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Serotonin transporter binding and genotype in the nonhuman primate brain using [C-11]DASB PET

B.T. Christian *, A.S. Fox, J.A. Oler, N.T. Vandehey, D. Murali, J. Rogers, T.R. Oakes, S.E. Shelton, R.J. Davidson, N.H. Kalin

Department of Psychiatry, Harlow Primate Center, Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, USA
Department of Medical Physics, Harlow Primate Center, Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin-Madison, USA

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ABSTRACT

The length polymorphism of the serotonin (5-HT) transporter gene promoter region has been implicated in altered 5-HT function and, in turn, neuropsychiatric illnesses, such as anxiety and depression. The nonhuman primate has been used as a model to study anxiety-related mechanisms in humans based upon similarities in behavior and the presence of a similar 5-HT transporter gene polymorphism. Stressful and threatening contexts in the nonhuman primate model have revealed 5-HT transporter genotype dependent differences in regional glucose metabolism. Using the rhesus monkey, we examined the extent to which serotonin transporter genotype is associated with 5-HT transporter binding in brain regions implicated in emotion-related pathology.

Methods: Genotype data and high resolution PET scans were acquired in 29 rhesus (*Macaca mulatta*) monkeys. [C-11]DASB dynamic PET scans were acquired for 90 min in the anesthetized animals and images of distribution volume ratio (DVR) were created to serve as a metric of 5-HT transporter binding for group comparison based on a reference region method of analysis. Regional and voxelwise statistical analysis were performed with corrections for anatomical differences in gray matter probability, sex, age and radioligand mass.

Results: There were no significant differences when comparing l/l homozygotes with s-carriers in the regions of the brain implicated in anxiety and mood related illnesses (amygdala, striatum, thalamus, raphe nuclei, temporal and prefrontal cortex). There was a significant sex difference in 5-HT transporter binding in all regions with females having 18%–28% higher DVR than males.

Conclusions: Because these findings are consistent with similar genotype findings in humans, this further strengthens the use of the rhesus model for studying anxiety-related neuropathologies.

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Introduction

The serotonin system plays a central role in regulating mood and affect (Lucki, 1998). Dysfunction of the serotonin system is implicated in a variety of psychopathologies, including anxiety, depression and obsessive compulsive behavior. The serotonin transporter clears serotonin from the synaptic cleft and thereby plays a major role in serotonergic neurotransmission. The gene encoding the 5-HT transporter (5-HTT) contains a functional length polymorphism in the promoter region (referred to as 5-HTTLPR) that is associated with the development of emotional traits and psychopathology (Lesch et al., 1996). Carriers of the short allele (s), combinations of either s/s or s/l, have reduced *in vitro* gene transcription of 5-HTT mRNA and protein (Heils et al., 1997) compared to homozygous carriers for the long allele (l/l). In humans, there is considerable evidence suggesting that s-carriers demonstrate higher levels of anxiety-related traits and have increased susceptibility for depression (Lesch et al., 1996; Caspi et al. 2003; Kendler et al. 2005; Ebstein 2006; Rutter et al. 2006; Hayden et al. 2007) (see Canli and Lesch, 2007 for review).

The rhesus monkey possesses a similar length polymorphism in the serotonin transporter gene promoter to that of humans (Lesch et al., 1996). In the same region (though not exactly same location) as the promoter polymorphism in humans, a 21 base pair insertion/deletion variant is found in the rhesus monkey (alternatively referred to as rh5-HTTLPR). As with humans, the s-allele is associated with alterations in serotonergic function, such as 5-HT metabolites present in CSF (Bennett et al., 2002), and in functions associated with the serotonergic system, including alterations in stress responsivity (Barr et al., 2004b) and behavior (Barr et al., 2004a; Champoux et al., 2002).

The nonhuman primate model for studying the underlying mechanisms of human anxiety has been developed to further

* Corresponding author. Fax: +1 608 262 9440.
E-mail address: bchristian@wisc.edu (B.T. Christian).

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characterize the anxious endophenotype using behavioral (Kalin and Shelton, 1989), endocrine (Kalin et al., 1998) and neuroimaging (Fox et al., 2005) testing. These studies have identified regional differences in metabolic activity based upon anxiety-related behaviors (Kalin et al., 2005). Using FDG high resolution PET scanning, Kalin et al. (2008) demonstrated that rhesus monkey s-carriers displayed increased regional metabolic activity in response to stressful situations (relocation and threat) compared to (l/l)-homozygotes, similar to functional imaging studies in humans using fearful stimuli (Hariri et al., 2002). The region of the brain affected (with s-carriers showing increased FDG metabolism) was dependent on the context of the eliciting stressor, with the amygdala activated in response to relocation and the bed nucleus of the stria terminalis activated in response to threat. Such findings illustrate a context by genotype interaction which affects separate neural networks involved in the mediation of emotion. It is conceivable that these networks are also characterized by differences in neurochemical endophentoypes, such as the distribution of the serotonin transporter.

In this study, the relation between 5-HTT receptor binding availability and the serotonin transporter length polymorphism was examined in a large cohort of rhesus monkeys. PET scans were acquired with a high resolution small animal PET scanner using [C-11] DASB to assay 5-HTT receptor binding. In humans, PET and SPECT studies examining this relation have reported mixed results, using a variety of radiotracers, methodologies and allelic variants for the 5-HTT (Heinz et al., 2000; Jacobsen et al., 2000; Kalbitzer et al., 2009; Parsey et al., 2006; Praschak-Rieder et al., 2007; Reimold et al., 2007; Shioe et al., 2003; Van Dyck et al., 2004; Willeit et al., 2001), although the majority of these studies find no difference between l/l homozygotes and s-carriers. The studies herein were conducted using a highly selective 5-HTT PET radiotracer in a well characterized cohort of rhesus monkeys, thus minimizing potential variability due to suboptimal radioligand kinetics and heterogeneous populations. Because there is only limited data on [C-11]DASB binding in the nonhuman primate model (Ichise et al., 2006) (Banks et al., 2008), we also present the regional variation of [C-11]DASB binding in several midbrain and cortical structures, particularly those implicated in anxiety-related behaviors.

Materials and methods

Subjects

29 *Macaca mulatta* (rhesus monkey) underwent [C-11]DASB PET scans. This cohort has been described in detail in our previous work (Fox et al., 2008; Kalin et al., 2008; Oakes et al., 2007). It consisted of 18 females, 11 males; mean age 4.4 ± 0.6 years; mean weight 6.0 ± 1.2 kg. None of the rhesus monkeys were closely related in this cohort. The average kinship across these animals is 0.003, which is equivalent to two individuals separated by six generations. All monkeys were mother-reared (mean age of weaning was 8.4 months, no significant difference between groups) and housed at the Harlow Primate Laboratory or the Wisconsin National Primate Research Center. Animals from both groups went through periods of individual, pair- and group- housing. Animal housing and experimental procedures were in accordance with institutional guidelines. Environmental conditions (e.g. temperature, lighting, feeding) were unchanged during the 3 month acquisition period of this protocol to control for potential seasonal effects.

Genotyping for the serotonin transporter promoter was performed as described in Kalin et al. (2008). From this cohort, there were 20 l/l homozygotes and 9 s-carriers (2 s/s and 7 s/l). Because the s-allele is believed to have a dominant mode of action, the homo- and heterozygote s-carriers were grouped for analysis (Lesch et al., 1996).

MRI scanning

Magnetic resonance imaging (MRI) data were acquired on all of the monkeys for the process of coregistration, spatial normalization and gray matter probability analysis. Before undergoing MRI acquisition, the monkeys were anesthetized with intramuscular ketamine (15 mg/kg). Data were collected using a GE Signa 3 T scanner (GE Medical Systems, Milwaukee, Wisconsin) with a standard quadrature birdcage headcoil. Whole brain anatomical images were acquired using an axial 3D T1-weighted inversion-recovery fast gradient echo sequence (TR = 9.4 ms, TE 2.1 ms, FOV = 14 cm, flip angle = 10°, NEX = 2, matrix = 512×512, voxel size = .3 mm, 248 slices, slice thickness = 1 mm, slice gap = 0.05 mm, prep time = 600, bandwidth = 15.63, frequency = 256, phase = 224).

Radiosynthesis of [C-11]DASB

The [C-11] for the radiolabeling was produced with a National Electrostatics 9SDH 6 MeV Van de Graff tandem accelerator (Middleton, WI). [C-11]Methane was created *in situ* by proton irradiation of a 90% N_2/10% H_2, pressurized to 120 psi in an electroplated stainless steel target (Barnhart, 2004). Following a 40-minute irradiation (up to 70 μA), the [C-11]methane was removed from the carrier gas mixture and converted to [C-11]CH_3I via the recirculation method (Larsen et al., 1997). The converted [C-11]CH_3I was then reacted with 1 mg precursor (ABX, Germany) in dimethylformamide and heated at 90 °C for 4 min. The product was then purified using semi-preparative HPLC and trapped using a C18 Sep-Pak similar to methods reported by others (Wilson et al., 2000). The C18 Sep-Pak was rinsed with 10 mL of sterile water and the product [C-11]DASB was recovered with a 1 mL ethanol rinse. The ethanol solution was diluted with 9 mL saline and filtered through a 0.2 μm filter (Millex-LG, 25 mm).

PET scanning

The PET data were acquired using a Concorde microPET P4 scanner (Tai et al., 2001). The monkeys were initially anesthetized with ketamine (15 mg/kg IM) at $t = 55.4 \pm 17.3$ min prior to injection and maintained on 0.75%–1.5% isoflurane for the duration of the entire imaging session. The animals were also administered atropine sulfate (0.27 mg IM) to minimize secretions during the course of the experiment. The head of the subject was positioned downward in the prone position using a steriotaxic headholder to maintain consistent orientation for all scanned monkeys. [C-11]DASB was administered with injected mean activity of 4.9 ± 1.1 mCi of [C-11]DASB, and a specific activity of 360 ± 150 mCi/μmol at time of injection. Transmission scans were completed using standard vendor-supplied equipment (a [Co-57] point source) for a duration of 8.6 min before each emission scan. List mode data were acquired beginning with injection of radioligand and lasting for a scanning duration of 90 min.

Emission and transmission events were binned into 3D sinograms with a span of 3 and a ring difference of 31 using the system software (version 2.3.3.6). No scatter correction or smoothing were applied during the binning process but default corrections for deadtime and randoms corrections were incorporated. The list mode files were binned into five 2-minute frames and eight 10-minute frames for the 90-minute study. A μ-map of attenuation coefficients was created based on segmentation of the reconstructed transmission data and subsequently forwarded projected to create the attenuation sinogram.

The 3D sinograms were first rebinned to 2D by the system FORE algorithm. The data were reconstructed using a ramp projection filter (Nyquist cutoff: 0.5 mm^{-1}), a 1.5× image zoom, and no offsets to a matrix size of $128 \times 128 \times 63$ with voxel dimensions of $1.26 \times 1.26 \times 1.21$ mm^3. Corrections were made for scatter (direct calculation), attenuation, and normalization during reconstruction.

Table 1
Description of experimental variables.

	l/l homozygotes ($n=20$; 15 female)	s-carriers (s/l and s/s) ($n=9$; 5 female)	p-value
Age (years)	4.4 ± 0.6	4.5 ± 0.6	0.56
Weight (kg)	5.7 ± 0.9	6.4 ± 1.5	0.26
Ketamine timing[a] (minutes)	57 ± 21	53 ± 5	0.75
Injected activity (mCi)	4.9 ± 0.7	5.0 ± 0.9	0.86
DASB mass (μg/kg)	1.12 ± 0.62	0.78 ± 0.44	0.27

[a] Duration between ketamine administration and [C-11]DASB injection.

Data analysis

The dynamic PET time series were transformed into parametric images with each voxel representing the distribution volume ratio (DVR) serving as an index of receptor binding (Innis et al., 2007). The DVR is defined as:

$$\mathrm{DVR} = \mathrm{BP_{ND}} + 1 = f_{\mathrm{ND}} B_{\mathrm{avail}} / K_{\mathrm{D}} + 1$$

where $\mathrm{BP_{ND}}$ is the binding potential (uncorrected for the free fraction of ligand in the nondisplaceable tissue compartment, f_{ND}), B_{avail} is the density of receptors available for binding (in units of nM) and K_{D} is the equilibrium dissociation constant (nM) of the ligand for the receptor site. The cerebellum was used as a reference region, representing an area of the brain with negligible specific receptor binding. Multiple, circular (5 mm diameter) ROIs were carefully placed over the cerebellar gray matter region on the PET images for each scan (total sampling volume = 1.11 cm^3), avoiding outer edges of the cerebellar lobes. The ROIs were applied to the entire PET time series to generate the cerebellar time activity curves (TACs).

Distribution volume ratio (DVR) parametric images were produced for each reconstruction using a multilinear reference tissue model (MRTM) (Ichise et al., 2002, 2003). The DVR for each voxel within the brain was estimated using linear least squares to create a final parametric volume of DVR values, using a period of linearization, t^*, of 0 (i.e. time of injection). To reduce noise at the voxel-based level, the images from each time frame were spatially smoothed using a 3 × 3 (in-plane) voxel median filter, similar to techniques proposed by Zhou et al. (2003).

To facilitate intersubject comparisons, each subject's DVR image was transformed into a standard space defined by the rhesus monkey atlas of Paxinos et al., (2000). Integrated images of [C-11]DASB uptake were first coregistered to the T1-weighted MRI scans for each subject using a 6-parameter (rigid body) fit via the FSL-Flirt software (Smith et al., 2004), and were subsequently spatially transformed into stereotaxic space using previously reported methods (Kalin et al., 2005). The spatially normalized DVR images were then smoothed using a 4 mm Gaussian filter (Fox et al., 2008). Brain analyses controlled for individual variations in anatomy by statistically covarying for differences in gray matter probability across subjects as described in Oakes et al. (2007). Voxelwise t-tests were performed to examine differences in 5-HTT binding between monkeys carrying l/l versus s-carrier rh5-HTTLPR alleles. To provide cohort averages of regional binding, region of interest (ROI) analysis was performed over structures implicated in emotion disregulation, including thalamus (region of medial dorsal nuclei, 95 mm^3), raphe nuclei (region of dorsal raphe, 95 mm^3), striatum (bilateral–caudate and putamen, 312 mm^3), amygdala (bilateral, 190 mm^3), temporal cortex (bilateral–region of superior gyrus, 580 mm^3) and prefrontal cortex (bilateral, 850 mm^3). The circular ROIs were placed over the central region of radiotracer uptake on the PET images and within the boundaries of each structure. Regression analysis was first performed for both the voxelwise and ROI-based analysis to examine the effects of age, sex, weight, DASB injected mass and ketamine timing (time between administration and radioligand injection) on [C-11]DASB DVR.

Results

Table 1 provides a summary of the experimental data related to the genotype groups. There were no significant group differences in any of these experimental parameters. A cohort average [C-11]DASB DVR parametric image is shown in Fig. 1 to highlight the elevated binding in the dorsal raphe region of the midbrain. Regression analyses across the cohort revealed no significant effect of age, weight, or ketamine timing on DVR values in the regions of interest. Given the relatively large range of specific activity for injected [C-11]DASB, we expected this to be an important covariate in the analysis; however, there was also no significant negative correlation between injected DASB mass and DVR (threshold of $p<0.05$). Because no effects of competing DASB were observed throughout the high and medium 5-HTT density

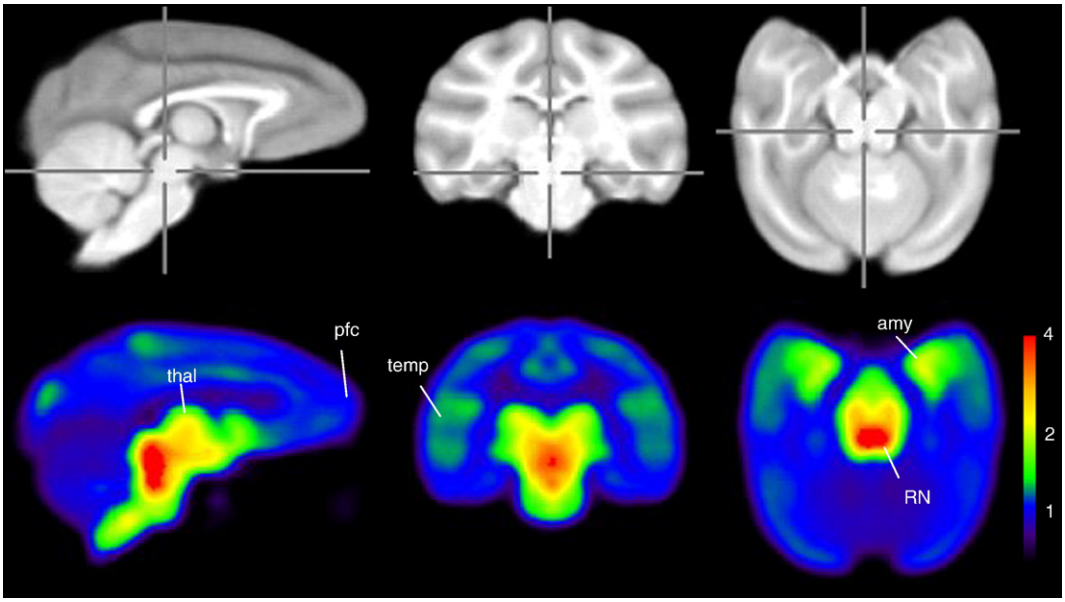

Fig. 1. Binding of [C-11]DASB in the rhesus brain. The MRI image (top row) is coregistered to the [C-11]DASB DVR image (bottom row). Binding is highest in the raphe nucleus (RN) region of the midbrain, with elevated levels of binding also seen in the thalamus (thal), amygdala (amy), lateral temporal cortex (temp) and prefrontal cortex (pfc).

Table 2
Cohort regional [C-11]DASB DVR ROI values.

Region	Female $n=18$ (mean ± s.d.)	Male $n=11$	s-carriers $n=9$	l/l homozygotes $n=20$
Raphe nuclei	4.19 ± 0.75	3.47 ± 0.83	3.85 ± 1.25	3.95 ± 0.65
Thalamus	2.92 ± 0.38	2.55 ± 0.33	2.75 ± 0.60	2.79 ± 0.27
Amygdala	2.56 ± 0.36	2.20 ± 0.37	2.40 ± 0.61	2.42 ± 0.28
Striatum	1.81 ± 0.13	1.57 ± 0.15	1.73 ± 0.24	1.72 ± 0.15
Temporal c.	1.51 ± 0.21	1.54 ± 0.10	1.57 ± 0.13	1.46 ± 0.13
Prefrontal c.	1.38 ± 0.12	1.34 ± 0.09	1.36 ± 0.13	1.37 ± 0.10

All of the brain regions contained subregions with significant sex differences based upon the voxelwise analysis ($p<0.05$, spatial extent ≥ 95 mm^3). There were no subregions with significant genotype differences.

regions of the brain, further analysis did not control for injected DASB mass. A significant sex effect was observed. Significantly higher DVR values were observed in females throughout portions (spatial extent ≥ 95 mm^3) of all regions in the brain with medium to high 5-HTT density, including the raphe nuclei (+22% higher in females), thalamus (+18%), striatum (+28%), amygdala (+22%) and temporal cortex (+20%) based on a $p<0.05$ threshold of the voxelwise analyses. The regional mean and standard deviations of DVR taken from the larger ROI volumes are shown in table 2, grouped according to sex. The coefficient of variation (=s.d./mean) for both sexes was highest in the raphe nuclei, 18% and 23% for females and males, respectively, and lowest in the striatum (7%, females) and temporal cortex (6%, males). There were no significant sex differences in the nondisplaceable component of the PET signal, indexed as cerebellar area under curve (in units of % injected dose · min/cc), between males and females ($p=0.40$).

The voxelwise comparison between the l/l and s-carrier groups (controlling for gray matter probability and sex) revealed no significant difference in DVR in the brain regions implicated in emotion regulation, such as the anterior cingulate, amygdala and insula cortex. The only significant difference was seen in the occipital cortex (V1 and V2) with the l/l group having higher binding than the s-carrier group using a threshold of $p<0.005$ (one-tailed, uncorrected) $t=3.07$ (peak), volume = 134 mm^3.

Included in table 2 are the ROI cohort averages of [C-11]DASB binding throughout regions of the brain with high to medium 5-HTT receptor density grouped according to genotype and sex. The COV is higher in the s-carrier group, which is attributed to a smaller group size. The s/s homozygotes (1 male, 1 female) had DVR values that were within close range of the mean (<1 s.d.) across all regions of the brain.

Discussion

The nonhuman primate model serves an invaluable role in studying the behavioral, biochemical and genetic underpinnings of psychiatric illnesses in humans. For anxiety-related disease, the rhesus monkey has been shown to demonstrate large and quantifiable behavioral inhibition in response to stressful situations, providing a model for excessive anxiety in humans which has strong face-validity along with similar neurochemical and neuroantomical substrates (Fox et al., 2008; Kalin and Shelton, 1989; Kalin and Shelton, 2003). In humans, the literature strongly supports a link between anxiety-related psychopathology and allelic differences in the promoter region of the serotonin transporter gene (Canli and Lesch, 2007). The positive identification of a similar allelic difference in the rhesus monkey significantly strengthens the use of this animal model for studying the genetic components of psychiatric illnesses implicating the serotonergic system (Barr et al., 2004a; Barr et al., 2004b; Lesch et al., 1996; Lesch et al., 1997). In addition to the ability to do invasive research, the nonhuman primate model provides an excellent model in which potentially confounding factors can be held constant. For example, although recent work in humans has highlighted the influence of seasonal variations (Praschak-Rieder et al., 2008) and dietary conditions (Attenburrow et al., 2003) in altering brain serotonergic systems, both the length of daylight and diet were held constant in this study, thereby eliminating any environmental effects associated with these factors. More importantly for genetic studies, the rhesus model provides the opportunity to eliminate or isolate epigenetic effects that may adversely alter the serotonergic system. It is possible to control for sex-specific environmental variables that are associated with increased susceptibility to anxiety and depression, such as social insubordination and social rejection stress (Altemus, 2006), which are extremely difficult to control for in human studies. Similarly, rearing conditions, such as time of weaning or being peer-raised, can be altered to create stressful environments that may potentially disrupt serotonergic development. The subjects used in this cohort were closely matched in age, and there were no differences due to seasonal effects or dietary intake and all subjects had similar rearing and socialization conditions. In summary, many of the confounding factors often present in similar human studies of 5-HTTLPR/5-HTT binding were removed or minimized in this cohort.

While there is compelling evidence linking emotional traits to genetic variation in the serotonin system, our findings support Hariri and Holmes' hypothesis (Hariri and Holmes, 2006) that the effects of the serotonin transporter genotype on behavior and brain function may not be mediated through the expression of the serotonin transporter. For imaging studies it is important to consider the sensitivity of the selected metrics for detecting significant changes. Based on the variability of [C-11]DASB DVR in the amygdala across the entire cohort (s.d./mean = 15%), we can estimate that for a power of 0.8 and $p<0.05$ (one sided), a 15% change in DVR between groups would be required for the ability to detect an effect. This range of an effect size in serotonin transporter binding has been reported in humans using [C-11]DASB PET for group differences of subjects with depression (Meyer et al., 2004; Reimold et al., 2008). In our study, the DVR in the high 5-HTT binding regions of the s-carrier group revealed a larger variance, which is attributed to a smaller sample size. Because the mean DVRs between genotype groups are approximately the same, we believe that it is unlikely that a larger sample size would reveal group differences, given typical intersubject variations of 10%–20% in PET neuroreceptor studies (Christian et al., 2009; Costes et al., 2005; Ito et al., 2008; Rabiner et al., 2002).

Despite the lack of association between genotype and *in vivo* 5-HTT binding in the rhesus monkey reported here, there is evidence that the length polymorphism in the promoter region of the serotonin transporter gene is associated with phenotypes related to emotionality. Several studies in the rhesus monkey support the relation between this genotype and endophenotypes for psychopathology. It has been reported that (s/s)-animals displayed significantly reduced performance in several tests measuring cognitive flexibility and socioemotional behavior (Izquierdo et al., 2007). Bethea et al. found a higher measure of anxiety-related behavior in (s/s)-homozygote rhesus monkeys (Bethea et al., 2004). Our previous work using FDG imaging in the rhesus monkey demonstrated a context dependent pattern of regional brain metabolism that differed between s-carriers compared to (l/l)-homozygotes (Kalin et al., 2008). When presented with the stressful situation of relocation, the s-carrier animals displayed increased amygdala reactivity (via increased FDG metabolism), and in response to threat using the human intruder paradigm (Kalin and Shelton, 1989) an increase in FDG metabolism was measured in the bed nucleus of the stria terminalis (BNST), which is an area implicated in anxiety (Davis et al., 1997). In extending this work, we recently demonstrated that metabolic activity within the amygdala and BNST in response to these stressors correlates with a composite measure (behavioral and endocrine) of anxious temperament (Fox et al., 2008). Future studies will be aimed at elucidating the functional relationship between 5-HTT binding and metabolic

activity within the brain circuitry hypothesized to mediate anxious temperament, and the risk to develop associated psychopathology.

There is a growing body of evidence supporting the involvement of rhesus serotonin transporter genotype and developmental experience on behavioral and endocrine responsivity outcomes, providing a valuable model for studying gene × environment interactions. Using a cohort of peer-reared (PR) monkeys as a condition of chronic stress, PR animals with the s-allele yielded elevated levels of CSF 5-hydroxy-3-indoleacetic acid (5-HIAA) compared to l-carriers and control s-carriers (Bennett et al., 2002). Subsequent studies in the rhesus monkey also demonstrated significant s-allele dependent affects on neonatal response (Champoux et al., 2002), alcohol sensitivity (Barr et al., 2003) and HPA axis stress reactivity (Barr et al., 2004b) only in s-allele PR animals, but not s-allele monkeys reared by their mothers. In sexual maturation, subordinate female carriers of the s-allele were significantly delayed in puberty compared to (l/l)-homozygote carriers, independent of neonatal growth hormone secretion (Wilson and Kinkead, 2008). In examining the stress associated with social status within the environment of the rhesus colony, female s-carriers displayed an increased vulnerability to the stress accompanying subordinate status compared to l/l animals (Jarrell et al., 2008). Using a colony of rhesus monkeys it was found that prenatal alcohol exposed carriers of the s-allele exhibited increased neonatal irritability and increased adrenocorticotropic hormone and cortisol compared to (l/l)-homozygotes independent of prenatal alcohol exposure (Kraemer et al., 2008). These findings support the body of literature in human gene × environment studies that s-carriers demonstrate higher levels of anxiety-related traits and have increased susceptibility for depression (Caspi et al., 2003; Ebstein, 2006; Hayden et al., 2007; Kendler et al., 2005; Lesch et al., 1996; Rutter et al., 2006). In summary, the literature suggests that this polymorphism has functional relevance, however, further research is needed to identify the mechanisms of their involvement.

Sex differences in 5-HTT binding

Sex-based differences in serotonergic function have long been implicated in anxiety and mood related disorders as supported by epidemiological studies (Cosgrove et al., 2007). Females have been reported to have increased vulnerability to phobias and anxiety disorders (Altemus, 2006) and there is ongoing research into understanding the underlying mechanisms of these serotonergically linked disorders. In this cohort, the females displayed elevated [C-11]DASB binding and the greatest difference was in the striatum, including regions of the caudate and putamen, with females having a DVR of 28% higher than males. Inconsistent sex differences in 5-HTT binding have been reported in healthy humans, one study reporting higher binding in females ($n=21$) (Staley et al., 2001) and another study, which imaged females only during the follicular phase of menstruation, found higher binding in males ($n=28$) (Jovanovic et al., 2008). In the present study, we did not control for the monkeys' phase of the estrous cycle, and we cannot further dissociate its effect on 5-HTT expression and/or function.

Methodological considerations: ketamine and DASB mass effects

Previous studies have reported a reduction in 5-HTT binding following high dose (333–400 mg/kg) ketamine administration with [H-3]-(S)-citalopram in rats, possibly acting as a competitive inhibitor to the 5-HTT (Elfving et al., 2003). For the experiments herein, there was an average of 55 min ± 17 min between the pre-anesthesia induction with ketamine and the injection of [C-11]DASB. The animals were placed under isoflurane within 5–10 min following ketamine. Across the entire cohort there was no significant relation detected between ketamine timing and [C-11]DASB binding, as shown in Fig. 2A, suggesting the competitive

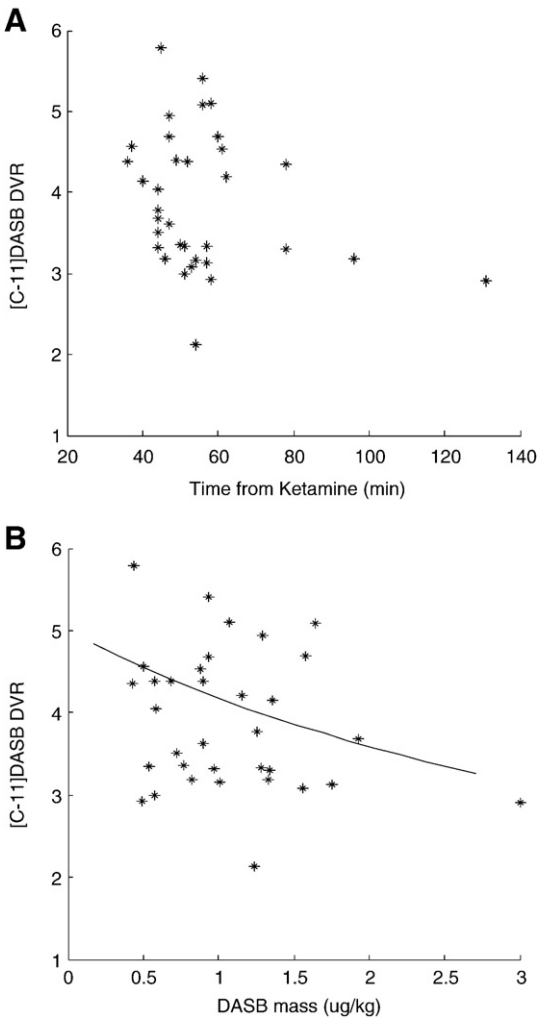

Fig. 2. Distribution volume ratio (DVR) in the raphe nucleus as a function of the time between ketamine and [C-11]DASB injection (A) and injected DASB mass (B). The line in panel B represents the theoretical relationship between free DASB concentration (represented by DASB injected mass/kg) and DVR. There is no statistically significant relationship between either of these experimental conditions and the measured DVR in the cohort.

effects of ketamine for 5-HTT are either too small at this dose (15 mg/kg) or not variable within the time frame reported here.

The groups were also closely matched for injected DASB ligand mass. Two animals were scanned on separate occasions with varying specific activity to obtain an estimate of the apparent K_D. For the first subject, the injected masses were 200 nmol and 26 nmol with a change in DVR-1 of 80% and the other subject with 79 nmol and 16 nmol and a change in DVR-1 of 59%. From these data and using the methods originally described by Farde et al. (1986), we can approximate the apparent K_D of [C-11]DASB to be 10 nM. Based upon this estimation and using the equilibrium relationship:

$$B/B_{max} = \frac{F}{K_D^{app} + F},$$

we can approximate the effects of competing ligand occupancy (B/B_{max}) on our outcome variable (DVR) by assuming the free ligand concentration (F) is proportional to the injected mass for each study. Fig. 2B shows the relationship between DVR and injected DASB mass, along with the theoretical curve. In the high binding region of the raphe nuclei, the coefficient of variation due to DASB mass effect can be approximated as 8%, i.e. if a single subject were to be scanned on 29

occasions using the injected masses for this cohort, then the COV in DVR due to just mass effects would be 8%. This DVR variability due to competing mass is lower for the regions with reduced [C-11]DASB, being approximately 3% in the temporal cortex. Based upon exploratory voxelwise analysis, there were no areas in the brain regions of interest where mass effects could be significantly identified above intersubject variability ($p<0.05$).

Conclusions

Further supporting the use of the nonhuman primate as a model for studying human anxiety, our findings are in agreement with the majority of human PET studies of 5-HTTLPR length polymorphisms that suggest there is not a significantly detectable relationship between *in vivo* 5-HTT binding and s-allele carrier status (Jacobsen et al., 2000; Parsey et al., 2006; Shioe et al., 2003; Van Dyck et al., 2004; Willeit et al., 2001 also though see Praschak-Rieder et al., 2008). A significant difference in 5-HTT binding between males and females was found, which is of interest because anxiety-related studies have a higher prevalence in females. There is strong evidence that this polymorphism is important in mediating the risk to develop stress associated psychopathology (Canli and Lesch, 2007) and may interact with other components of the 5-HT network, such as the expression of the 5-HT_{1A} receptor subtype (David et al., 2005). However, our work in the rhesus monkey, and that of others in humans, calls into question whether this increased risk is mediated by changes in the expression of the number of serotonin transporter molecules.

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References

Altemus, M., 2006. Sex differences in depression and anxiety disorders: potential biological determinants. Horm. Behav. 50 (4), 534–538.

Attenburrow, M.J., Williams, C., Odontiadis, J., Powell, J., Van de Ouderaa, F., Williams, M., Cowen, P.J., 2003. The effect of a nutritional source of tryptophan on dieting-induced changes in brain 5-HT function. Psychol. Med. 33 (8), 1381–1386.

Banks, M.L., Czoty, P.W., Gage, H.D., Bounds, M.C., Garg, P.K., Garg, S., Nader, M.A., 2008. Effects of cocaine and MDMA self-administration on serotonin transporter availability in monkeys. Neuropsychopharmacology 33 (2), 219–225.

Barnhart, T.E., 2004. Radiotracer Methodology with Applications to Functional PET/TMS. University of Wisconsin-Madison (Thesis), Madison.

Barr, C.S., Newman, T.K., Becker, M.L., Champoux, M., Lesch, K.P., Suomi, S.J., Goldman, D., Higley, J.D., 2003. Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. Alcohol. Clin. Exp. Res. 27 (5), 812–817.

Barr, C.S., Newman, T.K., Lindell, S., Shannon, C., Champoux, M., Lesch, K.P., Suomi, S.J., Goldman, D., Higley, J.D., 2004a. Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. Arch. Gen. Psychiatry 61 (11), 1146–1152.

Barr, C.S., Newman, T.K., Shannon, C., Parker, C., Dvoskin, R.L., Becker, M.L., Schwandt, M., Champoux, M., Lesch, K.P., Goldman, D., Suomi, S.J., Higley, J.D., 2004b. Rearing condition and rh5-HTTLPR interact to influence limbic–hypothalamic–pituitary–adrenal axis response to stress in infant macaques. Biol. Psychiatry 55 (7), 733–738.

Bennett, A.J., Lesch, K.P., Heils, A., Long, J.C., Lorenz, J.G., Shoaf, S.E., Champoux, M., Suomi, S.J., Linnoila, M.V., Higley, J.D., 2002. Early experience and serotonin transporter gene variation interact to influence primate CNS function. Mol. Psychiatry 7 (1), 118–122.

Bethea, C.L., Streicher, J.M., Coleman, K., Pau, F.K.Y., Moessner, R., Cameron, J.L., 2004. Anxious behavior and fenfluramine-induced prolactin secretion in young rhesus macaques with different alleles of the serotonin reuptake transporter polymorphism (5HTTLPR). Behav. Genet. 34 (3), 295–307.

Canli, T., Lesch, K.P., 2007. Long story short: the serotonin transporter in emotion regulation and social cognition. Nat. Neurosci. 10 (9), 1103–1109.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301 (5631), 386–389.

Champoux, M., Bennett, A., Shannon, C., Higley, J.D., Lesch, K.P., Suomi, S.J., 2002. Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. Mol. Psychiatry 7 (10), 1058–1063.

Christian, B.T., Vandehey, N.T., Fox, A.S., Murali, D., Oakes, T.R., Converse, A.K., Nickles, R.J., Shelton, S.E., Davidson, R.J., Kalin, N.H., 2009. The distribution of D2/D3 receptor binding in the adolescent rhesus monkey using small animal PET imaging. Neuroimage 44 (4), 1334–1344.

Cosgrove, K.P., Mazure, C.M., Staley, J.K., 2007. Evolving knowledge of sex differences in brain structure, function, and chemistry. Biol. Psychiatry 62 (8), 847–855.

Costes, N., Merlet, I., Ostrowsky, K., Faillenot, I., Lavenne, F., Zimmer, L., Ryvlin, P., Le Bars, D., 2005. A F-18-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. J. Nucl. Med. 46 (12), 1980–1989.

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo, M.R., Johnstone, E.C., Jacob, R., Walton, R.T., Grasby, P.M., 2005. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J. Neurosci. 25 (10), 2586–2590.

Davis, M., Walker, D.L., Lee, Y., 1997. Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 352 (1362), 1675–1687.

Ebstein, R.P., 2006. The molecular genetic architecture of human personality: beyond self-report questionnaires. Mol. Psychiatry 11 (5), 427–445.

Elfving, B., Bjornholm, B., Knudsen, G.M., 2003. Interference of anaesthetics with radioligand binding in neuroreceptor studies. Eur. J. Nucl. Med. Mol. Imaging 30 (6), 912–915.

Farde, L., Hall, H., Ehrin, E., Sedvall, G., 1986. Quantitative-analysis Of D2 dopamine receptor-binding in the living human-brain by PET. Science 231 (4735), 258–261.

Fox, A.S., Oakes, T.R., Shelton, S.E., Converse, A.K., Davidson, R.J., Kalin, N.H., 2005. Calling for help is independently modulated by brain systems underlying goal-directed behavior and threat perception. Proc. Natl. Acad. Sci. U. S. A. 102 (11), 4176–4179.

Fox, A.S., Shelton, S.E., Oakes, T.R., Davidson, R.J., Kalin, N.H., 2008. Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One 3 (7), e2570.

Hariri, A.R., Holmes, A., 2006. Genetics of emotional regulation: the role of the serotonin transporter in neural function. Trends Cogn. Sci. 10 (4), 182–191.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin transporter genetic variation and the response of the human amygdala. Science 297 (5580), 400–403.

Hayden, E.R., Dougherty, L.R., Maloney, B., Durbin, C.E., Olino, T.M., Nurnberger, J.I., Lahiri, D.K., Klein, D.N., 2007. Temperamental fearfulness in childhood and the serotonin transporter promoter region polymorphism: a multimethod association study. Psychiatr. Genet. 17 (3), 135–142.

Heils, A., Mossner, R., Lesch, K.P., 1997. The human serotonin transporter gene polymorphism — basic research and clinical implications. J. Neural Transm. 104 (10), 1005–1014.

Heinz, A., Jones, D.W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., Linnoila, M., Weinberger, D.R., 2000. A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biol. Psychiatry 47 (7), 643–649.

Ichise, M., Toyama, H., Innis, R.B., Carson, R.E., 2002. Strategies to improve neuroreceptor parameter estimation by linear regression analysis. J. Cereb. Blood Flow Metab. 22 (10), 1271–1281.

Ichise, M., Liow, J.S., Lu, J.Q., Takano, T., Model, K., Toyama, H., Suhara, T., Suzuki, T., Innis, R.B., Carson, T.E., 2003. Linearized reference tissue parametric Imaging methods: application to [C-11]DASB positron emission tomography studies of the serotonin transporter in human brain. J. Cereb. Blood Flow Metab. 23 (9), 1096–1112.

Ichise, M., Vines, D.C., Gura, T., Anderson, G.M., Suomi, S.J., Higley, J.D., Innis, R.B., 2006. Effects of early life stress on [C-11] DASB positron emission tomography imaging of serotonin transporters in adolescent peer- and mother-reared rhesus monkeys. J. Neurosci. 26 (17), 4638–4643.

Innis, R.B., Cunningham, V.J., Delforge, J., Fujita, M., Giedde, A., Gunn, R.N., Holden, J., Houle, S., Huang, S.C., Ichise, M., Lida, H., Ito, H., Kimura, Y., Koeppe, R.A., Knudsen, G.M., Knuuti, J., Lammertsma, A.A., Laruelle, M., Logan, J., Maguire, R.P., Mintun, M.A., Morris, E.D., Parsey, R., Price, J.C., Slifstein, M., Sossi, V., Suhara, T., Votaw, J.R., Wong, D.F., Carson, R.E., 2007. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J. Cereb. Blood Flow Metab. 27 (9), 1533–1539.

Ito, H., Takahashi, H., Arakawa, R., Takano, H., Suhara, T., 2008. Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography. Neuroimage 39 (2), 555–565.

Izquierdo, A., Newman, T.K., Higley, J.D., Murray, E.A., 2007. Genetic modulation of cognitive flexibility and socioemotional behavior in rhesus monkeys. Proc. Natl. Acad. Sci. U. S. A. 104 (35), 14128–14133.

Jacobsen, L.K., Staley, J.K., Zoghbi, S., Seibyl, J.P., Kosten, T.R., Innis, R.B., Gelernter, J., 2000. Prediction of dopamine transporter binding availability by genotype: a preliminary report. Am. J. Psychiatr. 157 (10), 1700–1703.

Jarrell, H., Hoffman, J.B., Kaplan, J.R., Berga, S., Kinkead, B., Wilson, M.E., 2008. Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. Physiol. Behav. 93 (4–5), 807–819.

Jovanovic, H., Lundberg, J., Karlsson, P., Cerin, A., Saijo, T., Varrone, A., Halldin, C., Nordstrom, A.L., 2008. Sex differences in the serotonin 1A receptor and serotonin

transporter binding in the human brain measured by PET. Neuroimage 39 (3), 1408–1419.
Kalbitzer, J., Frokjaer, V.G., Erritzoe, D., Svarer, C., Cumming, P., Nielsen, F.A., Hashemi, S.H., Baaree, W.F.C., Madsen, J., Hasselbalch, S.G., Kringelbach, M.L., Mortensen, E.L., Knudsen, G.M., 2009. The personality trait openness is related to cerebral 5-HTT levels. Neuroimage 45 (2), 280–285.
Kalin, N.H., Larson, C., Shelton, S.E., Davidson, R.J., 1998. Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. Behav. Neurosci. 112 (2), 286–292.
Kalin, N.H., Shelton, S.E., 1989. Defensive behaviors in infant rhesus-monkeys — environmental cues and neurochemical regulation. Science 243 (4899), 1718–1721.
Kalin, N.H., Shelton, S.E., 2003. Nonhuman primate models to study anxiety, emotion regulation, and psychopathology. Roots of Mental Illness In Children 1008, 189–200.
Kalin, N.H., Shelton, S.E., Fox, A.S., Oakes, T.R., Davidson, R.J., 2005. Brain regions associated with the expression and contextual regulation of anxiety in primates. Biol. Psychiatry 58 (10), 796–804.
Kalin, N.H., Shelton, S.E., Fox, A.S., Rogers, J., Oakes, T.R., Davidson, R.J., 2008. The serotonin transporter genotype is associated with intermediate brain phenotypes that depend on the context of eliciting stressor. Mol. Psychiatry 13, 1021–1027.
Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A., Riley, B., 2005. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression — a replication. Arch. Gen. Psychiatry 62 (5), 529–535.
Kraemer, G.W., Moore, C.F., Newman, T.K., Barr, C.S., Schneider, M.L., 2008. moderate level fetal alcohol exposure and serotonin transporter gene promoter polymorphism affect neonatal temperament and limbic–hypothalamic–pituitary–adrenal axis regulation in monkeys. Biol. Psychiatry 63, 317–324.
Larsen, P., Ulin, J., Dahlstrom, K., Jensen, M., 1997. Synthesis of [C-11]iodomethane by iodination of [C-11]methane. Appl. Radiat. Isot. 48 (2), 153–157.
Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274 (5292), 1527–1531.
Lesch, K.P., Meyer, J., Glatz, K., Flugge, G., Hinney, A., Hebebrand, J., Klauck, S.M., Poustka, A., Poustka, F., Bengel, D., Mossner, R., Riederer, P., Heils, A., 1997. The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. J. Neural Transm. 104 (11–12), 1259–1266.
Lucki, I., 1998. The spectrum of behaviors influenced by serotonin. Biol. Psychiatry 44 (3), 151–162.
Meyer, J.H., Houle, S., Sagrati, S., Carella, A., Hussey, D.F., Ginovart, N., Goulding, V., Kennedy, J., Wilson, A.A., 2004. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography — effects of major depression episodes and severity of dysfunctional attitudes. Arch. Gen. Psychiatry 61 (12), 1271–1279.
Oakes, T.R., Fox, A.S., Johnstone, T., Chung, M.K., Kalin, N., Davidson, R.J., 2007. Integrating VBM into the general linear model with voxelwise anatomical covariates. Neuroimage 34 (2), 500–508.
Parsey, R.V., Hastings, R.S., Oquendo, M.A., Hu, X.Z., Goldman, D., Huang, Y.Y., Simpson, N., Arcement, J., Huang, Y.Y., Ogden, R.T., Van Heertum, R.L., Arango, V., Mann, J.J., 2006. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. Am. J. Psychiatr. 163 (1), 48–51.