Imaging the serotonin transporter during major depressive disorder and antidepressant treatment

Jeffrey H. Meyer, MD

Neurochemical Imaging Program in Mood Disorders, PET Centre, Centre for Addiction and Mental Health, Toronto, Ont.

2005 CCNP Young Investigator Award Paper

This paper focuses on serotonin transporter 5-HTT imaging to investigate major depressive disorder (MDD) and antidepressant occupancy. Such investigations have only recently been possible as a result of major advances in ligand development. The state of the art method is [^{11}C] DASB PET or [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile) positron emission tomography. [^{11}C]DASB is a breakthrough for brain imaging 5-HTT. Compared with previous radioligands, [^{11}C]DASB offers both high selectivity and a favourable ratio of specific binding relative to free and nonspecific binding. These characteristics contribute to valid, reliable quantitation of the 5-HTT binding potential (BP). The 5-HTT BP can be viewed as an index of 5-HTT density in a medication free state, or unblocked 5-HTT density in a medication-treated state.During major depressive episodes with no other axis I comorbidity, either no difference in regional 5-HTT BP or a trend toward elevated 5-HTT BP is typically found. During major depressive episodes (of MDD) with more severe symptoms of pessimism (dysfunctional attitudes), regional 5-HTT BP is elevated. In subjects with major depressive episodes and comorbid axis I psychiatric illnesses, decreased regional 5-HTT BP is often reported. With selective serotonin reuptake inhibitor (SSRI) treatment at doses that distinguish from placebo in the treatment of major depressive episodes, 5-HTT occupancy is approximately 80%, and there is a strong relation between plasma level and occupancy that is not predictable based on affinity alone. Implications of 5-HTT imaging findings for understanding major depressive disorder and antidepressant treatment will be discussed.

Cet article porte sur l'utilisation de l'imagerie de la 5-HTT, transporteur de la sérotonine, pour étudier le trouble dépressif majeur (TDM) et l'occupation des récepteurs d'antidépresseurs. Une telle étude n'a que récemment été rendue possible par les grands progrès dans le domaine des ligands. La méthode de pointe dans ce domaine est la tomographie par émission de positons avec le [^{11}C]DASB ou [^{11}C]-3-amino-4-(2-diméthylaminométhyl-phénylsulfanyl)-benzonitrile). Le [^{11}C]DASB représente une percée pour l'imagerie cérébrale de la 5-HTT. Comparativement aux radioligands du passé, il se caractérise tant par une haute sélectivité que par un rapport favorable entre liaison spécifique et libre liaison non spécifique. Ce sont ces caractéristiques qui contribuent à une quantification valable et sûre du potentiel de fixation (PF) de la 5-HTT. On peut voir dans ce potentiel de fixation un indice de la densité de la 5-HTT dans un état de nonmédication ou de sa densité sans blocage dans un état de médication. Dans les épisodes dépressifs majeurs sans autre comorbidité axe I, il n'y a habituellement aucune différence de PF régional ou on constate ordinairement une tendance à l'élévation de ce potentiel. Dans les épisodes dépressifs majeurs (de TDM) avec des symptômes aggravés de pessimisme (attitudes dysfonctionnelles), le potentiel de fixation régional de la 5-HTT. Chez les sujets en proie à de tels épisodes dépressifs avec troubles psychiatriques de comorbidité axe I, on signale souvent une diminution du potentiel régional. Dans le traitement d'épisodes dépressifs majeurs à l'inhibiteur spécifique du recaptage de la sérotonine (ISRS) à des doses distinctes du placebo, l'occupation des récepteurs de la 5-HTT est d'environ 80 %; on note une étroite relation entre la concentration plasmatique et l'occupation qui ne saurait être prévue uniquement par l'affinité. Il sera question des conséquences de ces données d'imagerie de la 5-HTT sur le plan de la compréhension du trouble dépressif majeur et de son traitement aux antidépresseurs.

Correspondence to: Dr. Jeffrey Meyer, Neurochemical Imaging Program in Mood Disorders, PET Centre, Centre for Addiction and Mental Health, 250 College St., Toronto ON M5T 1R8; fax 416 979-4656; jeff.meyer@camhpet.ca

Medical subject headings: serotonin; serotonin transporter; depression; antidepressant; PET; positron emission tomography

J Psychiatry Neurosci 2007;32(2):86-102.

Submitted Aug. 30, 2006; Revised Oct. 20, 2006; Accepted Oct. 22, 2006

What properties of the serotonin transporter are important for major depressive disorder?

The serotonin transporter 5-hydroxy-tryptamine (5-HTT) is a 630 amino acid long receptor with 12 transmembrane domains.[1,2] The human 5-HTT gene is localized on chromosome 17, centred at 17q11.2.[3] Most 5-HTT are located at outer cell membranes, either perisynaptically or along axons.[4] In the human brain, the density of 5-HTT varies by region: Superior and inferior raphe nuclei > hypothalamus > thalamus (depending on the nucleus) ~ amygdala > putamen > caudate ~ hippocampus > insular cortex > prefrontal cortex > white matter > cerebellar cortex (except vermis).[5–7]

The serotonin transporter is coupled to sodium, chlorine and potassium transport.[3] However, the physiological role of interest of 5-HTT in major depressive disorder (MDD) and antidepressant treatment is its influence on extracellular serotonin levels. It is clear that many antidepressant drugs that bind to the serotonin transporter raise extracellular serotonin, and 5-HTT knockout mice have elevated extracellular serotonin, confirming the role of the serotonin transporter in modulating extracellular serotonin levels in vivo.[8–14]

Methods of imaging serotonin transporter in vivo

The following is a critical comparison of all 5-HTT imaging methods that have been applied in humans, with an emphasis on data relevant to humans (See Table 1). Previous comparisons have largely emphasized comparisons in baboons.[15] Although this information is valuable during radiotracer development,[16] it does not fully correspond to radioligand performance during human brain imaging because 5-HTT density can vary between animal species,[7] and the brain pharmacokinetics of 5-HTT radiotracers can differ between baboons and humans.[15,17–21]

These methods are used to derive the binding potential (BP). There are different versions of the BP but the one that is typically used is defined as follows: $BP = f_2 \times B_{max}/K_d$. f_2 is a fraction of free and nonspecific radiotracer that interacts with the specific binding compartment. B_{max} is receptor density and K_d is the dissociation constant. BP tends to be viewed as an index of B_{max} and, in the medication treated condition, it tends to be viewed as an index of receptor density not blocked by medication.

In the medication-treated state, a related measure is the 5-HTT occupancy, which can be defined as 5-HTT occupancy = (5-HTT BP_1- 5-HTT BP_2)/ 5-HTT $BP_1 \times 100\%$. 5-HTT BP_1 is the BP found in the untreated state and 5-HTT BP_2 is the BP found in the treated state.

[^{123}I]2-β-carbomethoxy-3-β-(4-iodophenyl)-tropane (β-CIT) single photon emission tomography (SPECT) was once the only technique developed for measuring the 5-HTT binding potential in humans.[37,38,44] This radiotracer has almost equal affinity for the dopamine transporter, compared with the serotonin transporter.[22,23] Because dopamine transporter density is high in the substantia nigra,[45] one cannot determine whether any changes in specific binding in the midbrain in an experimental paradigm are due to 5-HTT binding in superior raphe nuclei or dopamine transporter binding in substantia nigra. That there are specific binding sites that are not 5-HTT is consistent with the low 5-HTT occupancy estimates for selective serotonin reuptake inhibitors found with this method,[30,31] compared with 5-HTT occupancy estimates with selective 5-HTT binding radiotracers.[34,35] To the best of my knowledge, there are no reliability estimates of binding potential found in the midbrain with this method. Typically, this radiotracer is used for measuring dopamine transporter BP in the striatum in humans.[44]

The PET radiotracer [^{11}C](+)McN5652 (trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]-pyrrolo-[2,1-a]-isoquioline) shows greater selectivity for the serotonin transporter, compared with other monoamine transporters. It is estimated that this radiotracer has 1 or 2 orders of magnitude greater affinity for the serotonin transporter over the norepinephrine transporter and at least 2 orders of magnitude greater affinity for the serotonin transporter over the dopamine transporter.[24,25] [^{11}C](+)McN5652 has a low ratio of specific binding relative to free and nonspecific binding, which combined with modest reversibility, makes valid and reliable quantitation

Table 1: Comparison of radioligands for imaging of 5-HTT in humans

Properties	[^{123}I]β-CIT SPECT	[^{11}C](+)McN5652 PET	[^{11}C]DASB PET	[^{123}I]ADAM SPECT
Selectivity	Nonselective, near 1:1 affinity for 5-HTT to DAT[22,23]	Likely selective 10:1 to 100:1 affinity for 5-HTT over NET[24,25]	Highly selective 1000:1 affinity for 5-HTT over NET or DAT* [26,27]	Highly selective 1000:1 affinity for 5-HTT over NET or DAT[28,29]
Displaceability of specific binding	Incomplete[30,31]	In most, but not all, reports[19,32,33]	Highly displaceable[26,27,34,35]	Highly displaceable[28,29,36]
Reversibility†	Good[37,38]	Not adequate to adequate, depending on region‡ [18,19,39]	Adequate in midbrain, good to very good in other regions[20,21,40]	Adequate in midbrain[36,41]
Brain uptake	Adequate[37,38]	Good[19,39]	Very good[20,21,40]	Adequate[36,41]
Specific binding to free and nonspecific binding ratio†	Good	Not adequate in most regions, adequate in thalamus[39,42]	Adequate to very good‡ [20,21]	Not adequate in most regions; adequate in midbrain[36,41]
Reliability of 5-HTT BP†	Not measured	Modest[32]	Very good to excellent[34,43]	Most regions reasonable[41]
5-HTT BP measurability in multiple regions	Brainstem only[37,38]	Measurable in thalamus,[39] not measurable in cortex[19]	Yes[20,21]	Measurable in midbrain; unclear for other regions[36,41]

*[^{11}C]DASB is also highly selective for 5-HTT over several other targets tested in vitro.
†For humans (radiotracer performance differs across species.)
‡Depending on brain region.

difficult in regions other than the thalamus, and impossible in the human cortex.[18,19,32,39] Applications of this radiotracer in illness and in treatment have mostly focused on the thalamus, using the cerebellum as a reference region with noninvasive models.[33,39,46] However, some investigators use arterial sampling to measure 5-HTT BP in other subcortical brain regions to obtain a total distribution volume (an index of total radiotracer binding) and use the cerebellar cortex region to obtain an index of free and nonspecific binding.[32]

The radiotracer [^{11}C] 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (DASB) was a major advance because of its selectivity, reversibility, greater specific binding relative to free and nonspecific binding and reliability.[20,21,26,27,34,35,40,43,47] This radiotracer was found to be 3 orders of magnitude more selective for the 5-HTT than for the monoamine transporters and was highly selective for the 5-HTT, compared with several other screened targets.[26,27] Moreover, 92% to 95% of the specific binding to 5-HTT is displaceable by 5-HTT binding medications in animal models.[26,27] In humans, [^{11}C]DASB has good brain uptake[20,40]; its ratio of specific binding relative to free and nonspecific binding is good and the latter has low between–subject variability.[20,21] Multiple brain regions may be assessed with noninvasive methods,[20,21,26,27,34,35,40,43,47] and the reliability of regional 5-HTT BP measures is good.[34,35,43,48] The 5-HTT BP measures are low in the cortex, but with standardized region of interest methods, good reliability of 5-HTT BP in the human cortex may be obtained.[34,35,43,48] In summary, [^{11}C]DASB PET imaging is the state of the art method in quantifying 5-HTT in humans.

[^{123}I] ADAM (2-((2-((dimethylamino)methyl)-phenyl) thio)-5-iodophenylamine) SPECT is a fourth brain imaging method that has recently been applied to investigate 5-HTT BP in humans. It has a clear advantage of selectivity over [^{123}I] β-CIT SPECT, since most of the specific binding in most brain regions is displaceable in animal models, and it is selective for the 5-HTT over several other binding sites, including other monoamine transporters.[28,29] [^{123}I] ADAM has been modelled in baboons but not yet in humans.[49] The specific binding relative to free and nonspecific binding in humans is not optimal,[41] likely limiting the use of this method to assessing midbrain 5-HTT BP. However, reliability in the midbrain for 5-HTT BP measurement is good.[41]

[^{11}C]MADAM (^{11}C-N,N-Dimethyl-2-(2-amino-4 methylphenylthio)benzylamine) is a recently developed PET radiotracer that shows excellent selectivity over other monoamine transporters in vitro and good displacability in animal models.[50,51] Time activity curves presented show good reversibility potentially similar to [^{11}C]DASB but appear to have somewhat greater variability, particularly for the raphe.[20,52,53] Initial reports of reliability are also promising, although the scatter in repeated-measurement (standard deviation of percent difference in repeated-measure) appears greater than what has been reported for [^{11}C]DASB.[34,35,43,48,52,54]

What is the optimal method of applying [^{11}C]DASB PET for research protocols?

For selecting regions of interest, my group recommends automated region of interest approaches with visual validation, such as those involving subroutines from linear transformations and/or nonlinear deformations applied in the spatial normalization procedure from statistical parametric mapping.[55,56] Reliability of 5-HTT BP measurement is typically excellent when such applications are applied.[35,43,54,57] For subcortical regions, manual drawing upon coregistered MRI also has excellent reliability.[34]

For a reference region, my group recommends selecting the posterior half of the cerebellar cortex, excluding vermis, excluding white matter and keeping at least one full width half the maximum from the venous sinuses and from occipital cortex. At a distance of one full width half maximum, spillover from the occipital cortex (which possesses specific binding) or venous sinuses is negligible. White matter is excluded because [^{11}C]DASB has different kinetics in this tissue, compared with grey matter. The vermis is excluded because it has [^{11}C]DASB kinetics compatible with significant specific binding. We routinely use these methods.[34,35,43,58,59]

For selecting models for region of interest methods, we endorse reference tissue approaches.[20,21,34,35,43,58,59] By applying a linear regression between 5-HTT density and total distribution volume, we estimate that the reference tissue of posterior cerebellar cortex is composed of 93% free and nonspecific binding and 7% specific binding.[6] Knowing that the true BP = distribution volume of specific binding in region of interest divided by the distribution volume of free and nonspecific binding in the cerebellar cortex, the effect of specific binding in the cerebellar cortex is quite subtle. Disease influences of even 50% magnitude on the specific binding in reference tissue translate to 3.5% changes in the distribution volume estimate of free and nonspecific binding, which ultimately results in a 3.5% bias for between–group comparisons. For occupancy studies, the nature of the occupancy equation is such that the bias from a 7% underestimate during untreated conditions is translated into a lesser bias in the overall occupancy measure (less than 2%). For example, if the striatal 5-HTT BP has a true value of 1 in the untreated condition and 0.2 in the SSRI-treated condition, the true 5-HTT occupancy is ([1–0.2]/1) = 0.8 or 80% (5-HTT occupancy = (5-HTT BP_1–5-HTT BP_2)/ 5-HTT $BP_1 \times 100\%$). Taking into account the slight specific binding of reference tissue, the measured striatal 5-HTT BP, respectively, would be 0.93 in the untreated condition and 0.197 in the SSRI-treated condition (most of the 7% specific binding in reference tissue is blocked during treatment), leading to a measured 5-HTT occupancy of ([0.93–0.1972]/0.93) = 0.79 or 79%.

For [^{11}C]DASB PET, arterial methods offer no advantage for identifying subcompartments of free and nonspecific binding, because [^{11}C]DASB kinetics fit a single tissue compartment model in all regions.[21,60] Arterial methods do permit measurement of total distribution volume in the cerebellum, but this value is assumed to represent free and nonspecific binding, so as to quantitate binding potential measures in other regions. Thus, when arterial sampling is done, a very similar set of assumptions as compared with reference tissue models are applied.

Among the reference tissue methods, the noninvasive logan,[61] simplified reference tissue model 2 and multilinear

reference tissue model 2[21] have excellent reliability.[34,35,43,54,58,59] The latter two also avoid underestimating.[21] The Logan has some underestimate but correlates highly with the ratio of the distribution volume in regions with specific binding to the distribution volume in the cerebellum.[35] Moreover, with the Logan, the coefficient of variation is very low, and it has less assumptions (i.e., it does not require the single tissue compartment model).[61,62] For region of interest measurement in disease processes, we favour all 3 methods, but for drug- treated conditions, we prefer the Logan (to avoid requiring the single tissue compartment assumption across different levels of 5-HTT occupancy).[62]

What is the key evidence for low extracellular serotonin in untreated MDD?

Direct evidence that serotonin is low in MDD is unavailable for 2 main reasons: Brain serotonin cannot be directly measured in vivo and it is likely, based on animal simulations of postmortem delay, that serotonin levels are unstable, even within 24 hours of death.[63] Moreover, postmortem investigations of serotonin levels (previously listed by Mann[64]) have not sampled medication-free subjects with MDD in the midst of a major depressive episode (MDE).

Therefore, arguments that extracellular serotonin in the brain is likely to be low during MDE are based on the reversal of symptoms after serotonin-raising antidepressant drugs,[65–68] lowering of mood during paradigms that lower brain serotonin,[69–77] and changes in indices of serotonin 2 receptor density in suicide and MDD.[78–90]

This latter argument can be further clarified. An important property of 5-HT_2 receptors is that 5-HT_2 receptor density has an inverse relation to extracellular serotonin levels, such that the density of 5-HT_2 receptors in the cortex increases after chronic serotonin depletion and decreases after chronically raising extracellular serotonin.[91–94] Therefore, investigations of indices of 5-HT_2 density would be expected to report elevations in the midst of MDE. Postmortem investigations sometimes report elevated 5-HT_2 density in the prefrontal cortex of suicide victims,[78–88] and several of the investigations that found elevated prefrontal 5-HT_2 receptor density investigated subjects with MDD.[81,84] Such studies could be considered supportive of low extracellular serotonin in the prefrontal cortex of subjects with MDD.

Studies of 5-HT_2 receptors in the brain cortex usually measure 5-HT_{2A} receptors because ligand binding to 5-HT_{2C} receptors in the cortex is extremely low[95,96] and mRNA of 5-HT_{2B} receptors is extremely low in the cortex.[97]

At first review, there appears to be a contradiction between the results of postmortem and brain imaging studies of cortex 5-HT_2 receptors in MDD. Most of the imaging studies listed in Table 2 report a regional decrease in 5-HT_2 BP. The discrepancy can be resolved partly by the observation that most of these studies sampled subjects recently treated with serotonin-raising antidepressant drugs.

Upon further review of Table 2, the studies sampling subjects who recently had antidepressant treatment tend to report decreased regional 5-HT_2 BP, whereas the 2 studies not sampling subjects who recently had antidepressant treatment find no difference between subjects with depression and healthy subjects.[101,102] The study by Meyer and colleagues applied [^{18}F]setoperone PET. [^{18}F]setoperone is a good radioligand for imaging 5-HT_{2A} receptors because of its specific binding in the cortex, reversibility and favourable ratio of specific binding to free and nonspecific binding[106–110] (see Table 3 for properties of [^{18}F]setoperone). It is also insensitive to acute paroxetine-induced changes in extracellular 5-HT in humans.[118] The study by Meyer and colleagues sampled medication-free (> 6 mo), early-onset depression subjects with no comorbid psychiatric illnesses and found no difference in the prefrontal cortex 5-HT_2 BP, compared with healthy control subjects.[101] An investigation using [^{18}F] altanserin PET in older subjects with depression who were medication free similarly found no difference in 5-HT_2 BP between patients and healthy control subjects.[102] After considering medication-free status, there was still a lesser discrepancy, such that 5-HT_2 density was often elevated in the prefrontal cortex in postmortem studies of suicide victims, yet prefrontal 5-HT_2 BP was not changed in medication-free subjects with depression.

To resolve this lesser discrepancy, a more complicated model of low-cortex serotonin during MDEs was hypothesized. This hypothesis was that extracellular serotonin loss is

Table 2: Imaging studies of 5-HT_{2A} receptors in major depressive disorder

Study	Method	No. subjects (with depression, healthy)	Medication status	Result
Dh aenen et al[98]	[^{123}I]ketanserin SPECT	19,10	Medication free (7 d)	Greater in parietal cortex
Biver et al[99]	[^{18}F]altanserin PET	8,22	Medication free (10 d)	Lower in orbitofrontal cortex
Attar-Levy et al[100]	[^{18}F]setoperone PET	7,7	Taking benzodiazepines	Lower in prefrontal cortex
Meyer et al[101]	[^{18}F]setoperone PET	14,14	Medication free (3 mo plus, 5 half-lives)	No difference
Meltzer et al[102]	[^{18}F]altanserin PET	11,11	Untreated	No difference
Yatham et al[103]	[^{18}F]setoperone PET	20,20	Medication free (2 wk)	Decrease in all cortex
Messa et al[104]	[^{18}F]setoperone PET	19,19	Taking benzodiazepines	Decrease in all cortex
Meyer et al*[89]	[^{18}F]setoperone PET	22,22	Medication free (6 mo)	Positive association with dysfunctional attitude severity in cortex
Mintun et al†[105]	[^{18}F]altanserin PET	46,29	Medication free (4 wk)	Decrease in hippocampus

*Subjects enrolled in the study by Meyer et al 1999 were also included in the expanded study by Meyer et al.
†Findings largely appear driven by a single healthy subject with very high 5-HT_2 binding potential.

heterogenous during depressive episodes and that the loss is most severe in people with a greater severity of particular symptoms.

The symptom chosen in this hypothesis was elevated pessimism (dysfunctional attitudes) observed during MDEs. (There is a modest level of dysfunctional attitudes that increase during depressive episodes.) The rationale for choosing elevated dysfunctional attitudes is that raising extracellular serotonin after administering intravenous d-fenfluramine is associated with a strong shift in dysfunctional attitudes toward optimism 1 hour later in healthy individuals.[89] This suggests that, among the many roles of serotonin, one of them is to modulate dysfunctional attitudes in humans.

Dysfunctional attitudes can be measured with the dysfunctional attitudes scale (DAS), a measure sensitive for detecting negative thinking in the midst of depressive episodes[119,120] that has good internal consistency (Cronbach's α = 0.85 to 0.87)[121,122] and high test-retest reliability.[122,123]

If the hypothesis were true that extracellular serotonin in the cortex is variably reduced in the midst of depressive episodes, with the largest reductions in people with the most severe dysfunctional attitudes (pessimism), one would expect the highest prefrontal 5-HT_2 BP in subjects with the most severe dysfunctional attitudes (pessimism). This is based on the finding that 5-HT_2 receptor density increases after long-term serotonin depletion.[92,93] In support of the hypothesis, a strong correlation was observed between severity of dysfunctional attitudes (pessimism) and elevation in cortex 5-HT_2 BP.[89] Moreover, cortex 5-HT_2 BP was significantly elevated in subjects with severe depression with severe pessimism. For example, in the prefrontal cortex region centered on Brodman's area 9, 5-HT_2 BP was elevated 29% in depression subjects with dysfunctional attitude scores higher than the median for the group (Fig. 1). Thus the 2 parts of the study combined argue that extracellular serotonin is low in depression subjects who have a greater severity of pessimism.[89]

This investigation[89] is the first imaging study to show consistency with postmortem investigations reporting increased 5-HT_2 receptor density in the prefrontal cortex: The dysfunctional attitudes scale is well correlated with hopelessness, as measured with the Beck Hopelessness Scale.[124–127] Given that hopelessness is a risk factor for suicide,[128,129] it is plausible that investigations of suicide victims reporting increased 5-HT_2 BP sampled depression subjects with a greater severity of pessimism. A recent report has replicated the relation between cortex 5-HT_{2A} BP and severity of dysfunctional attitudes in recovered depression patients.[130]

Serotonin receptor binding that relates to extracellular serotonin in subcortical regions is also important. An interesting postmortem study by Stockmeier and colleagues[90] found elevated [^{3}H]OH-DPAT binding in the dorsal raphe nucleus of subjects in the midst of depressive episodes. Replication of this finding has been reported in suicide victims.[131] [^{3}H]OH-DPAT, being a 5-HT_{1A} agonist, would be expected to have elevated binding when serotonin is low.[132] In this interpretation, the result would support a hypothesis of lower extracellular

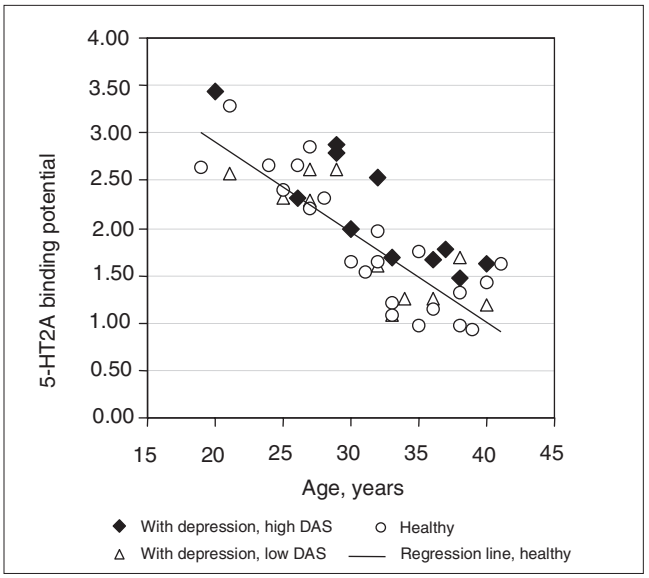

Fig. 1: 5-HT_{2A} receptor binding potential in averaged bilateral middle frontal gyrus (Brodmann's area 9) is plotted against age to show the relation between depressed and healthy subjects. The 22 depressed patients were divided into high and low dysfunctional attitudes scale (DAS) groups, depending on whether their DAS scores were above or below the median DAS score for depression patients. This median score was 166. Patients with high DAS scores had significantly higher 5-HT_{2A} receptor binding potential, compared with healthy subjects (ANCOVA [age covariate], diagnosis, $F_{1,19}$ = 11, p = 0.003). Reprinted with permission from the *American Journal of Psychiatry.*

Table 3: Properties of [^{18}F] Setoperone

Properties	[^{18}F] Setoperone
Selectivity and displaceability of specific binding	– 100:1 greater affinity for 5-HT_{2A} receptors over 5-HT_{2C} receptors, and a 1:10 to 1:50 greater affinity of 5-HT_{2A} receptors over D_2 receptors.[111] Since D_2 receptors are 10-fold less dense in human cortex[82–84,112] the combined relative effects of affinity and density correspond to an estimate that at least 99% of the specific binding signal in cortex is attributable to 5-HT_{2A} receptors.
	– No effect of the D_2 antagonist amisulpride on [^{18}F] setoperone binding in human cortex[113]
	– 5-HT_2 antagonists fully block specific binding in animal and human cortex[106,107,114,115]
	– Not selective in striatum where D_2 receptor density is high[107]
Reversibility	Very good with peak in cortex between 10 and 30 min[107,110]
Brain uptake	High[106,107,109]
Specific binding to free and nonspecific binding ratio	Very good; binding potential approximately 2 to 3 in age range18–40 yr[89,107,110]
Reliability of 5-HT_{2A} BP	Excellent[116]
Metabolites	Radioactive metabolites unable to cross blood–brain barrier[117]

serotonin in the dorsal raphe region (although this finding could also be viewed as a mechanism that enhances inhibition of the dorsal raphe by nearby extracellular serotonin[90]).

Possible disease models and the serotonin transporter in MDD

If extracellular serotonin is low during MDEs, with greater severity of dysfunctional attitudes, then it is logical to consider serotonin transporter function in the removal of extracellular monoamines. There are at least 4 plausible ways to understand how 5-HTT BP, an index of 5-HTT density and affinity, could be altered in a disease that lowers brain serotonin, described below. Prior to investigations of [^{11}C]DASB imaging during depressive episodes, model 1 and model 3 were the most plausible.

Model 1

The first possible model is a lesion model. If serotonin nerve terminals were destroyed in MDD, then one could expect that there would be less release of serotonin. This model is observed in neurotoxin exposure[133] and late in Parkinson's disease.[134,135] If there were less nerve terminals in MDD, then one would expect a reduction in regional 5-HTT BP.

Model 2

A second model to consider would be whether a lowering of extracellular serotonin (via a process unrelated to 5-HTT sites) would have a secondary effect on 5-HTT density. Acute reductions in serotonin have repeatedly shown reductions in 5-HTT mRNA.[136–138] However, longer-term reductions or elevations in serotonin typically show no influence on regional 5-HTT density.[139–141] The is not comparable to other monoamine transporters. For example, for the dopamine transporter, the evidence is much stronger to support a relation between long-term reductions in extracellular dopamine and a lowering of striatal dopamine transporter density.[142–145] This second model seems unlikely.

Model 3

A third model is increased clearance of serotonin via greater density of 5-HTT. There is an inverse relation between functioning 5-HTT and extracellular serotonin. For example, it has been demonstrated through antidepressant occupancy and 5-HTT knockout models that less functioning 5-HTT are associated with greater extracellular serotonin.[9–14,146] It is possible that, under conditions of greater 5-HTT density, greater extracellular serotonin loss may occur. With this model, greater 5-HTT BP would be associated with more severe serotonin loss.

Model 4

A fourth model to consider is endogenous displacement. Endogenous displacement refers to the property, found in a minority of PET radiotracers under physiological conditions, to have increased binding potential measures after a reduction in endogenous neurotransmitter.[147] The name originates from the initial explanation for this phenomenon, that the neurotransmitter itself prevented access of the radiotracer to receptors. For [^{11}C]DASB, endogenous displacement may occur with large magnitude changes in extracellular 5-HT but would not be expected to occur with extracellular 5-HT changes that are physiologically relevant for humans. In an animal study with [^{11}C]DASB, after raising extracellular serotonin with an intraperitoneal injection of 10 mg/kg of tranylcypromine, a MAO-A and MAO-B inhibitor, a reduction in 5-HTT BP was observed.[148] Similar work has been replicated with similarly substantial doses of tranylcypromine.[149] Notably, the rise in extracellular serotonin with high doses of tranylcypromine is enormous, with a several hundred to thousand percent rise being typical.[150–152] Humans cannot tolerate 1/10 this dose of tranylcypromine, even with lengthy titrations and oral administration. Thus this magnitude of serotonin change may exceed what is physiologically relevant in humans. In 14 humans, we examined the effect of tryptophan depletion upon 5-HTT and found no effect, demonstrating that endogenous serotonin occupancy is unlikely to appreciably influence [^{11}C]DASB[43] under physiologically tolerable conditions. Talbot and colleagues reported similar results in 8 humans.[153] Hence, the fourth model is unlikely to apply to PET imaging studies with [^{11}C]DASB in humans.

Studies of the serotonin transporter in MDD

There are only 2 postmortem investigations of 5-HTT density in subjects with recent symptoms of depressive episodes. In these 2 studies, no changes in 5-HTT density were found in either the dorsal raphe or the locus coeruleus.[154,155] Other postmortem studies of 5-HTT density sampled subjects with a history of a depressive episode (not necessarily recent) and typically focused on prefrontal cortex regions. These studies tend to report either decreased 5-HTT density[156–159] or no difference in 5-HTT density.[160–165] In several of these studies, subjects were medication free, based on clinical history and toxicological screening.[158,160,161] For many of these investigations, average postmortem delays were less than a day.[154–156,158,159,162]

Most of these studies were recently reviewed in detail by Stockmeier and colleagues.[88] However, most postmortem studies are not representative of a depressive episode, since only 2 studies of 5-HTT density sampled subjects who recently had symptoms.[154,155] Further, the postmortem studies are not completely selective for MDD because all include comorbid axis I psychiatric illnesses[156–162] and some also sample bipolar disorder.[158,160,165] A third key issue with sampling is that all studies include both early- and late-onset MDD.[156–162]

The first imaging study of 5-HTT in vivo used [^{123}I]β-CIT SPECT to measure specific binding of [^{123}I]β-CIT in the hypothalamus-midbrain region in depressed patients and healthy control subjects.[166] See Table 4 for a descriptive list of imaging studies of the 5-HTT. A reduction in the binding potential was found. Interestingly, subjects with major depression were

subdivided based on recency of medication use, and this did not affect the results. The following methodological issue makes this finding difficult to interpret: Because [^{123}I]β-CIT has similar affinity for both 5-HTT and DAT transporters[166] and because both the raphe and substantia nigra are within this region sampled, it is not clear whether specific binding of β-CIT reflects 5-HTT binding or DAT binding. The DAT downregulates in striatum after long-term dopamine depletion.[142–145] If DAT in substantia nigra downregulate similarly and if there is a monoamine lowering process in the midbrain during depressive episodes, it would be expected that an index of specific binding (to serotonin and dopamine transporters) would be lower.

The next study of 5-HTT applied [^{11}C](+)McN5652 PET to measure thalamus 5-HTT BP in 7 subjects with MDD.[46] The data from these subjects were added to data from 6 subjects who had bipolar disorder. Ichimiya found that, in subjects with either MDD or bipolar disorder, 5-HTT BP in the thalamus is elevated.[46] A strength of the study was that subjects were medication free, and a more selective radioligand was used. A disadvantage of the study is that it may be incorrect to assume that unipolar MDEs and bipolar MDEs have a common serotonin transporter abnormality. This study did not investigate 5-HTT BP during a current MDE because only 5 subjects with a current MDE and MDD were enrolled in the study.[46]

The next study was the first application of [^{11}C]DASB PET imaging to MDD. It sampled 20 subjects with MDE and 20 healthy controls.[170] This sample had a number of advantages because it was reasonably large, subjects were medication free for at least 3 months, and they had no other comorbid axis I illnesses, were nonsmoking, and had early onset depression. As a result of the technical advantages of [^{11}C]DASB, 5-HTT BP could be reliably measured in multiple brain regions, including cortex. There was no evidence to support a hypothesis of a difference in 5-HTT BP as no difference in regional 5-HTT BP was found between MDE and healthy subjects in any brain region.[170]

However, in this same study of [^{11}C]DASB PET,[170] there was highly significant support for the hypothesis that greater regional 5-HTT BP would occur during MDE with severe, pessimistic dysfunctional attitudes. This hypothesis was based on the interpretation of 3 findings which argue that extracellular serotonin is lowest during MDE with severe, pessimistic, dysfunctional attitudes: The first finding is the acute shift toward optimism in humans after raising extracellular serotonin with d-fenfluramine which argues for a role of serotonin in modulating pessimism/optimism in humans.[89] The second finding is that cortex 5-HT$_2$ BP is greater during MDE with severe pessimism.[89] This can occur when extracellular serotonin is low, according to the third set of findings: 5-HT$_2$ receptor density increases when extracellular serotonin is chronically lowered[92,93] (for more detail, see Key Evidence For Low Extracellular Serotonin in Untreated Major Depressive Disorder in this article). The subgroup of MDE subjects with severely pessimistic dysfunctional attitudes had significantly higher 5-HTT BP, compared with healthy subjects, in brain regions sampling serotonin nerve terminals (prefrontal cortex, anterior cingulate, thalamus, bilateral caudate, bilateral putamen) (see Fig. 2). On average, 5-HTT BP was 21%

Table 4: Imaging investigations of the serotonin transporter in untreated major depressive episodes

Study	Ligand	No. subjects (with MDE, healthy)	Illness	Medication status	Main finding
Malison et al[166]	[^{123}I]β-CIT SPECT	15, 15	MDD and/or comorbid disorders	6 medication naive, 9 medication free for 3 wk	Lower BP in brainstem
Ichimiya et al[46]	[^{11}C](+)McN5652 PET	7,* 15	MDD only (pooled with BD only)	All medication free for > 6 wk	↑ 5-HTT BP in thalamus; no change in midbrain in pool of MDD and BD subjects
Meyer et al[47]	[^{11}C]DASB PET	20, 20	MDD only	All medication free for > 3 mo and 14 also antidepressant naive	No change in 5-HTT BP in MDE; in MDE with severe pessimism, greater 5-HTT BP in all regions except midbrain
Newberg et al[167]	[^{123}I]ADAM SPECT	7, 6	MDD only	All medication free for > 2 wk, 2 antidepressant naive	No change in 5-HTT BP in thalamus and striatum; lower 5-HTT BP in midbrain
Parsey et al[168]	[^{11}C](+)McN5652 PET	25, 43	MDD, *n* = 19 with comorbid anxiety disorders	All potentially exposed to benzodiazepines. All antidepressant free for > 2 wk; 12 antidepressant naive	No change in putamen, thalamus, hippocampus, or anterior cingulate ↓ 5-HTT BP in midbrain and amygdala†
Herold et al[169]	[^{123}I]ADAM SPECT	21, 13	MDD only	All medication free for > 2 mo	Trend toward greater midbrain 5-HTT BP

MDD = major depressive disorder; MDE = major depressive episode.
*Ichimiya et al sampled 21 subjects with mood disorders, 14 of whom had bipolar disorder.
†Findings were natural log-transformed before analysis, after a quantity was added.

greater in these regions in MDE subjects with severely pessimistic dysfunctional attitudes (see Fig. 3). Moreover, within the MDE group, greater 5-HTT BP was strongly associated with more negativistic dysfunctional attitudes in the same brain regions. The interpretation was that serotonin transporters have an important role in influencing extracellular serotonin during MDEs: Greater regional 5-HTT BP can provide greater vulnerability to low extracellular 5-HT and symptoms of extremely negativistic dysfunctional attitudes.[170]

It is premature to conclude upon the etiology of an elevation in 5-HTT BP in the subset of MDE subjects with more severe dysfunctional attitudes, because one could consider both genetic[171] and environmental influences.[172] The simplest explanation is that the subgroup with greater pessimism happened to inherit a greater 5-HTT density. Under this explanation, it would be expected that inheriting a greater 5-HTT could increase the risk of acquiring a MDD with more severe pessimism during MDEs.

The relation between genotypes such as the 5-HTTLPR and/or 5-HTT LPR (L_A/L_G) and brain 5-HTT density or binding potential is an area of ongoing study.[59,158,173–178] Some investigators interpret the genotype associated with greater 5-HTT synthesis in cell lines as being reflective of 5-HTT density in the brain. The genotype associated with greater 5-HTT synthesis is sometimes associated with greater clinical response[179–182] and better long-term outcome.[183] It is well known that better antidepressant responsiveness predicts long-term outcome.[184–187] This body of literature does not need to be inconsistent with the finding of greater 5-HTT BP in depression subjects with more severe dysfunctional attitudes.[170] From a theoretical perspective, it could certainly be that subjects with depression with the highest 5-HTT binding potential and the lowest levels of extracellular serotonin could have more severe pessimism, yet they could be more responsive to SSRI treatment and have better long-term outcomes as a result of being more responsive to SSRI treatment.

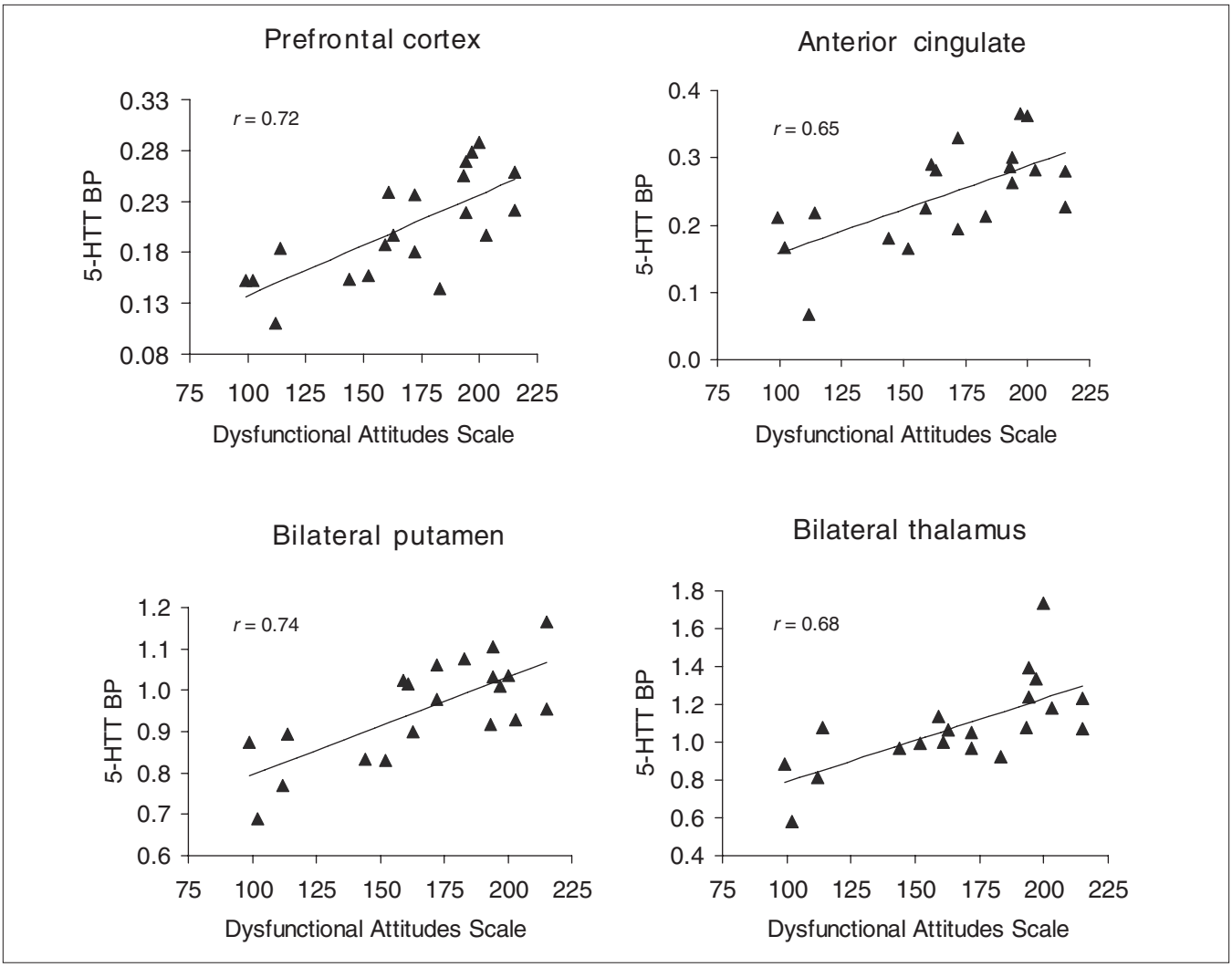

Fig. 2: Correlations between dysfunctional attitudes and serotonin transporter binding potential (5-HTT BP) in some of the larger regions in depression subjects. Highly significant correlations were found: prefrontal cortex ($p = 0.0004$), anterior cingulate ($p = 0.002$), bilateral putamen ($p = 0.0002$), bilateral thalamus ($p = 0.001$). Reprinted with permission from the *Archives of General Psychiatry*.

The other [^{11}C]DASB PET imaging study in mood disorder sampled depression subjects with bipolar disorder.[188] Since the idea of increased 5-HTT BP being associated with illness or severity of symptoms is still new, it is interesting that 5-HTT BP was significantly greater at the uncorrected level in 5 of 8 predefined regions of interest (with a similar trend in a sixth region). On a voxel level analysis, medial prefrontal cortex, thalamus, caudate and insula had significantly greater 5-HTT BP after accounting for multiple comparisons. No region had a significant decrease in 5-HTT BP after correcting for multiple comparisons.

A fourth study of brain 5-HTT in depression applied [^{123}I]ADAM SPECT to study 7 subjects with MDEs and 6 healthy control subjects.[167] Thalamus, striatum and midbrain regions were assessed. No difference in 5-HTT BP was found in the thalamus and striatum; however, a reduction in midbrain 5-HTT BP was reported. Limitations of the study were that 2 subjects had selective serotonin reuptake inhibitors as recently as 3 weeks previously and the small sample size.

A fifth study applied [^{11}C](+)McN5652 PET to study depressed and healthy subjects.[168] A strength of the study was that the sample size was reasonable and a subdivision of antidepressant naive subjects was gathered. The authors employed an approach of adding constants to data and applying the natural logarithm to 5-HTT binding potential values. It was reported that the 5-HTT binding potential was lower in the midbrain and amygdala, but it is unclear that this would have been the case had untransformed 5-HTT BP values been presented.

The sixth study applied [^{123}I]ADAM SPECT[169] in subjects with depression and in healthy subjects and found a trend toward increased midbrain 5-HTT BP. The main strengths of the study related to the sample of depression subjects selected: A reasonable number of medication-free subjects who had no comorbid illnesses were enrolled (n = 21) .

Summary interpretations of 5-HTT imaging studies in MDD

The following interpretations summarize key findings of 5-HTT imaging studies in MDD:

- Studies that exclude comorbid axis I illnesses (Meyer and others,[170] Ichimiya and others,[46] Herold and others[169]) tend to find either no change in regional 5-HTT BP or an increase in 5-HTT BP.
- The only study of [^{11}C]DASB PET with rigorously collected samples found no difference in regional 5-HTT BP.[170] This provides a strong argument against a degenerative model of loss of serotonin neurons (ruling out model 1 under possible disease processes section).
- The study of [^{11}C]DASB PET found significantly greater 5-HTT BP in depression subjects with more severe pessimism.[170] This finding argues that the contributing mechanism to extracellular serotonin loss is excessive 5-HTT (supporting model 3 under possible disease processes section).
- The [^{11}C]DASB PET study by Meyer and colleagues found no regional differences in 5-HTT BP between subjects with depression and healthy subjects. This study sampled depression subjects with MDD and no comorbid axis I psychiatric illnesses. Both imaging[151,172] and postmortem studies[156–159] that include other comorbid axis I illnesses in their sampling found some regional decreases in 5-HTT BP. It is possible that the findings in studies that sample comorbid axis I psychiatric illnesses reflect effects of common comorbid illnesses rather than MDD alone.

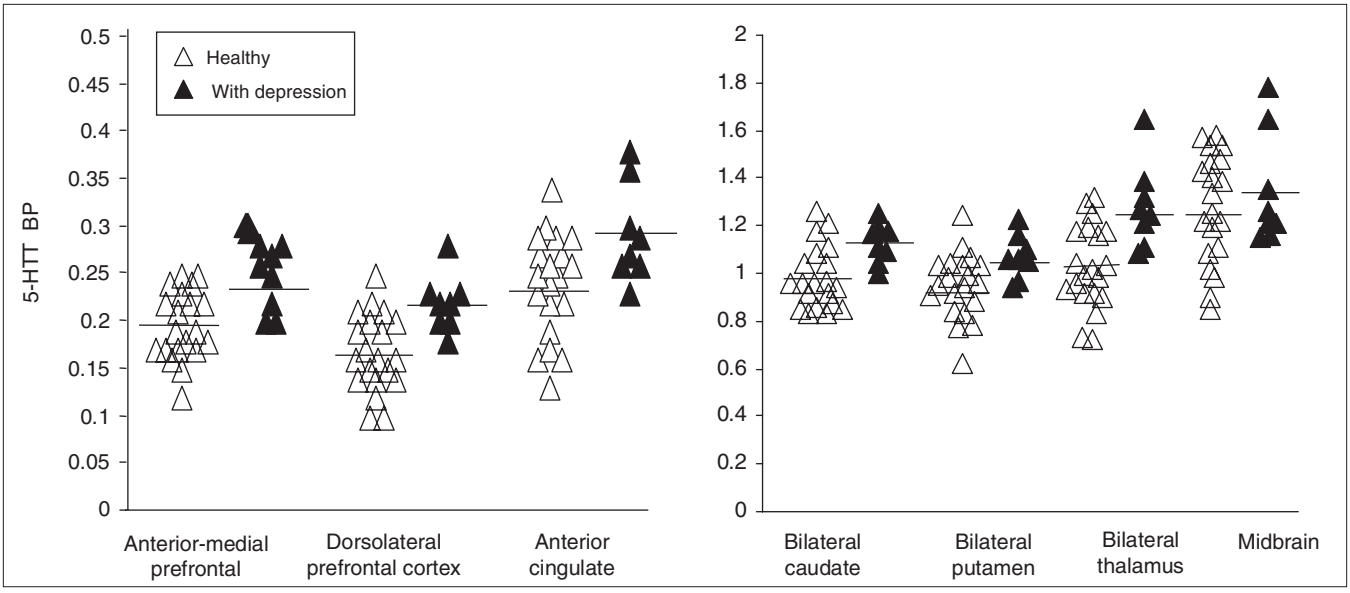

Fig. 3: Comparison of regional 5-HTT BP between 8 subjects with depression with severely negativistic dysfunctional attitudes (greater than 190) and 20 healthy subjects. For regions primarily sampling serotonergic nerve terminals (prefrontal cortex, anterior cingulate, caudate, putamen, thalamus) the 5-HTT BP was significantly greater in the depressed group ($F_{1,26}$ = 5.6 to 12.2, p = 0.03 to 0.002). The midbrain 5-HTT BP was not significantly different ($F_{1,26}$ = 0.5, p = 0.5). Reprinted with permission from the *Archives of General Psychiatry*.

Studies of serotonin transporter cccupancy

Prior to the first [^{11}C]DASB PET study, it was generally thought that 5-HTT occupancy of current SSRI was close to 100%, given the known plasma levels and known affinity of commonly prescribed SSRIs. The problem of using plasma levels as direct, identically proportionate estimates of brain levels of antidepressant drugs is that this method assumes

Table 5: 5-HTT occupancy imaging investigations of the serotonin transporter

Study	Ligand	Subjects	Medication	Regions	Main finding
Pirker et al[30]	[^{123}I]β-CIT SPECT	11 MDD, 1 BD, between subject design	Citalopram 20–60 mg/d; duration unclear	Hypothalamus-thalamus-midbrain only 1 region	46% occupancy*
Meyer et al[34]	[^{11}C]DASB PET	12 MDE, within subject design	Paroxetine 20 mg/d; citalopram 20 mg/d; 4 wk tx; 6–13 h after last dose	Whole striatum; also caudate, putamen, thalamus, prefrontal cortex, insula, anterior cingulate	80% occupancy in whole striatum and other regions
Kent et al[32]	[^{11}C](+)McN5652 PET	5 social phobia, within subject design	Paroxetine 20–40 mg/d; 3 to 6 mo tx	Striatum, thalamus, cingulate, hippocampus, midbrain, amygdala	Average occupancy 82%
Suhara et al[33]	[^{11}C](+)McN5652 PET	27 healthy; 10 patients	Fluvoxamine clomipramine healthy-acute dosing: 5 h after dose; patients: long-term dosing 1–10 h after last dose	Thalamus	80% occupancy at clinical dose Low-dose clomipramine has considerable occupancy Acute occupancy not very different from chronic
Meyer et al[35]	[^{11}C]DASB PET	37 healthy; 29 MDD; 16 MDD and anxiety disorder	Citalopram 1–60 mg/d; fluoxetine1–60 mg/d; sertraline 5–200mg/d; paroxetine 5–60mg/d; venlafaxine XR 2.4–225 mg/d; 4 wk tx; 6–13 h after last dose	Striatum, thalamus, anterior cingulate, prefrontal cortex, midbrain, bilateral cuneus	80% striatal occupancy at minimum clinical dose for SSRI; dose and plasma concentration highly predictive of 5-HTT occupancy; Regional occupancy in multiple regions correlated with striatum; significantly lower in thalamus and higher in midbrain; Striatal occupancy < 90% regardless of dose
Erlandsson et al[36]	[^{123}I]ADAM SPECT	16 healthy	Citalopram 10–60 mg over 2–7 d	Midbrain	Maximum occupancy 84%
Takano et al[189]	[^{11}C]DASB PET	15 healthy	Duloxetine 5–60 mg/d acutely (n = 12); 60 mg/d 1 wk (n = 3)	Thalamus	80% occupancy at 40 mg single dose 84 per occupancy after 1 wk, 60 mg/d half-life of brain occupancy apx. 78 h
Takano et al[48]	[^{11}C]DASB PET	6 healthy	Fluvoxamine 50 mg acutely	Frontal cortex, thalamus, striatum, hippocampus, amygdale	72% occupancy 5 h postdose 50% occupancy 26 h postdose 24% occupancy 53 h postdose
Parsey et al[190]	[^{11}C]DASB PET	17 healthy	Sertraline 25–100 mg/d; 4 d, 24 h after last dose	15 regions	Methods mostly dissimilar to other investigations Average occupancy 107% (large SDs)
Herold et al[169]	[^{123}I]ADAM SPECT	13 MDD	Citalopram 10 mg/d; 1 wk, 6–7 h after last dose	Midbrain	61% occupancy

BD = bipolar disorder; MDD = major depressive disorder; MDE = major depressive episode; SD = standard deviation; tx = treatment.
*[^{123}I]β-CIT was once the only method to image the serotonin transporter but it is probably not selective. This 5-HTT occupancy measure is believed to be a significant underestimate.

medications are equally and readily brain penetrant. These assumptions are tenuous because there are active transport processes that remove medication from the brain, and brain uptake is also related to lipophillicity. The initial work by Pirker and colleagues,[30] which had about 50% occupancy, was assumed to be inaccurate due to binding of β-CIT to DAT. However, they did demonstrate that citalopram entered the brain and had 5-HTT occupancy in humans. See Table 5 for a descriptive list of imaging studies of 5-HTT occupancy.

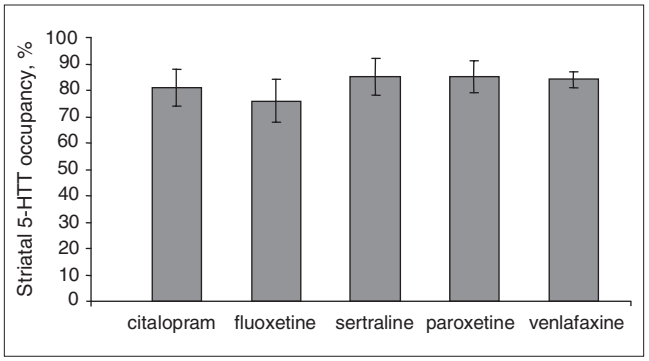

Fig. 4: 5–HTT occupancy at minimum therapeutic dose. Mean striatal serotonin transporter (5-HTT) occupancy for 5 selective serotonin reuptake inhibitors after 4 wk of minimum therapeutic dosing. The vertical ranges represent standard deviation. Subjects received citalopram 20–40 mg (n = 7), fluoxetine 20 mg (n = 4), sertraline 50 mg (n = 3), paroxetine 20 mg (n = 7), venlafaxine XR 75 mg (n = 4). Reprinted from the *American Journal of Psychiatry*.

In 2001, the first SSRI occupancy study with [^{11}C]DASB PET found an 80% occupancy in multiple regions after 4 weeks of antidepressant treatment, with doses of paroxetine and citalopram known to have clinical effects that distinguish from placebo.[34] Since that time, this result has been replicated in brain regions of reasonable size with fluvoxamine[33] as well as fluoxetine, sertraline and venlafaxine[35] (see Fig. 4 and Fig. 5). Interestingly, an 80% striatal 5-HTT occupancy occurs at minimum clinical dose, despite the 100-fold range in affinity of the 5 SSRIs for the serotonin transporter.[35] Further, the in vitro EC_{50} does not correlate with affinity.[35] This demonstrated that, although affinity is obviously a very important property of a drug, it cannot predict occupancy, even when plasma levels are known.[35]

Given the association between the clinically relevant dose and 5-HTT occupancy for all SSRIs, it is now generally believed that an 80% 5-HTT occupancy with an SSRI is therapeutically useful. Consequently, to develop antidepressant drugs with serotonin transporter binding, an 80% serotonin transporter occupancy is considered optimal. This practical approach can be applied to phase I trials to assess whether potential new antidepressant drugs are adequately brain penetrant and to guide dosing selection for subsequent phase II clinical trials.

The 2004 study[35] also studied the relation between 5-HTT

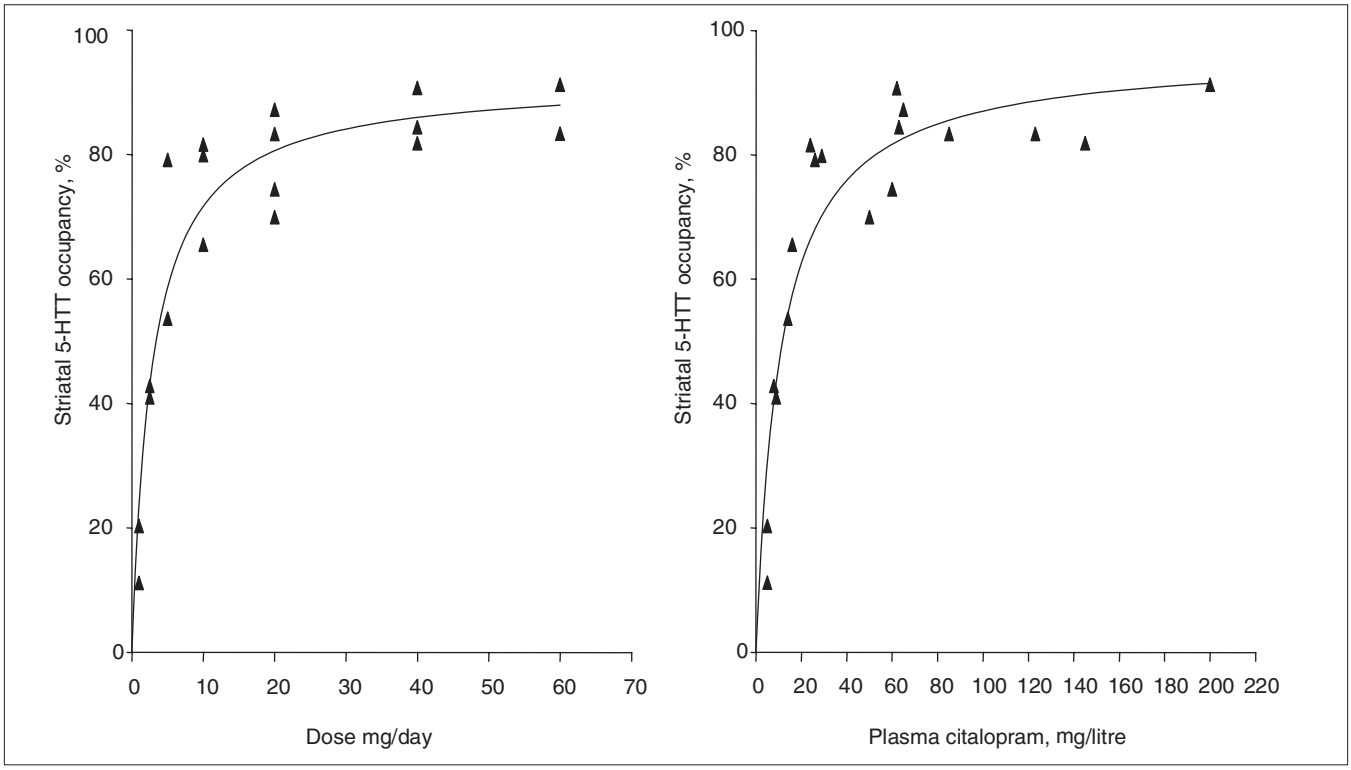

Fig. 5: Relation between striatal 5-HTT occupancy and dose* or plasma concentration† of citalopram. *†The data were fit using an equation of form f(x) = a*x/(b+x). *The relation between dose and occupancy was highly significant (f(x) = 92*x/(b+x), $F_{1,16}$ = 127, p < 0.0001). †The relation between plasma level and occupancy was highly significant (f(x) = 96*x/(b+x), $F_{1,16}$ = 103, p < 0.0001). Reprinted from the *American Journal of Psychiatry*.

occupancy and plasma level for 5 commonly prescribed SSRIs. There was increasing occupancy with increasing plasma levels, and occupancy plateaued at the higher doses and higher plasma levels. An important result of this study was that both dose and especially plasma level, had a very strong relation to 5-HTT occupancy. This has several practical clinical applications. First, it is unlikely that inadequate 5-HTT occupancy is a barrier to therapeutic response, because one may simply raise the dose of the SSRI to obtain adequate plasma levels. Second, in clinical circumstances, to estimate 5-HTT occupancy, a 5-HTT imaging study does not need to be completed. Instead, one may use the plasma level and the table in the study to estimate the 5-HTT occupancy of any SSRI.[35] Third, given the plateau of 5-HTT occupancy in the clinical dosing range, it is unlikely that 5-HTT occupancy has a strong relation to clinical response within current clinical dosing ranges (as was observed).[35]

In the [^{11}C]DASB studies of 2001 and 2004, 5-HTT occupancy of SSRIs did not exceed 90%. This raises the question as to whether there is a gap in current therapeutic development, such that SSRIs with extremely high 5-HTT occupancy are not available. Suhara and colleagues reported near 100% 5-HTT occupancy with clomipramine, using [^{11}C](+)McN5652 PET within clinical dosing ranges.[33] This has clinical implications because it suggests that clomipramine may be associated with high 5-HTT occupancy within clinical dosing ranges. This result is consistent with the clinical preference for clomipramine or high-dose SSRI for obsessive compulsive disorder[191] (for which greater 5-HTT occupancy is preferred).

To date, it seems that 5-HTT occupancy values after short-term dosings largely resemble the findings after 4 weeks of SSRI dosing.[33–36,48,169] This has implications for antidepressant development, because it is often desirable to study single - dose occupancy in early phase I investigations, before multiple dosing studies. Results by Parsey and colleagues[190] are similar in subcortical regions but may be discrepant in other regions; this may require further study.

Future 5-HTT occupancy investigations are likely to focus on novel antidepressant drugs that bind to both the serotonin transporter and other targets with high affinity.[189] An interesting question for future research will be whether an 80% occupancy in reasonably large brain structures is necessary for therapeutic effect when antidepressants target additional therapeutic sites.

Main Findings and Implications

The following are the key findings and/or implications from studies of 5-HTT occupancy:

- An 80% regional 5-HTT occupancy, particularly in striatum, typically occurs at doses of SSRIs known to distinguish from placebo in clinical trials of MDD.[33–35]
- Affinity values, even with accompanying blood plasma drug levels, cannot predict 5-HTT occupancy.[34,35] 5-HTT imaging methods are essential to predict 5-HTT occupancy.
- Inadequate 5-HTT occupancy alone cannot adequately explain treatment refractoriness, because 5-HTT occupancy is most strongly related to dose and plasma levels.[34,35]
- 5-HTT occupancy will be a useful tool for antidepressant development, either to develop antidepressant drugs for first-line treatment (ideally with near 80% 5-HTT occupancy[34,35]) or to develop antidepressant drugs with higher 5-HTT occupancy values[33] for treatment refractory depression.

Acknowledgements: I would like to thank Alan Wilson who discovered (first to synthesize and characterize) the [^{11}C]DASB compound. I would like to also thank Nathalie Ginovart and Sylvain Houle for their modeling efforts with [^{11}C]DASB. I would also like to thank members of the Toronto PET centre who have actively contributed to studies of serotonin receptors in depression or supporting technology, especially Armando Garcia, Sandra Sagrati, Anahita Carbonneau, Anna Carella, Verdell Goulding, Terry Bell, Ted Harris-Brandts, Alvina Ng and Doug Hussey. Funding support from the Canadian Institutes of Health Research, NARSAD and Eli Lilly Canada was also appreciated.

Competing interests: Dr. Meyer has acted as a paid consultant to Lundbeck and GlaxoSmithKline.

References

1. Lesch KP, Wolozin BL, Estler HC, et al. Isolation of a cDNA encoding the human brain serotonin transporter. *J Neural Transm Gen Sect* 1993;91:67-72.
2. Lesch KP, Wolozin BL, Murphy DL, et al. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. *J Neurochem* 1993;60:2319-22.
3. Blakely R, Ramamoorthy S, Qian Y, et al. Regulation of Antidepressant-Sensitive Serotonin Transporters. In: Reith M, editor. *Neurotransmitter Transporters: Structure, Function, and Regulation*. Totowa: Humana Press, 1997. p. 29-72.
4. Zhou FC, Tao-Cheng JH, Segu L, et al. Serotonin transporters are located on the axons beyond the synaptic junctions: anatomical and functional evidence. *Brain Res* 1998;805:241-54.
5. Cortes R, Soriano E, Pazos A, et al. Autoradiography of Antidepressant Binding Sites in the Human Brain: Localization Using [3H]Imipramine and [3H]Paroxetine. *Neuroscience* 1988;27:473-96.
6. Kish SJ, Furukawa Y, Chang LJ, et al. Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? *Nucl Med Biol* 2005;32:123-8.
7. Laruelle M, Vanisberg MA, Maloteaux JM. Regional and subcellular localization in human brain of [3H] paroxetine binding, a marker of serotonin uptake sites. *Biol Psychiatry* 1988;24:299-309.
8. Blier P, De Montigny C. Electrophysiological Investigations On The Effect of Repeated Zimelidine Administration on Serotonergic Neurotransmission in the Rat. *J Neurosci* 1983;3:1270-8.
9. Bel N, Artigas F. Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: an in vivo microdialysis study. *Eur J Pharmacol* 1992;229:101-3.
10. Bel N, Artigas F. Chronic Treatment With Fluvoxamine Increases Extracellular Serotonin in Frontal Cortex but Not in Raphe Nuclei. *Synapse* 1993;15:243-5.
11. Dreshfield LJ, Wong DT, Perry KW, et al. Enhancement of fluoxetine-dependent increase of extracellular serotonin (5-HT) levels by (-)-pindolol, an antagonist at 5-HT1A receptors. *Neurochem Res* 1996;21:557-62.

12. Moret C, Briley M. Effects of acute and repeated administration of citalopram on extracellular levels of serotonin in rat brain. *Eur J Pharmacol* 1996;295:189-97.

13. Bel N, Artigas F. In vivo evidence for the reversible action of the monoamine oxidase inhibitor brofaromine on 5-hydroxytryptamine release in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* 1995;351:475-82.

14. Mathews TA, Fedele DE, Unger EL, et al. Effects of serotonin transporter inactivation on extracellular 5-HT levels, in vivo microdialysis recovery, and MDMA-induced release of serotonin and dopamine in mouse striatum. *Soc Neurosci Abstr* 2000;30:624.

15. Huang Y, Hwang DR, Narendran R, et al. Comparative evaluation in nonhuman primates of five PET radiotracers for imaging the serotonin transporters: [11C]McN 5652, [11C]ADAM, [11C]DASB, [11C]DAPA, and [11C]AFM. *J Cereb Blood Flow Metab* 2002;22: 1377-98.

16. Frankle WG, Huang Y, Hwang DR, et al. Comparative evaluation of serotonin transporter radioligands 11C-DASB and 11C-McN 5652 in healthy humans. *J Nucl Med* 2004;45:682-94.

17. Szabo Z, Kao P, Scheffel U, et al. Positron Emission Tomography Imaging of Serotonin Transporters in the Human Brain Using [11C](+)McN5652. *Synapse* 1995;20:37-43.

18. Buck A, Gucker PM, Schonbachler RD, et al. Evaluation of serotonergic transporters using PET and [11C](+)McN-5652: assessment of methods. *J Cereb Blood Flow Metab* 2000;20:253-62.

19. Parsey RV, Kegeles LS, Hwang DR, et al. In vivo quantification of brain serotonin transporters in humans using [11C]McN 5652. *J Nucl Med* 2000;41:1465-77.

20. Ginovart N, Wilson AA, Meyer JH, et al. Positron emission tomography quantification of [(11)C]-DASB binding to the human serotonin transporter: modeling strategies. *J Cereb Blood Flow Metab* 2001;21:1342-53.

21. Ichise M, Liow JS, Lu JQ, et al. Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab* 2003;23:1096-112.

22. Carroll FI, Kotian P, Dehghani A, et al. Cocaine and 3 beta-(4'-substituted phenyl)tropane-2 beta-carboxylic acid ester and amide analogues. New high-affinity and selective compounds for the dopamine transporter. *J Med Chem* 1995;38:379-88.

23. Laruelle M, Giddings SS, Zea-Ponce Y, et al. Methyl 3 beta-(4-[125I]iodophenyl)tropane-2 beta-carboxylate in vitro binding to dopamine and serotonin transporters under "physiological" conditions. *J Neurochem* 1994;62:978-86.

24. Shank RP, Vaught JL, Pelley KA, et al. McN-5652: a highly potent inhibitor of serotonin uptake. *J Pharmacol Exp Ther* 1988;247:1032-8.

25. Kung MP, Hou C, Oya S, et al. Characterization of [(123)I]IDAM as a novel single-photon emission tomography tracer for serotonin transporters. *Eur J Nucl Med* 1999;26:844-53.

26. Wilson AA, Ginovart N, Schmidt M, et al. Novel radiotracers for imaging the serotonin transporter by positron emission tomography: synthesis, radiosynthesis, in vitro and ex vivo evaluation of [11]C-labeled 2-(phenylthio)araalkylamines. *J Med Chem* 2000;43: 3103-10.

27. Wilson AA, Ginovart N, Hussey D, et al. In vitro and in vivo characterisation of [11C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. *Nucl Med Biol* 2002;29:509-15.

28. Choi SR, Hou C, Oya S, et al. Selective in vitro and in vivo binding of [(125)I]ADAM to serotonin transporters in rat brain. *Synapse* 2000;38:403-12.

29. Oya S, Choi SR, Hou C, et al. 2-((2-((dimethylamino)methyl) phenyl)thio)-5-iodophenylamine (ADAM): an improved serotonin transporter ligand. *Nucl Med Biol* 2000;27:249-54.

30. Pirker W, Asenbaum S, Kasper S, et al. beta-CIT SPECT demonstrates blockade of 5HT-uptake sites by citalopram in the human brain in vivo. *J Neural Transm Gen Sect* 1995;100:247-56.

31. Tauscher J, Pirker W, de Zwaan M, et al. In vivo visualization of serotonin transporters in the human brain during fluoxetine treatment. *Eur Neuropsychopharmacol* 1999;9:177-9.

32. Kent JM, Coplan JD, Lombardo I, et al. Occupancy of brain serotonin transporters during treatment with paroxetine in patients with social phobia: a positron emission tomography study with 11C McN 5652. *Psychopharmacology (Berl)* 2002;164:341-8.

33. Suhara T, Takano A, Sudo Y, et al. High levels of serotonin transporter occupancy with low-dose clomipramine in comparative occupancy study with fluvoxamine using positron emission tomography. *Arch Gen Psychiatry* 2003;60:386-91.

34. Meyer JH, Wilson AA, Ginovart N, et al. Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. *Am J Psychiatry* 2001; 158:1843-9.

35. Meyer JH, Wilson AA, Sagrati S, et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. *Am J Psychiatry* 2004;161:826-35.

36. Erlandsson K, Sivananthan T, Lui D, et al. Measuring SSRI occupancy of SERT using the novel tracer [123I]ADAM: a SPECT validation study. *Eur J Nucl Med Mol Imaging* 2005;32:1329-36.

37. Brucke T, Kornhuber J, Angelberger P, et al. SPECT imaging of dopamine and serotonin transporters with [123I]beta-CIT. Binding kinetics in the human brain. *J Neural Transm Gen Sect* 1993;94:137-46.

38. Kuikka JT, Bergstrom KA, Vanninen E, et al. Initial experience with single-photon emission tomography using iodine-123-labelled 2 beta-carbomethoxy-3 beta-(4-iodophenyl) tropane in human brain. *Eur J Nucl Med* 1993;20:783-6.

39. Ikoma Y, Suhara T, Toyama H, et al. Quantitative analysis for estimating binding potential of the brain serotonin transporter with [11 C]McN5652. *J Cereb Blood Flow Metab* 2002;22:490-501.

40. Houle S, Ginovart N, Hussey D, et al. Imaging the serotonin transporter with positron emission tomography: initial human studies with [11C]DAPP and [11C]DASB. *Eur J Nucl Med* 2000;27:1719-22.

41. Catafau AM, Perez V, Penengo MM, et al. SPECT of serotonin transporters using 123I-ADAM: optimal imaging time after bolus injection and long-term test-retest in healthy volunteers. *J Nucl Med* 2005;46:1301-9.

42. Frankle WG, Lombardo I, New AS, et al. Brain serotonin transporter distribution in subjects with impulsive aggressivity: a positron emission study with [11C]McN 5652. *Am J Psychiatry* 2005;162:915-23.

43. Praschak-Rieder N, Wilson AA, Hussey D, et al. Effects of tryptophan depletion on the serotonin transporter in healthy humans. *Biol Psychiatry* 2005;58:825-30.

44. Innis RB, Seibyl JP, Scanley BE, et al. Single photon emission computed tomographic imaging demonstrates loss of striatal dopamine transporters in Parkinson disease. *Proc Natl Acad Sci U S A* 1993;90:11965-9.

45. Ciliax BJ, Drash GW, Staley JK, et al. Immunocytochemical localization of the dopamine transporter in human brain. *J Comp Neurol* 1999;409:38-56.

46. Ichimiya T, Suhara T, Sudo Y, et al. Serotonin transporter binding

in patients with mood disorders: a PET study with [11C](+)McN5652. *Biol Psychiatry* 2002;51:715-22.

47. Meyer JH, Houle S, Sagrati S, et al. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 2004;61:1271-9.

48. Takano A, Suhara T, Ichimiya T, et al. Time course of in vivo 5-HTT transporter occupancy by fluvoxamine. *J Clin Psychopharmacol* 2006;26:188-91.

49. Acton PD, Choi SR, Hou C, et al. Quantification of serotonin transporters in nonhuman primates using [(123)I]ADAM and SPECT. *J Nucl Med* 2001;42:1556-62.

50. Chalon S, Tarkiainen J, Garreau L, et al. Pharmacological characterization of N,N-dimethyl-2-(2-amino-4-methylphenyl thio)benzylamine as a ligand of the serotonin transporter with high affinity and selectivity. *J Pharmacol Exp Ther* 2003;304:81-7.

51. Halldin C, Lundberg J, Sovago J, et al. [(11)C]MADAM, a new serotonin transporter radioligand characterized in the monkey brain by PET. *Synapse* 2005;58:173-83.

52. Lundberg J, Halldin C, Farde L. Measurement of serotonin transporter binding with PET and [(11)C]MADAM: A test-retest reproducibility study. *Synapse* 2006;60:256-63.

53. Lundberg J, Odano I, Olsson H, et al. Quantification of 11C-MADAM binding to the serotonin transporter in the human brain. *J Nucl Med* 2005;46:1505-15.

54. Kim JS, Ichise M, Sangare J, et al. PET Imaging of Serotonin Transporters with [11C]DASB: Test-Retest Reproducibility Using a Multilinear Reference Tissue Parametric Imaging Method. *J Nucl Med* 2006;47:208-14.

55. Ashburner J, Friston KJ. Nonlinear spatial normalization using basis functions. *Hum Brain Mapp* 1999;7:254-66.

56. Ashburner J, Neelin P, Collins DL, et al. Incorporating prior knowledge into image registration. *Neuroimage* 1997;6:344-52.

57. Rusjan P, Mamo D, Ginovart N, et al. An automated method for the extraction of regional data from PET images. *Psychiatry Res* 2006;147:79-89.

58. Meyer J, Wilson A, Ginovart N, Houle S. Misunderstandings about how to choose a reference region. *Biol Psychiatry* 2007. In press.

59. Praschak-Rieder N, Kennedy J, Wilson A, et al. Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: An [11C] DASB Positron Emission Tomography study. *Biol Psychiatry*. In press.

60. Ginovart N, Wilson A, Meyer J, et al. PET Quantification of [^{11}C]-DASB binding to the serotonin transporter in humans, in *Abstracts of the 48th Annual Meeting of the Society of Nuclear Medicine*. Reston: SNM; 2001. p. 62.

61. Logan J, Fowler JS, Volkow ND, et al. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab* 1996;16:834-40.

62. Ichise M, Meyer JH, Yonekura Y. An introduction to PET and SPECT neuroreceptor quantification models. *J Nucl Med* 2001;42:755-63.

63. Kontur PJ, al-Tikriti M, Innis RB, et al. Postmortem stability of monoamines, their metabolites, and receptor binding in rat brain regions. *J Neurochem* 1994;62:282-90.

64. Mann J, Underwood M, Arango V. Postmortem Studies of Suicide Victims, in Biology of Schizophrenia and Affective Disorders. In: Watson SJ, editor. *Biology of Schizophrenia and Affective Disease.* Washington: American Psychiatric Press; 1996 p. 197-221.

65. Pare CM. Iproniazid in the treatment of depression. *Proc R Soc Med* 1959;52:585-7.

66. Owens MJ, Morgan WN, Plott SJ, et al. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther* 1997;283:1305-22.

67. Tatsumi M, Groshan K, Blakely RD, et al. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol* 1997;340:249-58.

68. Practice guideline for the treatment of patients with major depressive disorder (revision). American Psychiatric Association. *Am J Psychiatry* 2000;157(4 Suppl):1-45.

69. Quetsch RM, Achor RW, Litin EM, et al. Depressive reactions in hypertensive patients; a comparison of those treated with Rauwolfia and those receiving no specific antihypertensive treatment. *Circulation* 1959;19:366-75.

70. Delgado PL, Charney DS, Price LH, et al. Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry* 1990;47:411-8.

71. Delgado PL, Price LH, Miller HL, et al. Serotonin and the neurobiology of depression. Effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatry* 1994;51:865-74.

72. Leyton M, Young SN, Benkelfat C. Relapse of depression after rapid depletion of tryptophan [letter; comment]. *Lancet* 1997;349:1840-1.

73. Smith KA, Fairburn CG, Cowen PJ. Relapse of depression after rapid depletion of tryptophan. *Lancet* 1997;349:915-9.

74. Benkelfat C, Ellenbogen MA, Dean P, et al. Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* 1994;51:687-97.

75. Young SN, Smith SE, Pihl RO, et al. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)* 1985;87:173-7.

76. Smith K, Clifford E, Hockney R, et al. Effect of tryptophan depletion on mood in male and female volunteers: a pilot study. *Hum Psychopharmacol Clin Exp* 1997;12:111-7.

77. Neumeister A, Konstantinidis A, Stastny J, et al. Association between serotonin transporter gene promoter polymorphism (5HTTLPR) and behavioral responses to tryptophan depletion in healthy women with and without family history of depression. *Arch Gen Psychiatry* 2002;59:613-20.

78. Arango V, Ernsberger P, Marzuk PM, et al. Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry* 1990;47:1038-47.

79. Arango V, Underwood M, Mann J. Alterations in monoamine receptors in the brain of suicide victims. *J Clin Psychopharmacol* 1992;12:8S-12S.

80. Arora RC, Meltzer HY. Serotonergic measures in the brains of suicide victims: 5-HT2 binding sites in the frontal cortex of suicide victims and control subjects. *Am J Psychiatry* 1989;146:730-6.

81. Hrdina PD, Vu TB. Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT2 receptors in rat brain: an autoradiographic study. *Synapse* 1993;14:324-31.

82. Mann JJ, Stanley M, McBride PA, et al. Increased serotonin2 and beta-adrenergic receptor binding in the frontal cortices of suicide victims. *Arch Gen Psychiatry* 1986;43:954-9.

83. Stanley M, Mann JJ. Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* 1983;1:214-6.

84. Yates M, Leake A, Candy JM, et al. 5HT2 receptor changes in major depression. *Biol Psychiatry* 1990;27:489-96.

85. Stockmeier CA, Dilley GE, Shapiro LA, et al. Serotonin receptors in suicide victims with major depression. *Neuropsychopharmacology* 1997;16:162-73.

86. Turecki G, Briere R, Dewar K, et al. Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am J Psychiatry* 1999;156:1456-8.

87. Pandey GN, Dwivedi Y, Rizavi HS, et al. Higher expression of serotonin 5-HT(2A) receptors in the postmortem brains of teenage suicide victims. *Am J Psychiatry* 2002;159:419-29.

88. Stockmeier CA. Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J Psychiatr Res* 2003;37:357-73.

89. Meyer JH, McMain S, Kennedy SH, et al. Dysfunctional Attitudes and 5-HT(2) Receptors During Depression and Self-Harm. *Am J Psychiatry* 2003;160:90-9.

90. Stockmeier CA, Shapiro LA, Dilley GE, et al. Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. *J Neurosci* 1998;18:7394-401.

91. O'Regan D, Kwok RP, Yu PH, et al. A behavioural and neurochemical analysis of chronic and selective monoamine oxidase inhibition. *Psychopharmacology (Berl)* 1987;92:42-7.

92. Roth BL, McLean S, Zhu XZ, et al. Characterization of two [3H]ketanserin recognition sites in rat striatum. *J Neurochem* 1987;49:1833-8.

93. Stockmeier CA, Kellar KJ. In vivo regulation of the serotonin-2 receptor in rat brain. *Life Sci* 1986;38:117-27.

94. Todd KG, McManus DJ, Baker GB. Chronic administration of the antidepressants phenelzine, desipramine, clomipramine, or maprotiline decreases binding to 5-hydroxytryptamine2A receptors without affecting benzodiazepine binding sites in rat brain. *Cell Mol Neurobiol* 1995;15:361-70.

95. Hoyer D, Pazos A, Probst A, et al. Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT1C and 5-HT2 recognition sites. *Brain Res* 1986;376:97-107.

96. Marazziti D, Rossi A, Giannaccini G, et al. Distribution and characterization of [3H]mesulergine binding in human brain postmortem. *Eur Neuropsychopharmacol* 1999;10:21-6.

97. Schmuck K, Ullmer C, Engels P, et al. Cloning and functional characterization of the human 5-HT2B serotonin receptor. *FEBS Lett* 1994;342:85-90.

98. D'haenen H, Bossuyt A, Mertens J, et al. SPECT imaging of serotonin2 receptors in depression. *Psychiatry Res* 1992;45:227-37.

99. Biver F, Wikler D, Lotstra F, et al. Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. *Br J Psychiatry* 1997;171:444-8.

100. Attar-Levy D, Martinot J-L, Blin J, et al. The cortical serotonin2 receptors studied with positron emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. *Biol Psychiatry* 1999;45:180-6.

101. Meyer JH, Kapur S, Houle S, et al. Prefrontal cortex 5-HT2 receptors in depression: a [18F] setoperone PET imaging study. *Am J Psychiatry* 1999;156:1029-34.

102. Meltzer CC, Price JC, Mathis A, et al. PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. *Am J Psychiatry* 1999;156:1871-8.

103. Yatham LN, Liddle PF, Shiah IS, et al. Brain serotonin2 receptors in major depression: a positron emission tomography study. *Arch Gen Psychiatry* 2000;57:850-8.

104. Messa C, Colombo C, Moresco RM, et al. 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. *Psychopharmacology (Berl)* 2003;167:72-8.

105. Mintun MA, Sheline YI, Moerlein SM, et al. Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. *Biol Psychiatry* 2004;55:217-24.

106. Blin J, Pappata S, Kiyosawa M, et al. [18F] setoperone: a new high-affinity ligand for positron emission tomography study of the serotonin - 2 receptors in baboon brain in vivo. *Eur J Pharmacol* 1988;147:73-82.

107. Blin J, Sette G, Fiorelli M, et al. A method for the in vivo investigation of the serotonergic 5-HT2 receptors in the human cerebral cortex using positron emission tomography and 18F-labeled setoperone. *J Neurochem* 1990;54:1744-54.

108. Petit-Taboue MC, Landeau B, Osmont A, et al. Estimation of neocortical serotonin-2 receptor binding potential by single-dose fluorine-18-setoperone kinetic PET data analysis. *J Nucl Med* 1996; 37:95-104.

109. Maziere B, Crouzel C, Venet M, et al. Synthesis, affinity and specificity of 18F- setoperone, a potential ligand for in-vivo imaging of cortical serotonin receptors. *Int J Rad Appl Instrum B* 1988;15:463-8.

110. Fischman AJ, Bonab AA, Babich JW, et al. Positron emission tomographic analysis of central 5-hydroxytryptamine2 receptor occupancy in healthy volunteers treated with the novel antipsychotic agent ziprasidone. *J Pharmacol Exp Ther* 1996;279:939-47.

111. Seeman P. *Receptor tables vol. 2: Drug dissociation constants for neuroreceptors and transporters.* Toronto: SZ Research; 1993.

112. De Keyser J, Clayes A, DeBacker J-P, et al. Autoradiographic localization of D1 and D2 dopamine receptors in the human brain. *Neurosci Lett* 1988;91:142-7.

113. Trichard C, Paillere-Martinot ML, Attar-Levy D, et al. Binding of antipsychotic drugs to cortical 5-HT2A receptors: a PET study of chlorpromazine, clozapine, and amisulpride in schizophrenic patients. *Am J Psychiatry* 1998;155:505-8.

114. Kapur S, Zipursky RB, Remington G, et al. 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiatry* 1998;155:921-8.

115. Mamo D, Kapur S, Shammi CM, et al. A PET study of dopamine D2 and serotonin 5-HT2 receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone. *Am J Psychiatry* 2004;161:818-25.

116. Kapur S, Jones C, Da Silva J, Wilson A, et al. Reliability of [18F] setoperone in humans. *Nucl Med Commun* 1997;18:395-9.

117. Blin J, Crouzel C. Blood-cerebrospinal fluid and blood-brain barriers imaged by 18F- labeled metabolites of 18F-setoperone studied in humans using positron emission tomography. *J Neurochem* 1992; 58:2303-10.

118. Meyer JH, Cho R, Kennedy S, et al. The effects of single dose nefazodone and paroxetine upon 5-HT2A binding potential in humans using [18F]-setoperone PET. *Psychopharmacology (Berl)* 1999;144: 279-81.

119. Dohr KB, Rush AJ, Bernstein IH. Cognitive Biases and Depression. *J Abnorm Psychol* 1989;98:263-7.

120. Simons AD, Garfield SL, Murphy GE. The process of change in cognitive therapy and pharmacotherapy for depression. Changes in mood and cognition. *Arch Gen Psychiatry* 1984;41:45-51.

121. Cane DB, Olinger LJ, Gotlib IH, et al. Factor structure of the dysfunctional attitude scale in a student population. *J Clin Psychol* 1986;42:307-9.

122. Oliver JM, Baumgart EP. The dysfunctional attitude scale: psychometric properties and relation to depression in an unselected adult population. *Cognit Ther Res* 1985;9:161-7.

123. Weissman A. The Dysfunctional Attitude Scale: A validation study. *Dissertation Abstracts International* 1979; 40:1389B-1390B.

124. Bouvard M, Charles S, Guerin J, et al. [Study of Beck's hopelessness scale. Validation and factor analysis]. *Encephale* 1992;18:237-40.

125. Cannon B, Mulroy R, Otto MW, et al. Dysfunctional attitudes and poor problem solving skills predict hopelessness in major depression. *J Affect Disord* 1999;55:45-9.

126. DeRubeis RJ, Evans MD, Hollon SD, et al. How does cognitive therapy work? Cognitive change and symptom change in cognitive therapy and pharmacotherapy for depression. *J Consult Clin Psychol* 1990;58:862-9.

127. Norman WH, Miller W, Dow MG. Characteristics of depressed patients with elevated levels of dysfunctional cognitions. *Cognit Ther Res* 1988;12:39-51.

128. Beck AT, Steer RA, Kovacs M, et al. Hopelessness and eventual suicide: A 10-year prospective study of patients hospitalized with suicidal ideation. *Am J Psychiatry* 1985;142:559-63.

129. Beck AT, Brown G, Steer RA. Prediction of eventual suicide in psychiatric inpatients by clinical ratings of hopelessness. *J Consult Clin Psychol* 1989;57:309-10.

130. Bhagwagar Z, Hinz R, Taylor M, et al. Increased 5-HT2A Receptor Binding in Euthymic Medication Free Patients Recovered from Depression: A Positron Emission Tomography Study With [11C] MDL 100,907. *Am J Psychiatry* 2006; 163:1580-7.

131. Boldrinin M, Underwood MD, Martini A, et al. Brainstem raphe nucleus changes in suicide victims. *Psychiatr Danub* 2006; 18 (Suppl 1):120.

132. Compan V, Segu L, Buhot MC, et al. Differential effects of serotonin (5-HT) lesions and synthesis blockade on neuropeptide-Y immunoreactivity and 5-HT1A, 5-HT1B/1D and 5-HT2A/2C receptor binding sites in the rat cerebral cortex. *Brain Res* 1998;795: 264-76.

133. McCann UD, Szabo Z, Seckin E, et al. Quantitative PET studies of the serotonin transporter in MDMA users and controls using [11C]McN5652 and [11C]DASB. *Neuropsychopharmacology* 2005;30: 1741-50.

134. Bernheimer H, Birkmayer W, Hornykiewicz O. [Distribution of 5-hydroxytryptamine (serotonin) in the human brain and its behavior in patients with Parkinson's syndrome.] *Klin Wochenschr* 1961;39:1056-9.

135. Kish SJ. What is the evidence that Ecstasy (MDMA) can cause Parkinson's disease? *Mov Disord* 2003;18:1219-23.

136. Linnet K, Koed K, Wiborg O, et al. Serotonin depletion decreases serotonin transporter mRNA levels in rat brain. *Brain Res* 1995;697:251-3.

137. Xiao Q, Pawlyk A, Tejani-Butt SM. Reserpine modulates serotonin transporter mRNA levels in the rat brain. *Life Sci* 1999;64:63-8.

138. Yu A, Yang J, Pawlyk AC, et al. Acute depletion of serotonin down-regulates serotonin transporter mRNA in raphe neurons. *Brain Res* 1995;688:209-12.

139. Benmansour S, Cecchi M, Morilak D, et al. Effects of Chronic Antidepressant Treatments on Serotonin Transporter Function, Density and mRNA level. *J Neurosci* 1999;19:10494-501.

140. Dewar KM, Grondin L, Carli M, et al. [3H]paroxetine binding and serotonin content of rat cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum, and midbrain raphe nuclei region following p-chlorophenylalanine and p-chloroamphetamine treatment. *J Neurochem* 1992;58:250-7.

141. Graham D, Tahraoui L, Langer SZ. Effect of chronic treatment with selective monoamine oxidase inhibitors and specific 5-hydroxytryptamine uptake inhibitors on [3H]paroxetine binding to cerebral cortical membranes of the rat. *Neuropharmacology* 1987;26: 1087-92.

142. Gordon I, Weizman R, Rehavi M. Modulatory effect of agents active in the presynaptic dopaminergic system on the striatal dopamine transporter. *Eur J Pharmacol* 1996;298:27-30.

143. Han S, Rowell PP, Carr LA. D2 autoreceptors are not involved in the down-regulation of the striatal dopamine transporter caused by alpha-methyl-p-tyrosine. *Res Commun Mol Pathol Pharmacol* 1999;104:331-8.

144. Ikawa K, Watanabe A, Kaneno S, et al. Modulation of [3H]mazindol binding sites in rat striatum by dopaminergic agents. *Eur J Pharmacol* 1993;250:261-6.

145. Kilbourn MR, Sherman PS, Pisani T. Repeated reserpine administration reduces in vivo [18F]GBR 13119 binding to the dopamine uptake site. *Eur J Pharmacol* 1992;216:109-12.

146. Blier P, De Montigny C. Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J Neurosci* 1983;3:1270-8.

147. Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* 2000;20:423-51.

148. Ginovart N, Wilson AA, Meyer JH, et al. [11C]-DASB, a tool for in vivo measurement of SSRI-induced occupancy of the serotonin transporter: PET characterization and evaluation in cats. *Synapse* 2003;47:123-33.

149. Lundquist P, Wilking H, Hoglund AU, et al. Potential of [11C]DASB for measuring endogenous serotonin with PET: binding studies. *Nucl Med Biol* 2005;32:129-36.

150. Celada P, Artigas F. Monoamine oxidase inhibitors increase preferentially extracellular 5-hydroxytryptamine in the midbrain raphe nuclei. A brain microdialysis study in the awake rat. *Naunyn Schmiedebergs Arch Pharmacol* 1993;347:583-90.

151. Malyszko J, Urano T, Serizawa K, et al. Serotonergic measures in blood and brain and their correlations in rats treated with tranylcypromine, a monoamine oxidase inhibitor. *Jpn J Physiol* 1993;43:613-26.

152. Ferrer A, Artigas F. Effects of single and chronic treatment with tranylcypromine on extracellular serotonin in rat brain. *Eur J Pharmacol* 1994;263:227-34.

153. Talbot PS, Frankle WG, Hwang DR, et al. Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand 11C-DASB in healthy humans. *Synapse* 2005;55:164-75.

154. Bligh-Glover W, Kolli TN, Shapiro-Kulnane L, et al. The serotonin transporter in the midbrain of suicide victims with major depression. *Biol Psychiatry* 2000;47:1015-24.

155. Klimek V, Roberson G, Stockmeier CA, et al. Serotonin transporter and MAO-B levels in monoamine nuclei of the human brainstem are normal in major depression. *J Psychiatr Res* 2003;37:387-97.

156. Perry EK, Marshall EF, Blessed G, et al. Decreased imipramine

binding in the brains of patients with depressive illness. *Br J Psychiatry* 1983;142:188-92.

157. Crow TJ, Cross AJ, Cooper SJ, et al. Neurotransmitter receptors and monoamine metabolites in the brains of patients with Alzheimer-type dementia and depression, and suicides. *Neuropharmacology* 1984;23(12B):1561-9.

158. Mann JJ, Huang YY, Underwood MD, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch Gen Psychiatry* 2000;57:729-38.

159. Austin MC, Whitehead RE, Edgar CL, et al. Localized decrease in serotonin transporter-immunoreactive axons in the prefrontal cortex of depressed subjects committing suicide. *Neuroscience* 2002; 114:807-15.

160. Hrdina PD, Foy B, Hepner A, et al. Antidepressant binding sites in brain: autoradiographic comparison of [3H]paroxetine and [3H]imipramine localization and relationship to serotonin transporter. *J Pharmacol Exp Ther* 1990;252:410-8.

161. Lawrence KM, De Paermentier F, Cheetham SC, et al. Brain 5-HT uptake sites, labelled with [3H]paroxetine, in antidepressant-free depressed suicides. *Brain Res* 1990;526:17-22.

162. Little KY, McLauglin DP, Ranc J, et al. Serotonin transporter binding sites and mRNA levels in depressed persons committing suicide. *Biol Psychiatry* 1997;41:1156-64.

163. Arango V, Underwood MD, Boldrini M, et al. Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology* 2001;25:892-903.

164. Hendricksen M, Thomas AJ, Ferrier IN, et al. Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *Am J Psychiatry* 2004;161:1096-102.

165. Leake A, Fairbairn AF, McKeith IG, et al. Studies on the serotonin uptake binding site in major depressive disorder and control postmortem brain: neurochemical and clinical correlates. *Psychiatry Res* 1991;39:155-65.

166. Malison RT, Price LH, Berman R, et al. Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography. *Biol Psychiatry* 1998;44:1090-8.

167. Newberg AB, Amsterdam JD, Wintering N, et al. 123I-ADAM binding to serotonin transporters in patients with major depression and healthy controls: a preliminary study. *J Nucl Med* 2005;46:973-7.

168. Parsey RV, Hastings RS, Oquendo MA, et al. Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am J Psychiatry* 2006;163:52-8.

169. Herold N, Uebelhack K, Franke L, et al. Imaging of serotonin transporters and its blockade by citalopram in patients with major depression using a novel SPECT ligand [(123)I]-ADAM. *J Neural Transm* 2006;113:659-70.

170. Meyer JH, Houle S, Sagrati S, et al. Brain serotonin transporter binding potential measured with [11C]DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* 2004;61(12):1271-9.

171. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996;274:1527-31.

172. Filipenko ML, Beilina AG, Alekseyenko OV, et al. Increase in expression of brain serotonin transporter and monoamine oxidase a genes induced by repeated experience of social defeats in male mice. *Biochemistry (Mosc)* 2002;67:451-5.

173. Little KY, McLaughlin DP, Zhang L, et al. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am J Psychiatry* 1998;155:207-13.

174. Heinz A, Jones DW, Mazzanti C, et al. A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biol Psychiatry* 2000;47:643-9.

175. Willeit M, Stastny J, Pirker W, et al. No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biol Psychiatry* 2001;50:8-12.

176. Shioe K, Ichimiya T, Suhara T, et al. No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by PET. *Synapse* 2003;48:184-8.

177. Parsey RV, Hastings RS, Oquendo MA, et al. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. *Am J Psychiatry* 2006;163:48-51.

178. Lim JE, Papp A, Pinsonneault J, et al. Allelic expression of serotonin transporter (SERT) mRNA in human pons: lack of correlation with the polymorphism SERTLPR. *Mol Psychiatry* 2006;11:649-62.

179. Kim DK, Lim SW, Lee S, et al. Serotonin transporter gene polymorphism and antidepressant response. *Neuroreport* 2000;11:215-9.

180. Zanardi R, Benedetti F, Di Bella D, et al. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J Clin Psychopharmacol* 2000;20:105-7.

181. Pollock BG, Ferrell RE, Mulsant BH, et al. Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacology* 2000;23:587-90.

182. Kraft JB, Slager SL, McGrath PJ, et al. Sequence analysis of the serotonin transporter and associations with antidepressant response. *Biol Psychiatry* 2005;58:374-81.

183. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003;301:386-9.

184. Faravelli C, Ambonetti A, Pallanti S, et al. Depressive relapses and incomplete recovery from index episode. *Am J Psychiatry* 1986;143:888-91.

185. Georgotas A, McCue RE, Cooper TB, et al. How effective and safe is continuation therapy in elderly depressed patients? Factors affecting relapse rate. *Arch Gen Psychiatry* 1988;45:929-32.

186. Paykel ES, Ramana R, Cooper Z, et al. Residual symptoms after partial remission: an important outcome in depression. *Psychol Med* 1995;25:1171-80.

187. Paykel ES, Scott J, Teasdale JD, et al. Prevention of relapse in residual depression by cognitive therapy: a controlled trial. *Arch Gen Psychiatry* 1999;56:829-35.

188. Cannon DM, Ichise M, Fromm SJ, et al. Serotonin transporter binding in bipolar disorder assessed using [11C]DASB and positron emission tomography. *Biol Psychiatry* 2006;60:207-17.

189. Takano A, Suzuki K, Kosaka J, et al. A dose-finding study of duloxetine based on serotonin transporter occupancy. *Psychopharmacology (Berl)* 2006;185:395-9.

190. Parsey RV, Kent JM, Oquendo MA, et al. Acute occupancy of brain serotonin transporter by sertraline as measured by [11C]DASB and positron emission tomography. *Biol Psychiatry* 2006;59:821-8.

191. Fineberg NA, Gale TM. Evidence-based pharmacotherapy of obsessive-compulsive disorder. *Int J Neuropsychopharmacol* 2005;8:107-29.