Reduced Serotonin Receptor Subtypes in a Limbic and a Neocortical Region in Autism

Adrian Oblak, Terrell T. Gibbs, and Gene J. Blatt

Autism is a behaviorally defined, neurological disorder with symptom onset before the age of 3. Abnormalities in social-emotional behaviors are a core deficit in autism, and are characterized by impaired reciprocal–social interaction, lack of facial expressions, and the inability to recognize familiar faces. The posterior cingulate cortex (PCC) and fusiform gyrus (FG) are two regions within an extensive limbic-cortical network that contribute to social-emotional behaviors. Evidence indicates that changes in brains of individuals with autism begin prenatally. Serotonin (5-HT) is one of the earliest expressed neurotransmitters, and plays an important role in synaptogenesis, neurite outgrowth, and neuronal migration. Abnormalities in 5-HT systems have been implicated in several psychiatric disorders, including autism, as evidenced by immunology, imaging, genetics, pharmacotherapy, and neuropathology. Although information is known regarding peripheral 5-HT in autism, there is emerging evidence that 5-HT systems in the central nervous system, including various 5-HT receptor subtypes and transporters, are affected in autism. The present study demonstrated significant reductions in 5-HT_{1A} receptor-binding density in superficial and deep layers of the PCC and FG, and in the density of 5-HT_{2A} receptors in superficial layers of the PCC and FG. A significant reduction in the density of serotonin transporters (5-HTT) was also found in the deep layers of the FG, but normal levels were demonstrated in both layers of the PCC and superficial layers of the FG. This study provides potential substrates for decreased 5-HT modulation/innervation in the autism brain, and implicate two 5-HT receptor subtypes as potential neuromarkers for novel or existing pharmacotherapies. ***Autism Res*** *2013, 6: 571–583.* © 2013 International Society for Autism Research, Wiley Periodicals, Inc.

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Introduction

Autism is a neurodevelopmental disorder estimated to affect one in 88 eight-year-old children and one in 54 boys surveyed in the United States in 2008 [Baio, 2012]. Elevated platelet serotonin (5-HT) levels (i.e. hyperserotonemia) have been found in approximately one third of individuals with autism and/or their relatives [Abramson et al., 1989; Anderson et al., 1987; Cook & Leventhal, 1996; Cook et al., 1993; Lam, Aman, & Arnold, 2006; LeBoyer et al., 1999; Leventhal et al., 1990; McBride et al., 1998; Piven et al., 1991; Schain & Freedman, 1961; Singh et al., 1997], suggesting dysfunction of the 5-HT system in autism [Hranilovic et al., 2007].

Genetic studies have linked serotonin transporter gene (SLC6A4) polymorphisms, and genes regulating 5-HT neurotransmission to autism vulnerability and symptom severity [Brune et al., 2006; Sutcliffe et al., 2005; Wassink et al., 2007]. The SLC6A4 gene has been shown to modulate the function of social brain systems responsible for processing facial emotions in typically developing individuals [Surguladze et al., 2008]. This may have implications in autism, where in the social behavior domain, individuals exhibit a range of socio-emotional deficits, including impaired reciprocal–social interaction, lack of facial expression, and inability to recognize familiar faces [e.g. Adrien et al., 1992; Gillberg et al., 1990; Hoshino et al., 1987].

Thus, central 5-HT abnormalities are emerging as viable candidates and as contributing factors to many aspects of the disorder. Pharmacological studies have provided evidence for central serotonin dysfunction in autism, including the serotonin uptake sites or transporters [5-HTT; Lam et al., 2006]. Reductions in serotonin transporters in autism, as evidenced by single photon emission computed tomography (SPECT) and positron emission tomography (PET) studies, have been observed in the cortex, including the cingulate gyrus, further suggesting that alterations in serotonin may contribute to

From the Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts (A.O., G.J.B); Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, Massachusetts (T.T.G.)

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Address for correspondence and reprints: Adrian Oblak, Department of Anatomy and Neurobiology, Boston University School of Medicine, 700 Albany Street, W-701, Boston, MA 02118. E-mail: aoblak@bu.edu. ALO: Indiana University School of Medicine, Department of Pathology and Laboratory Medicine, 635 Barnhill Drive, MS A134, Indianapolis, IN 46202

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the symptomatology observed in autism [Makkonen, Riikonen, Kokki, Airaksinen, & Kuikka, 2008; Nakamura et al., 2010]. Recently, Azmitia, Singh, and Whitaker-Azmitia [2011] found increased 5-HT axons in the cortex of individuals with autism, suggesting increased release of serotonin from these terminals. Nakamura et al. [2010] used PET, and found reduced 5-HTT binding in the anterior and posterior cingulate cortices (PCC) associated with impairment in social cognition in individuals with high-functioning autism, and also found reduction of 5-HTT binding in the thalamus that correlated with obsessive behaviors and interests.

Despite this emerging evidence of central 5-HT dysfunction that has, for the large part, focused on the 5-HTT, recent discoveries have begun to reshape the focus of the etiology of 5-HT changes in autism. First, a SPECT imaging study by Murphy et al. [2006] in Asperger's syndrome patients used a ligand selective for 5-HT_{2A} receptors, and found a significant reduction in binding and the findings related to abnormal social communication. A few years later, Goldberg et al. [2009] published findings from a PET imaging study showing that parents of children with autism spectrum disorders (ASD) have significantly reduced 5-HT_2 binding, and that platelet 5-HT levels are inversely correlated to cortical 5-HT_2 binding potential. Then, in the same year, King et al. [2009] reported the outcome of a clinical trial for the selective serotonin reuptake inhibitor (SSRI) citalopram hydrobromide, which binds to 5-HTT. In 149 ASD patients who are 5–17 years of age from six academic centers, no significant improvement was observed and the treatment group experienced adverse effects, casting doubt on the efficacy of using SSRI to target the serotonin transporter.

With the recent evidence downplaying the 5-HTT as a major drug target site for effective treatment in autism, coupled with exciting evidence from imaging studies identifying the 5-HT_{2A} receptor as a possible target and related to social behavior impairments, we undertook the present study using postmortem tissue from adult autism and age-matched control cases, and used single-concentration ligand binding autoradiography to quantify the densities of two major 5-HT receptor subtypes as well as the 5-HT transporter. Specifically, the study focused on 5-HT_{1A} (located both pre- and postsynaptically) and 5-HT_{2A} receptors (located mostly postsynaptically) and 5-HTT (located presynaptically on 5-HT afferents) binding site densities in one limbic- and one neocortical area, the PCC and fusiform gyrus (FG), respectively. Postsynaptic receptors are located on the surface of neurons and bind their respective neurotransmitters that have been released into the synaptic cleft from the presynaptic neurons. Excess neurotransmitter is recovered by the presynaptic neuron and serves as a feedback system in signal transduction. Loss of presynaptic neurons would suggest that more neurotransmitter would be available at the synapse to bind, whereas loss of postsynaptic receptors suggests that the signal would be less likely to be transmitted appropriately. The PCC plays an important role in the normal brain's default network, and both structures are part of an extensive cortical network involving social functions, such as face recognition and interpretation of facial expressions. The goal of this investigation was to identify potential serotonergic neuromarkers for consideration for drug therapies, and to increase our understanding of the role of 5-HT within the functional circuitry of the autism brain.

Methods and Materials

Brain Tissue

Postmortem tissue was provided by The Autism Research Foundation, the Autism Tissue Program, Harvard Brain Tissue Resource Center, and the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. Fresh frozen brain tissue blocks were obtained from the PCC (Brodmann area 23) and the FG (Brodmann area 37). A combined total of 33 blocks from both regions were obtained (16 autism and 17 controls) and stored at -80°C in an ultra-low freezer. Seven autism and seven control cases were used in the PCC study; nine autism and ten controls were used in the FG study. A detailed list of case information is found in Tables 1 and 2.

Case Data

Tables 1 and 2 indicate the cases used from each region, diagnosis, age, postmortem interval (PMI), cause of death, and gender. The mean age for autism cases was 20.6 years (PCC study) and 24.0 years (FG study). The mean age for controls was 27.0 years (PCC study) and 26.1 years (FG study). The mean PMI for the autism cases was 16.5 hr (PCC) and 18.4 hr (FG); for controls, the average PMI was 19.1 (PCC) and 16.6 (FG). Note that seven cases from the autism group had a history of at least one seizure (AN04151, AN07948, 3845, AN08792, AN09730, AN07591, AN11989). All moderate-severe autism cases were diagnosed using either the Autism Diagnostic Interview (ADI) or Autism Diagnostic Observation Scale (ADOS). Any case diagnosed with high-functioning or Asperger's syndrome was excluded from the study to reduce variability in diagnosis.

Single-Concentration Ligand Binding Assays

Using a Hacker/Bright's motorized cryostat, tissue blocks were sectioned 20 μm thick at -20°C and thaw-mounted on 2×3 inch gelatin-coated glass slides. Two sections per case were used for each ligand to estimate "total binding." One section was used from each case per

Table 1. Posterior Cingulate Cortex Case Information

Case	Diagnosis	Age	PMI (hr)	Cause of death	Gender
AN08726	Autism	21	20.6	Sepsis	Female
AN04151*	Autism	19	15	Burns	Male
AN07948*[a]	Autism	19	9.5	Heart attack	Male
3845*[b]	Autism	30	28.4	Cancer	Male
3924	Autism	16	9	Seizure	Female
4099	Autism	19	3	Congestive heart failure	Male
AN15440	Autism	20	29.98	Unknown	Male
Mean		20.6	16.5		
AN02140	Control	43	23	Heart attack/disease	Male
AN11551	Control	24	5	Gunshot	Male
AN04629	Control	26	20	Accidental	Male
4268	Control	30	22	Heart attack/disease	Male
AN03206	Control	19	21	Epiglottitis	Male
4275	Control	20	16	Accidental	Male
4364	Control	27	27	Motor vehicle accident	Male
Mean		27.0	19.1		

Note. Cases with an asterisk (*) had a history of at least one seizure.
The following symbols indicate medication history:
[a]Klonopin, Mysoline, Phenobarbital, Thorazine.
[b]Dilantin, Mellaril, Phenobarbital.
PMI, postmortem interval.

Table 2. Fusiform Gyrus Case Information

Case	Diagnosis	Age	PMI (hr)	Cause of death	Gender
AN08842	Autism	20	15	Unknown	Male
4899	Autism	14	9	Drowning	Male
5027	Autism	37	26	Bowel obstruction	Male
AN00493	Autism	27	8.3	Drowning	Male
AN00764	Autism	20	23.7	Auto trauma	Male
AN08792*[a]	Autism	30	20.3	Gastrointestinal bleeding	Male
AN09730*	Autism	22	25	Choked	Male
AN07591*	Autism	16	22	Myocardial infarction	Male
AN11989*	Autism	30	16	Congestive heart failure	Male
Mean		**24.0**	**18.4**		
602	Control	27	15	Accident	Male
1026	Control	28	6	Congenital heart disease	Male
1365	Control	28	17	Multiple injuries	Male
4605	Control	29	18.3	Renal failure	Male
4642	Control	28	13	Cardiac arrhythmia	Male
4916	Control	19	5	Drowning	Male
AN19760	Control	28	23.3	Unknown	Male
AN15240	Control	36	18	Unknown	Female
AN17425	Control	16	26.2	Heart attack	Male
AN14368	Control	22	24.2	Unknown	Male
Mean		**26.1**	**16.6**		

Note. Cases with one asterisk (*) had a history of at least one seizure.
The following symbol indicates medication history:
[a]Cisapride, Clorazepate, Depakote, Dilantin, Mysoline, Phenobarbital.
PMI, postmortem interval.

ligand to determine nonspecific binding. Table 3 gives a detailed description of each radiolabeled ligand used in the study—all specific ligands obtained from Perkin Elmer, Inc. (Waltham, MA USA). Serotonin 1A receptors were labeled using ^{3}H-8-Hydroxy-N,N-dipropyl-2-aminotetralin (8-OH-DPAT; specific activity 170.2 Ci/mmol; 1nM); serotonin 2A receptors were labeled with ^{125}I-2,5-dimethoxy-4-iodoamphetamine (DOI; specific activity 2200 Ci/mmol; 0.15nM); and serotonin transporters were labeled with ^{3}H-citalopram (specific

Table 3. Specific Receptor-Binding Study Details and Film Exposure Time

Receptor	Buffer	Blocker	Pre-incubation	Incubation	Wash	Dip	Film exposure
5-HT_{1A}	170 mM Tris-HCl 4 mM $CaCl_2$ 0.01% ascorbic acid pH = 7.6	10^{-5} M Serotonin	Buffer 30 min at room temperature	60 min at room temperature	2 × 10 min in 4°C buffer	ddH_2O	12 weeks
5-HT_{2A}	50 mM Tris-HCl 4 mm $CaCl_2$ 0.1% ascorbic acid pH = 7.4	10^{-5} M Ritanserin	Buffer 30 min at room temperature	60 min at room temperature	3 × 5 min in 4°C buffer	ddH_2O	48 hr
5-HTT	50 mM Tris-HCl 120 mM NaCl 5 mM KCl pH = 7.4	10^{-5} M Imiprimine	Buffer 15 min at room temperature	60 min at room temperature	2 × 10 min in 4°C buffer	ddH_2O	16 weeks

activity 79.0 Ci/mmol; 1nM). Nonspecific binding was measured by adding a high concentration of a competitive displacer ligand to the buffer solution (see Table 3). Although specific details for each ligand's protocol differ (see Table 3), in general the following steps were performed: pre-incubation with buffer, incubation with ligand and blocker (only for nonspecific binding), three washes (5 min each) in buffer, and a dip in double deionized water. Slides for each experiment were processed in parallel using the same incubation solutions to reduce variability. Slides were dried under a stream of cool air overnight and loaded into X-ray cassettes with a ^{3}H or ^{125}I standard (General Electric, Pittsburgh, PA, USA, and American Radiolabeled Chemicals, St. Louis, MO, USA), and apposed to Kodak Scientific Imaging Biomax MR film (Fisher Scientific, Pittsburgh, PA, USA) for a known period of time (^{3}H-Hyperfilm, Kodak; see Table 3). The exposed films were developed for 4 min with Kodak D19 developer, fixed with Kodak Rapidfix (Fisher Scientific, Pittsburgh, PA, USA; 3 min) at room temperature, washed, and air-dried. Slides were stained with thionin to determine cytoarchitecture and laminar distribution of binding in the PCC and FG. In the PCC and FG, superficial layers corresponded to layers I–IV, and deep layers correspond to layers V–VI.

Data Analysis

The film autoradiograms were digitized using an Inquiry densitometry system (Loats Associates, Westminster, MD, USA) to gather quantitative measurements of optical density. Tissue sections were co-exposed with ^{3}H or ^{125}I polymer tissue autoradiography standards [Amersham Microscale; Geary, Toga, & Wooten, 1985], which were used to calibrate the autoradiograms to quantify the amount of ligand bound per milligram of tissue in specific tissue layers. A standard curve relating optical density to nanocurries (nCi) of radioactivity per milligram of protein was constructed by fitting optical density for the standards as a function of specific activity (corrected for radioactive decay) to the sensitometric equation [Palfi, Hatvani, & Gulya, 1998]: optical density = $B1 \times (1\text{-}10^{-k1(\text{specific activity})}) + B3$, by nonlinear least-squares minimization using the Solver tool of Excel (Microsoft Office Professional XP (Microsoft Corporation, Redmond, WA, USA)) to determine the values of the parameters k1, B1, and B3. Binding density was reported as femtomoles of ligand bound per milligram of tissue (fmol/mg) based on the specific activity of the ligand.

Note that pseudocolored images for each of the three illustrated figures were selected from only one autism and one control case, and present some perceived differences in background labeling. All sections from all autism and control cases were assayed with the same solutions and ligands from the same baths at the same time and developed at the same time. There is some inherent variability when comparing cases on different films, but that is why each film is equipped with its own set of standards. These standards are quantified separately for each film, and binding curves were constructed accordingly. The nonspecific binding, if present, was subtracted from total binding for each section, thereby minimizing variability between cases and groups.

Statistical Analyses

Student's *t*-test with unequal variances was performed to determine if there was a significant difference in the binding density of any of the ligands in autism vs. control cases in the superficial and deep layers of the PCC and FG. Mann–Whitney *U* nonparametric tests were used to determine if there were differences between the autism group with a history of seizure and autism group with no seizure history.

Results

In the PCC, no significant difference in age was found between autism (mean age 20.6 years ± 4.4 years, SD) and

control (mean age 27.1 years ± 8.1 years, SD) cases (Student's *t*-test, $P = 0.085$). Similarly, there was no difference in age in the FG study between autism (average age 25 years ± 7.3 years, SD) and control (average age 26.1 years ± 5.7 years, SD) cases (Student's *t*-test, $P = 0.72$) *or* in the PMI in either region between groups.

Serotonin 1A Receptor-Binding Density

Results from the single-concentration binding study using ^{3}H-8OH-DPAT revealed nearly significant reductions in the receptor-binding density in the superficial ($P = 0.055$) layers and significant reductions in the deep ($P = 0.024$) layers of the PCC in autism (Table 4). Similarly, a significant reduction in the density in both the superficial ($P = 0.011$) and deep ($P = 0.033$) layers of the FG in the autism group was also observed (Table 5). An example of pseudocolored images of sections from the FG study are found in Figure 1, with the scatterplots demonstrating the distribution of binding within the PCC and FG. Note that previous history of seizure did not significantly affect the binding density in either the superficial or deep layers of the PCC ($P = 0.27$, $P = 0.94$) or FG ($P = 0.15$, $P = 0.08$), respectively.

Serotonin 2A Receptor-Binding Density

Significant reductions in the density of ^{125}I-DOI labeled 5-HT$_{2A}$ receptors were found in the superficial ($P = 0.033$) layers of the PCC in autism (Table 4). A trend toward a reduction ($P = 0.064$) in the density of receptors in the deep layers of the PCC in autism was also found (Table 4). In addition, significant reductions in the receptor-binding density in the superficial layers ($P = 0.023$) of the FG in the autism group were found, with no change in the density of receptors in the deep layers (Table 5). Pseudocolored images of sections in the PCC are found in Figure 2A, and scatterplots of both the PCC and FG demonstrating differences in the superficial layers are illustrated in Figure 2B. Again, prior history of seizure did not significantly influence the reduction in receptor-binding density in the superficial or deep layers of the PCC ($P = 0.53$, $P = 0.84$) or FG ($P = 0.80$, $P = 0.08$).

Serotonin Transporter (5-HTT) Binding Density

In the PCC, the density of ^{3}H-Citalopram labeled serotonin transporters in the autism cases did not differ from the controls in either superficial or deep layers (Table 4). However, a significant reduction in 5-HTT density was found in the deep layers ($P = 0.022$) of the FG in the autism group, with no significant change in the superficial layers (Table 5). Figure 3 shows pseudocolored autoradiograms from sections in the PCC demonstrating similar-binding densities from representative sections of autism and control brains, as well as scatterplots demonstrating the distribution across cases of binding density in

Table 4. Receptor-Binding Density in the Posterior Cingulate Cortex

		5-HT$_{1A}$		5-HT$_{2A}$		5-HTT	
Case	Diagnosis	Super	Deep	Super	Deep	Super	Deep
AN08726	Autism	27.65	10.21	8.69	8.32	13.42	10.66
AN04151*	Autism	33.05	16.97	8.22	8.96	17.11	13.62
AN07948*[a]	Autism	31.2	10.65	10.37	6.45	19.22	11.98
3845*[b]	Autism	48.26	23.42	5.62	3.83	25.19	15.8
3924	Autism	34.97	18.86	11.01	4.33	15.88	12.86
4099	Autism	29.59	20.31	9.73	6.59	13	11.09
AN15440	Autism	33.13	19.96	7	5.06	8.98	8.79
Mean ± SEM		**33.98 ± 2.55**	**17.20 ± 1.89**	**8.66 ± 0.72**	**6.22 ± 0.74**	**16.11 ± 1.96**	**12.11 ± 0.85**
AN02140	Control	56.1	19.66	10.31	6.78	19.65	12.74
AN11551	Control	61.11	20.71	14.52	13.98	16.21	14.61
AN04629	Control	42.81	30.54	10.36	7.33	11.62	8.21
4268	Control	36.85	30.42	11.93	9.42	24.29	16.14
AN03206	Control			8.47	5.77		
4275	Control	56.57	26.99	9.91	12.06	8.11	6.67
4364	Control	62.27	19.81	13.12	7.9	11.77	10.73
Mean ± SEM		**56.62 ± 1.31**	**24.69 ± 2.14**	**11.23 ± 0.78**	**9.03 ± 1.13**	**15.27 ± 2.44**	**11.52 ± 1.5**
***P*-value (*t*-test)**		**0.055**	**0.024**	**0.033**	**0.064**	**0.79**	**0.74**
***P*-value (Mann–Whitney *U*)**		**0.27**	**0.94**	**0.53**	**.084**	**0.03**	**0.09**

Notes. Cases with an asterisk (*) had a history of seizure. Tissue for 5-HT$_{1A}$ and 5-HTT was not available from control case AN03206. All values in table are in fmol/mg tissue.

The following symbols indicate medication history:

[a]Klonopin, Mysoline, Phenobarbital, Thorazine.

[b]Dilantin, Mellaril, Phenobarbital.

SEM, Standard Error of the Mean.

Table 5. Receptor-Binding Density by Case in the Fusiform Gyrus

		5-HT_{1A}		5-$HT2_A$		5-HTT	
Case	Diagnosis	Super	Deep	Super	Deep	Super	Deep
AN08842	Autism	16.6	3.91	3.74	2.54	8.88	6.82
4899	Autism	18.31	5.54	4.84	7.15	2.03	4.7
5027	Autism	11.95	4.51	7.41	4.1	11.21	4.22
AN00493	Autism	20.97	6.59	3.22	5.05	20.66	3.24
AN00764	Autism	15.17	6.84	5.09	6.92	9.39	2.19
AN08792*[a]	Autism	10.01	3.82	4.82	3.72	24.7	4.52
AN09730*	Autism	2.22	1.99	5.95	2.58	10.2	5.91
AN07591*	Autism	14.44	3.45	2.89	2.32	1.15	0.91
Mean ± SEM		**13.71 ± 2.04**	**4.59 ± 0.58**	**4.77 ± 0.47**	**4.34 ± 0.60**	**11.03 ± 2.88**	**4.06 ± 0.68**
602	Control	14.09	5.86	7.16	5.7	38.62	12.53
1026	Control	12.69	4.58	7.25	2.41	8.93	3.59
1365	Control	33.71	6.24	6.32	3.84	13.02	7.68
4605	Control	39.14	5.65	4.91	3.15	6.55	3.13
4642	Control	31.67	9.42	5.12	3.41	16.12	8.58
4916	Control	29.32	8.27	9.6	9.61	12.87	10.05
AN19760	Control	23.84	7.14	6.2	3.03		
AN15240	Control	23	5.87	5.56	4.11	35.76	18.8
AN17425	Control	14.74	6.63	8.76	4.73	24.16	3.01
AN14368	Control	18.26	4.24	4.5	2.52	3.95	18.05
Mean ± SEM		**24.05 ± 2.90**	**6.39 ± 0.50**	**6.54 ± 0.53**	**4.25 ± 0.67**	**17.78 ± 4.16**	**9.49 ± 2.01**
***P*-value (*t*-test)**		**0.011**	**0.033**	**0.023**	**0.93**	**0.2**	**0.028**
***P*-value (Mann-Whitney *U*)**		**0.15**	**0.08**	**0.80**	**0.08**	**0.85**	**0.81**

Notes. Cases with an asterisk (*) had a history of seizure. All values in table are in fmol/mg tissue.
The following symbol indicates medication history:
[a]Cisapride, Clorazepate, Depakote, Dilantin, Mysoline, Phenobarbital.

the two regions. A prior history of seizure did not have an effect on the receptor-binding density observed in the present FG study. However, in contrast to the other binding studies presented, there was a significant effect of seizure on the ^{3}H-citalopram labeled serotonin transporter density in the superficial layers of the PCC, in that autism cases with seizure had a higher density of transporters compared with the autism cases with no seizure ($P = 0.03$). Given the small sample size (four cases with seizure, three cases with no seizure), this experiment should be repeated with larger cohorts. There was no significant difference in the deep layers of the PCC ($P = 0.09$) or either superficial or deep layers of the FG ($P = 0.85$, $P = 0.81$).

Discussion

The PCC and FG Are Two Cortical Regions Implicated in Autism Based on Neuropathological and Neurochemical Profiles, as well as Their Functional Roles and Connectivity

Based on previous findings in the autistic brain demonstrating alterations in neural circuitry, cortical lamination, and neuronal migration within these important social areas [Bailey et al., 1998; Bauman & Kemper, 1985; Casanova, 2004, 2006; Casanova, Buxhoeveden, & Gomez, 2003; Oblak, Kemper, Bauman, & Blatt, 2011; Simms, Kemper, Timbie, Bauman, & Blatt, 2009] and the use of SSRI to ameliorate the symptomatology of the disorder, we investigated serotonin receptor subtypes in postmortem tissue.

Imaging studies have found that autism is associated with abnormalities in the gray matter of the PCC that are correlated with impaired ADI communication scores [Uddin et al., 2011], as well as increased cerebral blood flow in the PCC [Pagani et al., 2012], and weaker connections between the PCC and other areas of the default network, including the retrosplenial cortex, lateral parietal cortex/angular gyrus, medial prefrontal cortex, superior frontal gyrus, temporal lobe, and parahippocampal gyrus [Weng et al., 2010]. The PCC in adolescent and adult postmortem autism brains contains altered cytoarchitecture, including irregularly distributed neurons, difficulty in discerning the borders of layers IV and V, and/or increased density of white matter neurons adjacent to layer VI, indicative of abnormal early developmental migratory patterns [Oblak, Kemper et al., 2011]. Reductions in gamma-Aminobutyric acid type A (GABA-A) receptors, its benzodiazepine binding site, and GABA-B receptors were also demonstrated in superficial and/or deep cortical layers, suggesting dysfunction of inhibitory circuitry [Oblak, Gibbs, & Blatt, 2010; Oblak, Gibbs, & Blatt, 2011].

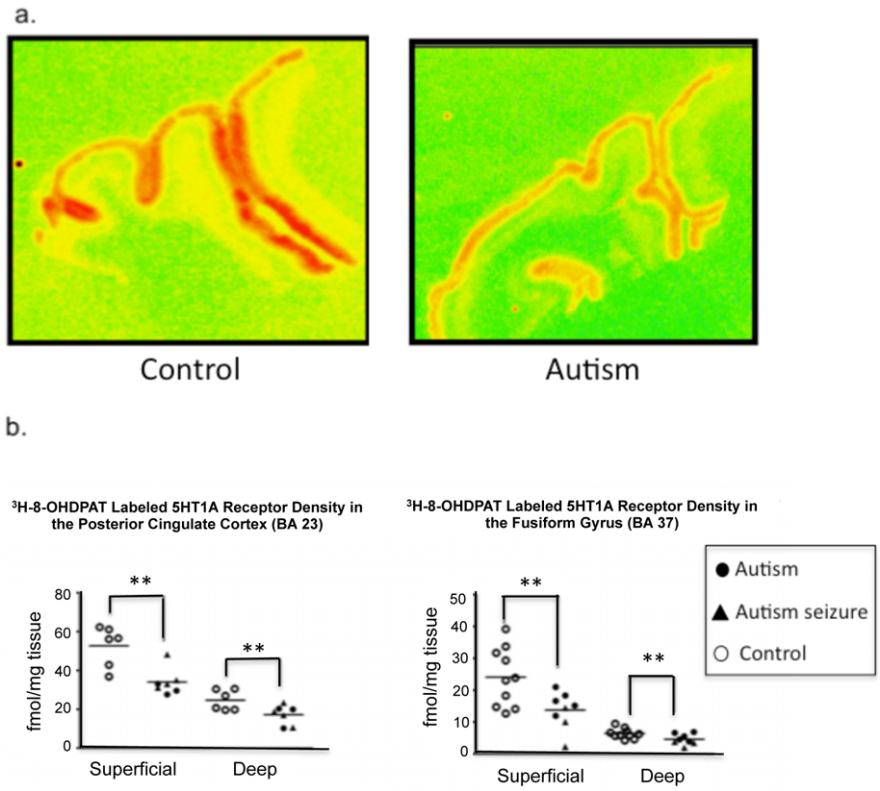

Figure 1. Serotonin 1A receptor-binding density in the PCC and FG. Examples of pseudocolored images of the FG (A) taken from film. Control is on the left and autism on the right. Red indicates high binding; yellow-green indicates low binding. Note the reduced density of binding in the autism case compared with the control case. Scatterplots displaying the density of serotonin receptor binding (B) in the PCC (left panel) and the FG (right panel). Significant reductions in the density of 5-HT_{1A} receptors were found in the superficial ($P = 0.055$) and deep ($P = 0.024$) layers of the PCC in autism. Significant reductions in the receptor-binding density of 5-HT_{1A} receptors were also observed in the superficial ($P = 0.011$) and deep ($P = 0.033$) layers of the FG in the autism group. PCC, posterior cingulate cortex; FG, fusiform gyrus.

The temporal lobe FG is a neocortical area that is multifunctional, participating in the processing of objects, color, words, numbers, and faces [Kanwisher, McDermott, & Chun, 1997; Nasr et al., 2011; Weiner & Grill-Spector, 2010]. Several groups have looked at the activation of the FG in autism, with results suggesting either no change in activation of the FG compared with controls or decreased activation of this area in autism [e.g. Dalton et al., 2005; Hadjikhani et al., 2004]. As abnormalities in face perception are a core feature of social dysfunction in autism, analyses of both imaging studies and quantitative neuropathological studies have recently been performed. van Kooten et al. [2008] demonstrated in seven postmortem autism brains compared with ten controls that the FG had significant reductions in neuronal densities in layer III, total neuron numbers in layers III, V, and VI, and mean perikaryal volumes of neurons in layers V and VI.

The decreases in the serotonin receptor densities found in the present study may result from a decrease in the density of neurons within this region; however, Oblak, Kemper et al. [2011] did not find significant density changes in either the PCC or FG in a subset of these cases. van Kooten et al. [2008] found a reduction in neurons within the FG. The discrepancy may be from the sampling scheme, superficial, and deep layer analysis in Oblak, Kemper et al. [2011] vs. layer-specific quantification in the van Kooten et al. [2008] paper. Reductions in the density of receptors without a difference in neuron number may reflect decreased functioning of the receptors resulting from altered molecular mechanisms. The method used here is limited by measuring the density of receptors that bind a particular ligand; the underlying binding properties cannot be obtained using the present method, and further molecular analysis of the receptors themselves should be performed.

Functionally, the layers of the cerebral cortex can be divided into three parts. The supragranular layers consist of layers I–III. The supragranular layers are the primary origin and termination of intracortical connections, which are either associational (i.e. with other areas of the same hemisphere) or commissural (i.e. connections to the opposite hemisphere, primarily through the corpus callosum). The supragranular portion of the cortex is highly developed in humans, and permits communication

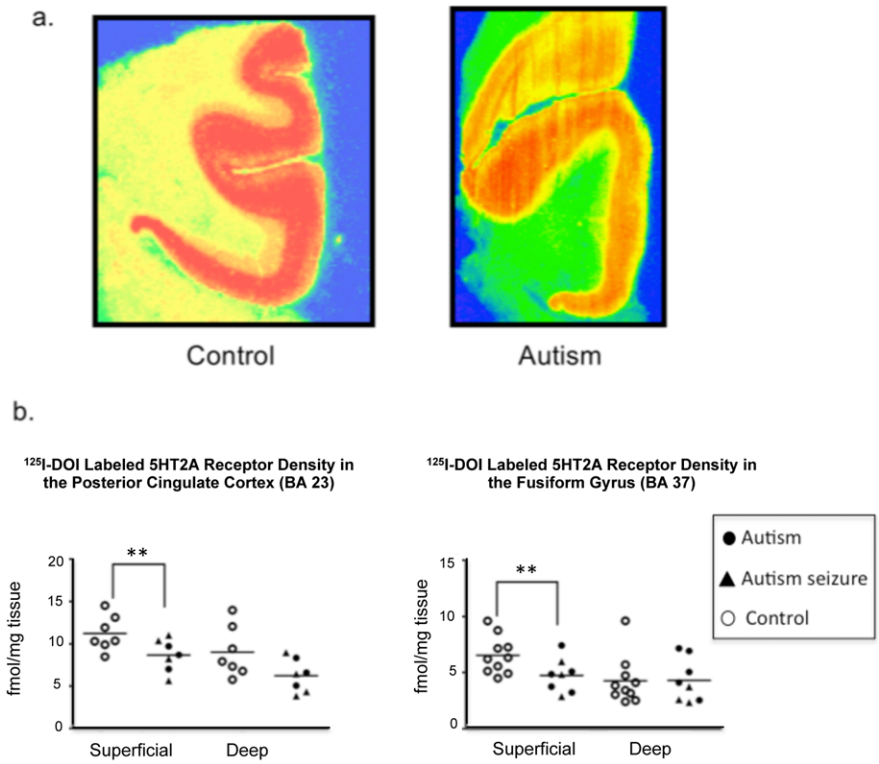

Figure 2. Serotonin 2A receptor-binding density in the PCC and FG. Examples of pseudocolored images of the PCC (A) taken from film. Control is on the left and autism on the right. Red indicates high binding; yellow-green indicates low binding. Note the reduced density of binding in the autism case compared with the control case. Scatterplots displaying the density of serotonin receptor binding (B) in the PCC (left panel) and the FG (right panel). Significant reductions in the density of 5-HT_{2A} receptors were found in the superficial ($P = 0.033$) layers and a trend toward a decrease in the deep ($P = 0.064$) layers of the PCC in autism. There was also a significant reduction in the binding density of 5-HT_{2A} receptors in the superficial layers ($P = 0.023$) of the FG, with no change in the deep layers. PCC, posterior cingulate cortex; FG, fusiform gyrus.

between one portion of the cortex and other regions [Brodmann, 1909]. The internal granular layer, layer IV, receives thalamocortical connections, especially from the specific thalamic nuclei. The infragranular layers, layers V and VI, primarily connect the cerebral cortex with subcortical regions. Layer V gives rise to all of the principal cortical efferent projections to basal ganglia, brain stem, and spinal cord. Layer VI, the multiform or fusiform layer, projects primarily to the thalamus [Brodmann, 1909]. Alterations in the balance of the inhibitory (5-HT_{1A}) and excitatory (5-HT_{2A}) receptors within these two regions with vast connections have implications for both cortical connections, as well as subcortical connections, and proper functioning. Thus, these receptor changes may result in aberrant signal modulation and/or transmission between local and long-distance social-communicative cortical regions.

Serotonin Receptor Subtypes as Neuromarkers in the Autism Brain

There are identified defects in the serotonin transporter gene in a number of individuals with autism [e.g. Brune et al., 2006; Sutcliffe et al., 2005; Wassink et al., 2007], and the SLC6A4 gene has been shown to modulate the function of social brain systems responsible for processing facial emotions in typically developing individuals [Surguladze et al., 2008]. In a 2010 study, PET imaging was used on high-functioning young adults with autism ($n = 20$, 18–26 years of age) to determine the occurrence of changes in 5-HTT [Nakamura et al., 2010]. Results found reduced 5-HTT binding in the anterior and PCC associated with impairment of social cognition in individuals with high-functioning autism, and a reduction of 5-HTT binding in the thalamus that correlated to repetitive and/or obsessive behaviors and interests [Nakamura et al., 2010]. In a SPECT study, in a very limited study sample of very high-functioning children and adolescents with autism (Leiter method IQ = 70–109), Makkonen et al. [2008] found normal 5-HTT binding levels in midbrain and temporal lobe areas but reduced binding in the medial frontal lobe, particularly in the adolescents. In adults with Asperger's syndrome, Murphy et al. [2006] used the 5-HT_{2A} receptor selective ligand ^{123}I-5-I-R91150 and found a significant reduction in receptor binding in select cortical areas, including the

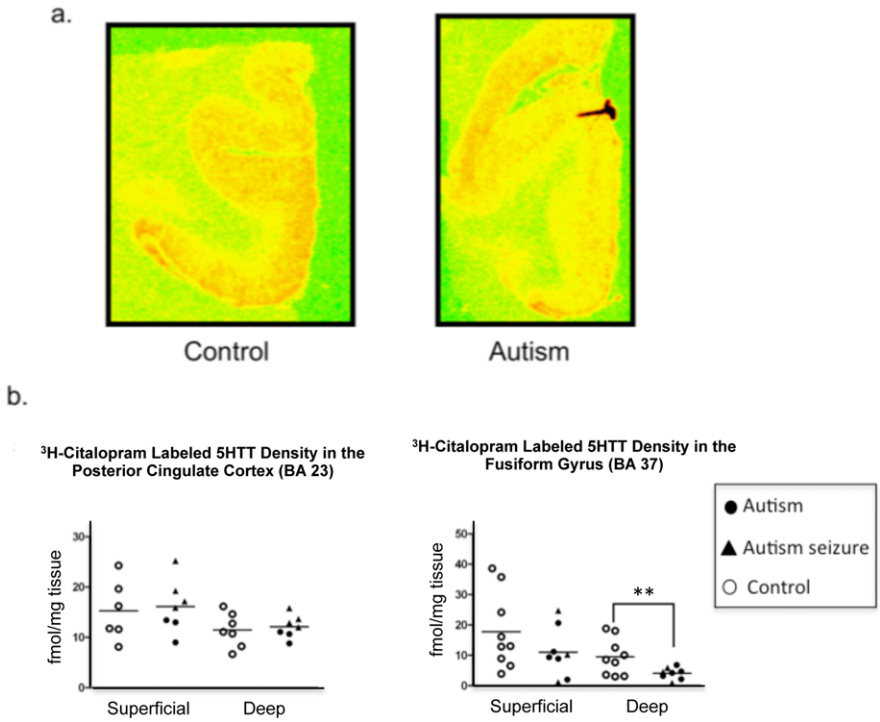

Figure 3. Serotonin transporter-binding density in the PCC and FG. Examples of pseudocolored images of the PCC (A) taken from film. Control is on the left and autism on the right. Red indicates high binding; yellow-green indicates low binding. Scatterplots displaying the density of transporters (B) in the PCC (left panel) and the FG (right panel). There were no significant reductions in the density of transporters in the PCC. There was a significant reduction in the density of transporters in the deep layers of the FG ($P = 0.028$) only. PCC, posterior cingulate cortex; FG, fusiform gyrus.

cingulate gyrus, and related findings to abnormal social communication. Most recently, Daly and colleagues demonstrated that individuals with ASD modulate the processing of facial expression of emotion by serotonin differently from control subjects using acute tryptophan depletion and functional magnetic resonance imaging [Daly et al., 2012].

The present single-concentration autoradiographic study is the first postmortem tissue-quantitative autoradiographic study of autism and normal brain tissue that provides precise histological localization within superficial and deep lamina of the presynaptic 5-HTT, the mainly postsynaptic 5-HT_{2A} receptors, as well as the 5-HT_{1A} receptor, which exists as an autoreceptor and postsynaptic receptor in the posterior PCC and the FG in autism. The reduced 5-HT_{2A} receptor densities in the PCC and FG in idiopathic autism patients agree with Murphy et al.'s [2006] imaging findings from Asperger's patients described above. The normal density of 5-HTT in both cortical areas differs from *in vivo* PET imaging results of Nakamura et al. [2010] in the cingulate cortex (reduced 5-HTT), suggesting that there may be differences between autism and Asperger's patients with respect to uptake mechanisms of serotonin, branching patterns of 5-HT axons, and/or differences in 5-HT release/neuromodulation that could affect receptor subtype density and distribution. This is further emphasized by Makkonen et al.'s [2008] imaging study that found variable 5-HTT densities in different brain regions of Asperger's patients. Alterations in 5-HT_{1A} receptor binding in humans are linked to fewer psychiatric disorders than 5-HT_{2A} receptors, but are commonly observed in studies of major depression and epilepsy [López-Figueroa et al., 2004; Merlet et al., 2004; Meschaks, Lindstrom, Halldin, Farde, & Savic, 2005]. 5-HT_{1A} knockout mice display lower seizure thresholds and higher lethality in response to kainic acid administration. Furthermore, 5-HT_{1A} knockout mice demonstrate impaired hippocampal-dependent learning and enhanced anxiety-related behaviors.

The present study found no significant differences in 5-HT_{1A} or 5-HT_{2A} receptor binding in the superficial and deep lamina in the PCC and FG between cases with a prior history of seizure or anticonvulsant therapy compared with those without. Due to the small sample sizes, such a relationship, however, cannot be ruled out, and it is also possible that the reduced serotonin receptor-binding density could act to increase the susceptibility to seizure in autism cases. Similarly, 5-HTT density in superficial and deep layers of the FG for autism cases with

seizure was not significantly different from those without, but in contrast, the PCC 5-HTT density was higher for cases with seizures than cases without seizure history but was not significantly different from controls. In future studies, there is a need for an increased number of postmortem autism cases to definitively determine what role, if any, prior seizures may have on the 5-HT receptor system, and to run assays on a variety of transmitter systems to achieve an overall neurochemical profile of each case. One additional experimental group might also include cases of dup15q syndrome that have a higher incidence of seizures with autism [Chifari et al., 2002].

Serotonin and Pharmacotherapy in Autism

Several SSRI, including fluoxetine, citalopram, and risperidone, have been prescribed for children with autism. These drugs have been used to treat several symptoms of autism for language improvement, to increase social interaction and attention, and to curb aggression, mood, anxiety, and repetitive behaviors [Couturier & Nicolson, 2002; DeLong, Teague, & McSwain Kamran, 1998; Hollander et al., 2005; King et al., 2009; Mandell et al., 2008; McDougle et al., 2005; Nagaraj, Singhi, & Malhi, 2006; Namerow, Thomas, Bostic, Prince, & Monuteaux, 2003; Oswald & Sonenklar, 2007; Pandina, Aman, & Findling, 2006; Posey, Erickson, Stigler, & McDougle, 2006; Shea et al., 2004]. Clinical trials for each of them have shown mixed results, suggesting that only a subset of individuals respond to a particular drug type, and only some of the symptoms are improved or ameliorated.

Risperidone and aripiprazole are the only Food and Drug Administration-approved pharmaceuticals for the treatment of irritable, aggressive, and self-stimulating behaviors in children with autism [Blankenship, Erickson, Stigler, Posey, & McDougle, 2010; West & Waldrop, 2006]. Both are members of the class of atypical antipsychotics. Risperidone is a nonspecific dopamine and serotonin receptor antagonist with a high affinity for serotonin receptors, while aripiprazole is a dopamine–serotonin system stabilizer acting as a partial agonist at D_2 and D_3 dopamine receptors, as well as 5-HT_{1A} serotonin receptors, and an antagonist at 5-HT_{2A} receptors [Tadori et al., 2005]. Several clinical trials of risperidone and aripiprazole have demonstrated moderate and clinically significant benefits in behavioral disturbances, including hyperactivity, stereotypy, and self-injury [Ching & Pringsheim, 2012; McDougle et al., 2005; Nagaraj et al., 2006; Pandina et al., 2006; Shea et al., 2004]. However, social and language impairments were only slightly modified by treatment, although results differed between studies.

The studies reviewed suggest that increasing the availability of serotonin at the synapse may play a role in the treatment of individuals with the disorder, but these treatments are often accompanied by side effects, including behavioral activation and aggression. Although the results of the present study provide stronger evidence for targeting 5-HT_{1A} and 5-HT_{2A} receptors in the treatment of individuals with autism, it is likely that many factors determine the efficacy of particular therapies, including interactions with other neurotransmitter systems, for example GABA and glutamate, as well as genetics, ethnicity, and age.

Conclusions

The present postmortem analysis of serotonin receptor subtypes and transporters in two areas that normally function in social-emotional/communicative behaviors provides further support for limbic and neocortical serotonin dysfunction in autism. Factors that may contribute to decreased 5-HT_{1A} and 5-HT_{2A} binding in these regions include an increase in serotonin during early life, alterations in development and organization of cells and neuropil in the cortical lamina, altered connectivity between areas within the social-emotional processing network, and/or disturbances in other neurotransmitter systems, such as GABA in both areas [Oblak, Gibbs, & Blatt, 2009; Oblak et al., 2010]. Postmortem analyses of serotonin receptors and transporters in other areas of the brain in autism should be examined to determine if 5-HT changes are widespread or limited to specific limbic and neocortical regions. The etiology of the 5-HT changes is still under investigation and includes possible genetic and/or epigenetic causes. The present analysis, which is currently being followed up with a multiple-concentration autoradiographic experiments in our laboratory to determine if the decrease in binding is due to a difference in the number of receptor subtypes and/or receptor-binding affinity, nevertheless provides strong postmortem evidence in young adult autism patients that 5-HT_{1A} and 5-HT_{2A} receptors should be considered viable targets for pharmacotherapies, in addition to or as an alternative to targeting the 5-HTT. Finally, although the respective brain banks are beginning to accumulate some specimens from a high-functioning cohort, adequate numbers even for pilot studies have not been available nor have some important clinical data, for example IQ and specific language impairments. The addition of more specimens and medical background of postmortem specimens will enable viable comparisons between functional ASD groups, and yield a better understanding of differences in morphology and connectivity, and ultimately behavior and targeted biomarkers for improved treatment specific for particular autism subtypes.

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