuals.

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