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Serotonin-1A receptors in major depression quantified using PET: controversies, confounds, and recommendations

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Abstract

The serotonin-1A (5-HT_{1A}) receptor is of particular interest in human positron emission tomography (PET) studies of major depressive disorder (MDD). Of the eight studies investigating this issue in the brains of patients with MDD, four reported decreased 5-HT_{1A} receptor density, two reported no change, and two reported increased 5-HT_{1A} receptor density. While clinical heterogeneity may have contributed to these differing results, methodological factors by themselves could also explain the discrepancies. This review highlights several of these factors, including the use of the cerebellum as a reference region and the imprecision of measuring the concentration of parent radioligand in arterial plasma, the method otherwise considered to be the `gold standard'. Other potential confounds also exist that could restrict or unexpectedly affect the interpretation of results. For example, the radioligand may be a substrate for an efflux transporter —like P-gp—at the blood-brain barrier; furthermore, the binding of the radioligand to the receptor in various stages of cellular trafficking is unknown. Efflux transport and cellular trafficking may also be differentially expressed in patients compared to healthy subjects. We believe that, taken together, the existing disparate findings do not reliably answer the question of whether 5-HT_{1A} receptors are altered in MDD or in subgroups of patients with MDD. In addition, useful meta-analysis is precluded because only one of the imaging centers acquired all the data necessary to address these methodological concerns. We recommend that in the future, individual centers acquire more thorough data capable of addressing methodological concerns, and that multiple centers collaborate to meaningfully pool their data for meta-analysis.

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Keywords

major depressive disorder (MDD); neuroimaging; Permeability-glycoprotein (P-gp); positron emission tomography (PET); radioligand; serotonin-1A (5-HT_{1A}) receptors

1. Introduction

The serotoninergic (5-HT) system is widely thought to be involved in the pathophysiology and treatment of major depressive disorder (MDD) (Schatzberg et al., 2002). Positron emission tomography (PET) has unsurpassed sensitivity for measuring specific proteins in the living brain, and has been widely used to measure several proteins that are selective biomarkers of 5-HT neurotransmission and that may be involved in the pathophysiology or treatment of MDD. Toward that end, many PET studies have examined the serotonin-1A (5-HT_{1A}) receptor, which plays a key role in maintaining stable 5-HT transmission and is likely involved in the mechanism of antidepressant treatment. To date, however, PET imaging studies of 5-HT_{1A} receptors in MDD have produced conflicting results, and no consensus exists as to the cause of these discrepant findings. The goals of this article are 1) to review the extant evidence from PET studies of 5-HT receptors in MDD; 2) to suggest the most likely interpretations of the evidence obtained to date; 3) to propose possible reasons for the discrepant findings; and 4) to suggest ways that future discrepancies can be avoided in studies imaging 5-HT_{1A} receptors and, by extension, other protein targets in brain.

2. Potential role of 5-HT_{1A} receptors in MDD

Many components of the 5-HT transmitter system are likely involved in the pathophysiology and treatment of MDD; however, evidence for the involvement of the 5-HT_{1A} receptor is perhaps the most extensive, and is based on electrophysiological studies, genetic modulation of the 5-HT_{1A} receptor in mice, and genetic association studies in humans. The proposed dual role of 5-HT_{1A} receptors derives, in part, from their presence both presynaptically and postsynaptically in brain. Presynaptic 5-HT_{1A} receptors, located on the soma and dendrites of 5-HT neurons in the dorsal raphe, act as inhibitory autoreceptors and decrease firing rate and serotonin release. Postsynaptic 5-HT_{1A} receptors, which are located in many brain regions including neocortex and hippocampus, help in 5-HT neurotransmission.

Electrophysiological studies in rats suggest that the 5-HT_{1A} receptor is at least partially responsible for the therapeutic effects of antidepressants as well as the typical delay in response associated with these medications (Lenox and Frazer, 2002). For instance, such studies have shown that the inhibitory autoreceptor desensitizes when selective serotonin reuptake inhibitors (SSRIs) are administered for at least two weeks (Blier et al., 1998). This desensitization then leads to increased 5-HT release, and its time dependence (i.e. more than two weeks) may be responsible for the delayed therapeutic response observed in patients with MDD.

Genetic modulation of 5-HT_{1A} receptors also strongly implicates this receptor in animal phenotypes of anxiety and depression. For instance, genetic knockout of the 5-HT_{1A} receptor in mice resulted in an anxiety-like phenotype, whereas genetic overexpression during early postnatal development reduced this phenotype (Kusserow et al., 2004). Furthermore, inactivating the 5-HT_{1A} receptor may depend on the age of the animal and may be brain region-specific. For example, inactivating 5-HT_{1A} postsynaptic receptors in the forebrain during development, but not in adulthood, produced anxiety-like behaviors in adult mice (Gross et al., 2002). In another rodent study, Richardson-Jones and colleagues showed that increasing and decreasing the density of 5-HT_{1A} autoreceptors by 30% in the

dorsal raphe during development had opposing effects on stress-induced behaviors and on the animals' response to SSRIs (Richardson-Jones et al., 2010). Within the limitations of animal models, these results strongly suggest that the 5-HT_{1A} receptor is involved in anxiety, stress response, and the mechanism of action of SSRIs.

One example of this comes from a meta-analysis that demonstrated that, in some individuals, an ethnicity-specific single nucleotide polymorphism (SNP)—C(−1019)G, located on the human promoter of the 5-HT_{1A} receptor—increased susceptibility to MDD, incidence of suicide, and response to treatment with SSRIs (Albert and Francois, 2010). Albert and colleagues (2010) listed seven independent studies demonstrating an association between the C(−1019)G polymorphism and MDD, increased incidence of suicide, or resistance to treatment with SSRIs. That study further showed that the homozygous G(−1019) allele was enriched two-fold and fourfold in depressed patients and suicide cases, respectively (Albert and Francois, 2010). The homozygous G(−1019) allele was noted to impair repression of the 5-HT_{1A} gene by the transcription factor nuclear deformed epidermal autoregulatory factor (NUDR/Deaf1), leading to overexpression of presynaptic 5-HT_{1A} receptors in dorsal raphe. Because this presynaptic receptor inhibits firing of 5-HT neurons, increased expression would be expected to decrease overall 5-HT transmission in the brain. Using PET, investigators further demonstrated that MDD patients had a threefold increase in G/G allele over controls, which correlated with increased 5-HT_{1A} binding in raphe (Parsey et al., 2006).

3. Controversies surrounding 5-HT_{1A} receptor imaging in MDD

Five independent groups using eight separate cohorts quantified 5-HT_{1A} receptors using PET and [*carbonyl*-^{11}C]WAY-100635 in patients with MDD (Table 1). As mentioned above, these studies have produced inconsistent results, a problem exacerbated by the current lack of consensus in the field regarding the `gold standard' in quantifying 5-HT_{1A} receptor binding. In addition, some methodological explanations remain hypothetical because not all studies collected data in sufficient detail to draw definitive conclusions. We should begin by noting that this review focuses on [*carbonyl*-^{11}C]WAY-100635—the radioligand used with the greatest frequency in studies of 5-HT_{1A} receptor binding in MDD. We do, however, offer limited results obtained using other closely related compounds that illustrate the types of discrepancies that can occur in such studies.

Patients with MDD have been found to have increased, decreased, or unaltered 5-HT_{1A} receptor binding (Table 1). It is our contention that methodological factors rather than clinical variables likely explain these discrepancies. Indeed, it would be difficult to parse out the exact clinical factors that could be responsible for these discrepancies, because most studies reported similar diagnostic criteria, illness severity of the patient population, and distribution of demographic variables (Table 1). As described in greater detail below, this variability in findings across studies is not random, but rather systematically determined by the outcome measures and reference regions used (Table 1). This review will attempt to resolve some of the discrepancies by examining potential methodological confounds.

Receptor binding can be expressed as specific binding normalized to a reference concentration. While specific binding is always measured from the brain region of interest, three different reference concentrations can be used for normalization: free (non-protein bound) radioligand concentration in plasma, total (free plus protein-bound) radioligand concentration in plasma, and radioactivity concentration in a receptor-free reference region in the brain (typically the cerebellum). Accuracy of normalizing specific binding will thus depend on the accuracy of measuring the reference uptake. As we will explain, inconsistency in measuring reference uptake may lead to discrepant findings in MDD.

Drevets and colleagues (1999) were the first to report globally lower 5-HT_{1A} binding in patients with MDD compared to healthy subjects (Drevets et al., 1999). They normalized specific binding to reference region concentration in brain, and later replicated these results (Drevets et al., 2007). Using similar methodology, Sargent and colleagues (2000) replicated the finding of lower 5-HT_{1A} binding in patients with MDD compared to healthy subjects; furthermore, they found that binding was not affected by antidepressant drug treatment (Sargent et al., 2000). Meltzer and colleagues (2004) later extended these findings to elderly patients with late-life depression, and noted that the most prominent decrease (about 40%) was found in the raphe nucleus (Meltzer et al., 2004). However, they normalized specific binding to total radioactivity concentration in plasma. Hirvonen and colleagues (2008) normalized specific binding to total radioactivity concentration in plasma, and found globally decreased binding in drug-naïve patients with MDD (Hirvonen et al., 2008). In contrast to previous studies, specific binding normalized to reference region in brain was not decreased, because reference region uptake itself was also lower. Finally, Mickey and colleagues (2008) found no differences in 5-HT_{1A} binding normalized to reference region in brain between patients with MDD and healthy controls (Mickey et al., 2008).

In striking contrast to prior studies showing decreased 5-HT_{1A} binding, Parsey and colleagues (2006) reported that medication-naïve patients with MDD had increased 5-HT_{1A} receptor binding, although binding in previously medicated patients was the same as in healthy subjects (Parsey et al., 2006). This finding was recently replicated by the same group (Parsey et al., 2010); two cohorts were included in the replication study—a new cohort (2010) independent of the cohort described in the 2006 study, and a combined cohort (2006-2010). These studies differed from previous ones because the investigators normalized specific binding to free (non-protein bound) radioligand in plasma. Remarkably, these authors were able to replicate lower specific binding normalized to reference region in brain (cerebellar gray matter), because uptake in the reference region itself was higher among patients with MDD. The results obtained by Parsey and colleagues suggest that choice of the reference concentration to normalize specific binding can directly contribute to the discrepant findings across studies. Below, we describe several methodological factors that may have caused such discrepant findings in PET studies of MDD.

4. Major confound: reference tissue

As mentioned above, specific binding can be normalized to three different reference concentrations: free (non-protein bound) radioligand concentration in plasma, total (free plus protein-bound) radioligand concentration in plasma, and radioactivity concentration in a receptor-free reference region in the brain (typically the cerebellum). Accuracy of this ratio depends heavily on the accuracy of the denominator (e.g., the reference concentration). Normalizing to free radioligand in plasma is considered the `gold standard' method because only free radioligand in plasma can enter the brain. However, this method requires measuring the parent (non-metabolized) radioligand concentration in plasma as well as protein binding; both measurements are inherently imprecise. The most convenient outcome measure is specific binding normalized to concentration in reference tissue in brain, but this outcome measure similarly depends on the accuracy of the reference tissue. Here, we discuss potential sources of error in measuring the denominator (i.e., the reference concentration used to normalize specific binding) and how these errors may have led to the discrepant findings in MDD.

4.1 Brain as reference tissue

Normalizing specific binding to radioactivity concentration in the reference region is attractive because it obviates the need to take arterial blood samples. In addition, using an arterial line is technically more difficult and risky than using a venous one. In the case of

studies investigating MDD, the cerebellum is assumed to reflect only non-specifically bound and free radioligand in brain. Violations of this assumption and inaccuracies in measuring cerebellar uptake have likely contributed to discrepant findings in MDD, as exemplified by the fact that conflicting results are obtained when specific binding is normalized to plasma or the cerebellum even in the same dataset (Parsey et al., 2010; Parsey et al., 2006). Three interrelated disadvantages exist in using the cerebellum as the reference region for [*carbonyl*-^{11}C]WAY-100635 studies: 1) uptake is too low to reliably estimate non-displaceable uptake; 2) cerebellar gray matter has specific binding; and 3) cerebellar signal may be disproportionally contaminated by radiometabolites.

After [*carbonyl*-^{11}C]WAY-100635 injection, radioactivity concentration in the cerebellum is very low. In this regard, [*carbonyl*-^{11}C]WAY-100635 is probably “too good” for use with a reference region in brain. That is, even a small absolute change in the concentration of radiometabolite in the cerebellum propagates a large proportional bias in specific binding normalized to cerebellum. Potential sources of such small absolute changes in the cerebellum include 1) spill-in of radioactivity from adjacent high-uptake regions (such as the occipital and temporal cortices) via partial volume effects; 2) accumulation of radioactive metabolites; 3) scattered photons from outside the field-of-view; 4) inaccuracies in estimating blood volume within the region of interest; and 5) small amounts of specific binding in cerebellar gray matter.

In vitro studies have shown that cerebellar gray matter has 5-HT$_{1A}$ receptors (Parsey et al., 2005). *In vivo*, specific binding in cerebellar gray matter was suggested by extremely high cerebellar uptake in a healthy subject (Hirvonen et al., 2007), and confirmed by significantly reduced cerebellar gray matter (~ 30%) binding after a pharmacological challenge with pindolol, a 5-HT$_{1A}$ antagonist (Parsey et al., 2010). Differences in 5-HT$_{1A}$ receptor density in cerebellar gray matter between subjects may therefore bias specific binding normalized to cerebellum. Parsey and colleagues (2010) found higher cerebellar gray matter, but not white matter, uptake in patients with MDD than in healthy subjects, which caused an artefactual decrease in specific binding normalized to cerebellum (Parsey et al., 2010). To avoid this bias, cerebellar white matter has been recommended as the reference region in [*carbonyl*-^{11}C]WAY-100635 studies (Hirvonen et al., 2007; Parsey et al., 2005), although using cerebellar white matter does not solve the problem of low uptake in the reference region. In addition, using white matter as the reference region may violate the theoretical assumption that reference and target regions are identical except for specific binding, because non-specific binding may differ between grey and white matter.

Finally, cerebellum may be disproportionally contaminated by radiometabolites. One such radiometabolite, [^{11}C]cyclohexanecarboxylic acid, only minimally enters monkey brain and binds non-specifically (Osman et al., 1998), and is therefore not likely to be a concern in target regions where the vast majority of the signal represents specific binding. Nevertheless, because of very low overall radioactivity concentrations in the cerebellum in humans, this small absolute contribution may constitute up to 20% of cerebellar radioactivity concentrations at later time points (Carson et al., 2003). Thus, differences between subjects in the rate of metabolism of [*carbonyl*-^{11}C]WAY-100635 into [^{11}C]cyclohexanecarboxylic acid may bias specific binding normalized to the cerebellum. It is important to note, however, that metabolic differences between patients and controls are speculative, and the cited studies report no such changes.

In summary, using cerebellum as a reference region for [*carbonyl*-^{11}C]WAY-100635 is problematic for many reasons: overall uptake is too low to be accurately measured, cerebellum has 5-HT$_{1A}$ receptors, and radiometabolites may disproportionately affect the cerebellum. Furthermore, no region other than cerebellum is large enough to measure

background uptake of radioligand and thus avoid spill-in of radioactivity from adjacent receptor-rich regions. As a result, normalizing specific binding to radioactivity concentration in plasma, rather than a reference region in brain, appears to be a more valid method in studies of patients with MDD. As we will see, however, measuring the concentration of parent radioligand in plasma may also be problematic because of measurement errors.

4.2 Radiometabolites

During PET imaging, radiometabolites that accumulate in the field of view by entering brain and/or accumulating in skull can be problematic for quantification (Pike, 2009). This is, indeed, the case for many PET radioligands for the 5-HT_{1A} receptor. Here, we briefly place this issue in context for [*carbonyl*-^{11}C]WAY-100635 and related radioligands.

Originally, WAY-100635 was labeled with carbon-11 in its methoxy position, and this radioligand—[*methoxy*-^{11}C]WAY-100635—was used to obtain the first PET images of 5-HT_{1A} receptors in human brain, albeit with only moderate signal contrast (Pike et al., 1995). Further investigation demonstrated that this radioligand was mainly metabolized by amide hydrolysis in primates to give a radioactive amine that readily entered brain, and which was likely responsible for a high level of non-specific binding (Osman et al., 1996). A method was therefore developed to label WAY-100635 in its carbonyl group (McCarron et al., 1996) with the expectation that the major radiometabolite would be changed to a poorly brain-penetrant and ionizable carboxylic acid, namely [^{11}C]cyclohexanecarboxylic acid. This new radioligand, [*carbonyl*-^{11}C]WAY-100635, was found to give much higher signal contrast in human studies due to lower non-specific binding in cerebellum (Pike et al., 1996), and [^{11}C]cyclohexanecarboxylic acid was confirmed to be the major radiometabolite (Osman et al., 1998) (Figure 1A). Experiments with [^{11}C]cyclohexanecarboxylic acid itself showed that relatively small amounts entered primate brain after intravenous administration but did not accumulate. Therefore, [*carbonyl*-^{11}C]WAY-100635 quickly became the preferred PET radioligand. Quantification methods with this radioligand were soon developed, based on use of cerebellum as a reference tissue or use of a measured arterial input function with full compartmental modeling (Farde et al., 1998; Gunn et al., 1998).

The fast metabolism of [*carbonyl*-^{11}C]WAY-100635 in human subjects may be considered a disadvantage; rapidly decreasing radioactivity in cerebellum or in plasma compromise accurate quantification by the reference tissue or compartmental models, respectively (Figure 2). Therefore, a new radioligand, dubbed [^{11}C]RWAY (McCarron et al., 2007), was developed with a reversed direction of the amide bond in an attempt to avoid rapid amide hydrolysis. While amide hydrolysis was avoided, this radioligand was metabolized by other processes that led to several radiometabolites; at least one of these appeared able to enter human brain and invalidated attempts at 5-HT_{1A} receptor quantification (Figure 1B) (Zhang et al., 2007). [^{11}C]RWAY therefore did not gain utility in human studies.

Radioligands with a fluorine-18 label are often sought because their two-hour physical half-life creates possibilities for their distribution and use at sites remote from their production. Replacement of one of the hydrogen atoms in the cyclohexyl ring of WAY-100635 with fluorine-18 produced a radioligand known as [^{18}F]FCWAY that had comparable pharmacology and promising utility for imaging human brain 5-HT_{1A} receptors (Carson et al., 2000). [^{18}F]FCWAY, like [*carbonyl*-^{11}C]WAY, is also rapidly metabolized by amide hydrolysis. This produces *trans* [^{18}F]4-fluorocyclohexanecarboxylic acid as a radiometabolite, which enters brain to some extent (Figure 1C) (Carson et al., 2003). In addition, this radioligand and its major radiometabolite are also radiodefluorinated to give [^{18}F]fluoride ion, which accumulates in skull, as readily seen in PET images that become quickly dominated by radioactivity in skull rather than brain (Figure 3A, 3C). This is problematic for receptor quantification in nearby brain, because of the `partial volume effect'

that `spills' radioactivity across the skull-brain boundary. Defluorination can be blocked by disulfiram, which markedly decreases uptake of [^{18}F]fluoride ion in skull and increases uptake of parent radioligand in brain (Ryu et al., 2007) (Figure 3B).

Thus, problems arising from the metabolism of radioligands are common, difficult to avoid completely, and must be taken into account in rigorous attempts at quantification.

4.3 Plasma as reference tissue

Presently, the only reliable way to resolve discrepancies associated with using a brain region like cerebellum as a reference tissue is to compare this technically easier outcome with the more complicated `gold standard', which requires measuring the concentration and plasma protein binding of parent radioligand in arterial plasma. This method entails calculating receptor density using compartmental modeling of serial PET measurements over time of concentrations of radioactivity in brain and the concentrations of parent radioligand in plasma. This well-accepted kinetic modeling method estimates receptor density as the ratio *at equilibrium* of the concentration of radioligand in brain to that in plasma. Because tracer doses of radioligand are administered, this equilibrium ratio (after subtraction of nonspecific binding) is linearly proportional to receptor density. However, to compare this ratio between individuals, it must be corrected for plasma protein binding of the radioligand, because only the free (unbound) radioligand has access to the brain. That is, brain uptake at equilibrium is determined relative to the free concentration of radioligand in plasma and not total concentration (free plus protein bound). For example, if a nonradioactive drug is administered concurrently with the radioligand and displaces it from the plasma proteins, brain uptake of the radioligand will increase in a manner directly proportional to its plasma free fraction. This effect also holds for therapeutic drugs given in pharmacological doses. Indeed, one case study found that patients who were concurrently prescribed hydroxychloroquine and digoxin had a 30% increase in free digoxin concentration in plasma (Leden, 1982). Similarly, the widely-used antiepileptic drug phenytoin strongly binds to plasma protein (90%); thus, even a slight increase in its free fraction concentration by drugs such as valproic acid can prove toxic (Soldin, 1999).

Among the investigators who reported 5-HT$_{1A}$ receptor density in MDD, only Parsey and colleagues used the method that measured concentration and protein binding of [*carbonyl*-^{11}C]WAY-100635 in arterial plasma. Although this method of using the arterial input function may introduce additional sources of error—for instance, inaccurate measurement of plasma free fraction—many experts would agree that using the arterial input function is the `gold standard' in brain PET imaging. Based on the theory of pharmacokinetic modeling, in our opinion the conclusion drawn by Parsey and colleagues that 5-HT$_{1A}$ receptors are elevated in several brain regions of patients with MDD may ultimately prove to be the `correct' answer. Furthermore, that series of studies analyzed the same dataset of patient and control subjects using both plasma and cerebellum as reference tissues and reproduced the apparently false finding of decreased 5-HT$_{1A}$ receptors with cerebellum. The discordant results (increased receptors using plasma but decreased receptors using cerebellum) were largely attributable to cerebellar uptake in patients being higher than in controls. Those studies therefore also provided a plausible explanation for the discrepant results—namely, that the cerebellum is not a valid reference region because, for unknown reasons, its uptake differs between groups.

Nevertheless, it is important to note that although the conclusions reached by Parsey and colleagues may be theoretically correct, they are also vulnerable in practice to errors. Such errors stem from the additional measurements of parent radioligand concentrations (which are relatively low, especially at late times of the scan) and of the free fraction of parent radioligand (which is also relatively low). Thus, a critical way to assess the accuracy of the

results obtained by Parsey and colleagues would be to determine whether others can replicate them, especially the key finding of increased cerebellar uptake in patients compared to control subjects.

After the initial publication and subsequent replication of these findings by Parsey and colleagues, several investigators studying 5-HT_{1A} receptors with PET discussed whether a consensus statement regarding patients with MDD could be generated by sharing data and performing a composite analysis. While this was a clearly reasonable solution, the task was impossible because no other group had measured both the concentration and protein binding of [*carbonyl*-^{11}C]WAY-100635 in plasma. In hindsight, this failure to reconcile the published results shows the value of groups collaborating as much as possible *a priori* to acquire data needed to resolve future discrepancies.

5. Other potential confounds

Although the results to date suggest that the reference tissue for [*carbonyl*-^{11}C]WAY-100635 should be plasma (assuming it is accurately measured), at least two other unresolved confounds may also bias these imaging results: efflux transporters at the blood-brain barrier and cellular trafficking of 5-HT_{1A} receptors.

5.1 Efflux transporters at the blood-brain barrier

Efflux transporters at the blood-brain barrier either block the entry into and/or enhance the removal of drugs that are substrates (Gillet and Gottesman, 2010). As such, these energy-dependent transporters change the equilibrium concentration of drugs in brain that would be achieved only by passive diffusion. Thus, if a radioligand is a substrate of an efflux transporter at the blood-brain barrier, its uptake will reflect the combined effects of receptor density and sensitivity to transport. For example, if a radioligand has less uptake in a particular brain region, does that reflect decreased receptor density or increased function of the efflux transporter? Some phenylpiperazine analogs of WAY-100635 are known to be substrates of efflux transporters and, as explained below, their sensitivity may well have confounded the effects of some past PET imaging studies of 5-HT_{1A} receptors.

The efflux transporters that most commonly affect psychotropic drugs are in the adenosine triphosphate (ATP)-binding cassette (ABC) family; the three most prevalent at the blood-brain barrier are ABCB1 (also called P-gp), ABCC1, and ABCG2 (Kannan et al., 2009). Of the three, P-gp is the most studied and tends to transport drugs that are lipophilic and carry a positive charge. Because almost all psychotropic drugs are lipophilic and many carry a positive charge at an amino group, all new central nervous system (CNS) drugs are now routinely screened to exclude those that are P-gp substrates. The full chemical characteristics that make a drug a P-gp substrate are unknown, but small molecular changes can significantly affect sensitivity. A broad spectrum exists in which some drugs may be very avid substrates while others remain totally unaffected. For instance, the anti-diarrheal agent loperamide is a very avid substrate; this potent opiate agonist has no CNS effects because P-gp almost completely blocks its entry into the brain.

Some compounds in the phenylpiperazine class of WAY-100635 are substrates for P-gp, and their sensitivity can vary between species (Zhang et al., 2007). For example, [^{18}F]MPPF, a selective antagonist for 5-HT_{1A} receptors, is a P-gp substrate in rodents (Passchier et al., 2000). P-gp inhibition by cyclosporin A (CsA) increased [^{18}F]MPPF uptake into rat brain about five- to ten-fold without changing the radioligand concentration in plasma. In P-gp knockout mice, [^{18}F]MPPF uptake increased two- to three-fold (Lacan et al., 2008; Passchier et al., 2000). [^{11}C]RWAY, a 5-HT_{1A} antagonist, is also a P-gp substrate in rats and mice (Liow et al., 2007) but not monkeys (Yasuno et al., 2006).

The sensitivity of WAY-100635 to P-gp transport has only been reported in rats where cyclosporin A treatment increased cerebral uptake two- to three-fold (Elsinga et al., 2005). Assuming that P-gp distribution is similar between the region of interest and the reference region, if WAY-100635 is also a P-gp substrate in humans, outcome measures obtained using free fraction would be questionable, because this would lead to greater variability compared to outcome measures that use reference tissue. Although P-gp function is probably distributed in a fairly uniform manner in a healthy brain, it may be modulated in selected regions demonstrating brain pathology. For example, P-gp is likely upregulated in regions surrounding the epileptogenic focus of man and rat and may cause resistance to antiepileptic medications, which are P-gp substrates (Bauer et al., 2008). In this regard, Drevets and colleagues reported that uptake of the antagonist [^{18}F]FCWAY was decreased in the area of the epileptogenic focus in human subjects with MDD (Theodore et al., 2007). Although the authors interpreted the results to reflect decreased 5-HT$_{1A}$ receptors in MDD, it could also reflect increased P-gp function surrounding the epileptogenic focus.

5.2 Affinity states and cellular trafficking of 5-HT$_{1A}$ receptors

5-HT$_{1A}$ receptors are G-protein coupled receptors (GPCRs) bound to heterotrimeric α, β, and γ G-protein subunits (Luttrell and Lefkowitz, 2002), and are believed to exist in two affinity states – high and low. The membranous and intracellular dynamics of GPCRs are rather complex. It is important to note, however, that both affinity states and the cellular trafficking of 5-HT$_{1A}$ receptors are unlikely candidates for the discrepant findings described above specifically because all the studies discussed in this review used the same radioligand —[*carbonyl*-^{11}C]WAY-100635—in imaging studies of MDD. However, if affinity states and cellular trafficking are differentially altered between MDD patients and healthy controls, this could potentially result in discrepant findings.

In vitro binding experiments confirmed that 5-HT$_{1A}$ receptors exist in high-affinity (coupled to G-proteins), and low-affinity (uncoupled) states. Whereas antagonists do not differentiate between these states, agonists preferentially bind to the high-affinity receptors (Aznavour et al., 2006; Stockmeier et al., 2009). A recent study showed that both G-protein (G_s) and an agonist were required to stabilize the active form of the β2 adrenoceptor, and that the affinity for β2AR increased nearly a hundred-fold in the presence of a binding protein similar to G_s (Rasmussen et al., 2011). High-affinity receptor states are functional, allowing for ligand binding, and activating and triggering secondary signaling cascades.

In light of these receptor affinity states, it is possible that imaging studies using [*carbonyl*-^{11}C]WAY-100635 may reflect differences in *total* 5-HT$_{1A}$ receptors without accounting for the functional states of the receptors, which may very well be altered in disease states like MDD. If the functional state of 5-HT$_{1A}$ receptors and/or the proportion of high- and low-affinity states are altered in individuals with MDD then, theoretically, an agonist 5-HT$_{1A}$ radioligand would be more sensitive for detecting changes in 5-HT$_{1A}$ receptors.

The cellular trafficking of 5-HT$_{1A}$ receptors regulates the availability of binding sites at the cell surface for PET ligands. Subsequent to 5-HT$_{1A}$ receptor activation, β-arrestin is recruited, thus desensitizing the receptor, which leads to recycling in two (short and long) distinct endosomal recycling pathways (Blier and de Montigny, 1990; Fichter et al., 2010). β-arrestin levels are decreased by chronic corticosterone and normalized by chronic fluoxetine treatment (David et al., 2009), suggesting that the internalization processes of 5-HT$_{1A}$ receptors in individuals with MDD may play a key role in this disorder. *In vivo* β microprobes, and *in vitro* immunoelectron microscopy data obtained by Zimmer and colleagues (2004), showed that a single dose of 8-OH-DPAT (0.5 mg/kg, i.v.) decreased [^{18}F]MPPF binding; this was attributed to 5-HT$_{1A}$ internalization (~30%) seen only for

raphe (autoreceptors), but not for hippocampus (heteroreceptors). Please note that radioligands, like [^{18}F]MPPF and [carbonyl-^{11}C]WAY-100635, that readily traverse the lipid bilayer of the blood-brain barrier would also be expected to rapidly traverse the cell membrane and have access to internalized receptors. Compared to membrane-bound receptors, those that are internalized may well have a different inonic environment, protein chaperones, or tertiary structure that would alter (i.e., decrease in the case of [^{18}F]MPPF) the affinity of the radioligand. It is also possible that the specific targeting of 5-HT$_{1A}$ receptors to the somatodendritic compartment in neurons may be dysregulated, thereby altering potential binding sites for PET ligands towards 5-HT$_{1A}$ receptors. One recent study found that the C-terminus of 5-HT$_{1A}$ receptors interacts with Yif1B, a member of the ER/Golgi trafficking machinery, and that this interaction plays a key role in specific targeting of 5-HT$_{1A}$ receptors to neuronal dendrites (Carrel et al., 2008). However, the regulation of Yif1B and other adaptor proteins towards 5-HT$_{1A}$ receptors in disease states and by antidepressant regimens remain to be studied.

The extant evidence suggests that the two-affinity states model and the cellular trafficking of 5-HT$_{1A}$ receptors are less plausible sources for the discrepant findings in PET studies of MDD; however, both certainly warrant further exploration.

6. Conclusion

The 5-HT transmitter system is widely thought to be involved in the pathophysiology and treatment of MDD, but findings from multiple PET studies describing changes in 5-HT$_{1A}$ receptor density in brain have yielded inconsistent results. Although most of these studies found evidence of decreased 5-HT$_{1A}$ receptor density in individuals with MDD, Parsey and colleagues noted increased 5-HT$_{1A}$ receptor density in these patients. This review highlighted the manner in which specific technical differences in data analysis could produce these disparate results. Notably, most 5-HT$_{1A}$ PET studies have used the reference tissue model, whose main advantage is that the arterial line is negated. This method, however, has the potential to yield inaccurate results because 1) nonspecific uptake in cerebellum (which was used as the reference region) is associated with too much variability; 2) cerebellar gray matter contains 5-HT$_{1A}$; and 3) radiometabolites accumulate in cerebellum over time. For these reasons, we propose that using the `gold standard' of arterial blood sampling (and reporting the free binding potential) is preferable for PET studies of 5-HT$_{1A}$ receptor density in individuals with MDD. We further propose that radioligands in general undergo more thorough evaluation of the effects of efflux transporters at the blood-brain barrier, which may alter radioligand uptake. Lastly, for receptors that exist in high- and low-affinity states, such as 5-HT$_{1A}$, we recommend using radioligands that distinguish between these states as a way to further our understanding of the pathophysiology of our targets.

It is also important to note that while clinical heterogeneity may well have contributed to the inconsistent PET imaging results, Parsey and colleagues were able to reproduce discrepant results by analyzing the same large sample size using different methods. Thus, methodological differences are an equally plausible cause of the discrepant findings. As a field, we are presently in the unfortunate situation of not knowing the true answer to this dilemma. Furthermore, because data from the multiple sites cannot be pooled, we cannot identify subgroups of MDD patients who may have altered 5-HT$_{1A}$ receptors. To place these arguments in a historical context, the issue of discrepant results outlined in this review echoes problems that occurred two decades ago in imaging studies of dopamine receptors in individuals with schizophrenia (for a review, see (Guillin et al., 2007)). At that time, the two main research groups investigating this issue used different radioligands and analysis methods in their studies, ultimately making it difficult to reconcile their data to reach a

definitive conclusion. We propose that, as a field, we can and should learn from such past mistakes. One key reason for resolving such problems mirrors, on a much smaller scale, recent difficulties associated with genetic studies in psychiatry. While such genetic studies continue to be very promising, it has become clear that they require much larger sample sizes in order to reach definitive conclusions. Similarly, we need larger sample sizes in PET studies in order to reach definitive conclusions about 5-HT_{1A} receptor density in MDD. Currently, the clinical relevance of altered 5-HT_{1A} receptor density of ±20% between MDD patients and healthy subjects is uncertain. Nonetheless, achieving definitive answers are important and will require pooling our data and this, in turn, will require that disparate studies have elements that make their data, for lack of a better word, “pool-able”. The planning and labor-intensive costs of achieving these goals suggest that future human PET studies will need to enter a more collaborative phase in order to clarify discrepancies and advance our knowledge in this key area of psychiatric research. While this is an important goal in its own right, working together now to resolve these issues would, by extension, also have profound implications for studies investigating other protein targets in the brain.

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Research Highlights

- PET imaging studies of patients with MDD have reported mixed findings on 5-HT_{1A} receptor density.
- 'Methodological' rather than 'clinical' factors likely explain these discrepancies.
- One such methodological confound is the use of the cerebellum as a reference region.
- A second confound is that measuring parent radioligand concentrations in arterial plasma is inherently imprecise.
- Other potential confounds include efflux transporter (P-gp), radiometabolites, 5-HT_{1A} affinity states, and cellular trafficking.

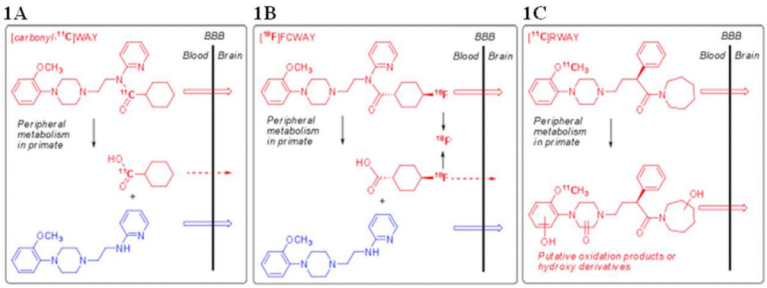

Figure 1. Peripheral metabolism of radioligands for imaging brain 5-HT$_{1A}$ receptors in humans: [*carbonyl*-^{11}C]WAY-100635, [^{18}F]FCWAY, and [^{11}C]RWAY

Radioactive species are shown in red and non-radioactive species in blue. **(A)** [*carbonyl*-^{11}C]WAY-100635 is primarily metabolized by hydrolysis of its amide bond giving a non-radioactive amine and [^{11}C]cyclohexanecarboxylic acid as a radiometabolite (Osman et al., 1998). The radioactive acid is able to enter brain from blood, but only to a small extent. Overall non-specific binding of radioactive species is therefore low. **(B)** [^{18}F]FCWAY is also metabolized by amide bond hydrolysis, yielding the same non-radioactive amine as well as [^{18}F]4-fluorocyclohexanecarboxylic acid (Carson et al., 2003; Ryu et al., 2007), a radioactive acid also capable of entering brain (Carson et al., 2003). Defluorination of [^{18}F]FCWAY and of its acid radiometabolite occur to give [^{18}F]fluoride ion that cannot penetrate into brain but is avidly accumulated by bone, including skull. **(C)** In [^{11}C]RWAY, the direction of the amide bond is reversed, thereby preventing hydrolysis (McCarron et al., 2007). However, this radioligand is now prone to metabolism by other mechanisms, including ring hydroxylation, piperazine oxidation, and dephenylation, and the resultant radiometabolites may enter human brain (Zhang et al., 2007).

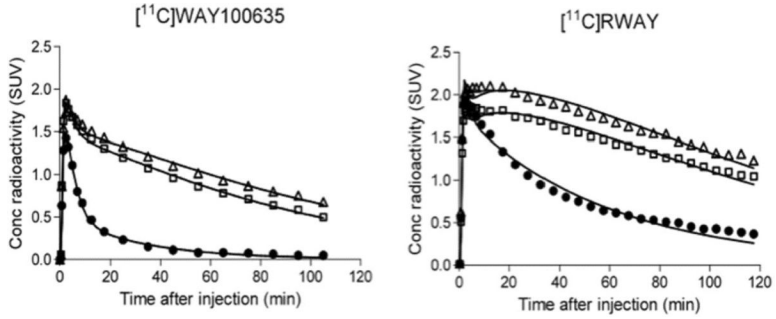

Figure 2. Time-activity curves in healthy human subjects for [*carbonyl*-^{11}C]WAY-100635 and [^{11}C]-RWAY, two different radioligands that bind to 5-HT$_{1A}$ receptors

As a measure of nonspecific binding, cerebellar uptake of [^{11}C]-WAY-100635 is much lower than that of [^{11}C]-RWAY. For example, cerebellar uptake at 100 minutes is 0.05 standardized uptake value (SUV) for [^{11}C]-WAY-100635, and 0.42 SUV for [^{11}C]-RWAY (SUV is a measure of concentration, and is normalized for injected activity and body weight). If the outcome measure is total binding in target regions like forebrain, then [^{11}C]-WAY-100635 is superior to [^{11}C]-RWAY because [^{11}C]-WAY100635 has less nonspecific binding—namely, the total binding of [^{11}C]-WAY-100635 has a higher percentage of specific (receptor) binding. However, the very low cerebellar uptake becomes problematic if the primary outcome measure is a ratio of target region to cerebellum. For example, the ratio at 100 minutes of uptake in temporal cortex to that in cerebellum is 13.6 for [^{11}C]-WAY-100635 and 3.3 for [^{11}C]-RWAY. The cerebellar uptake is so low that small errors in its absolute measurement propagate large percentage errors in the ratio of uptake in target to background regions. (Δ) temporal cortex; (□) frontal cortex; (•) cerebellum

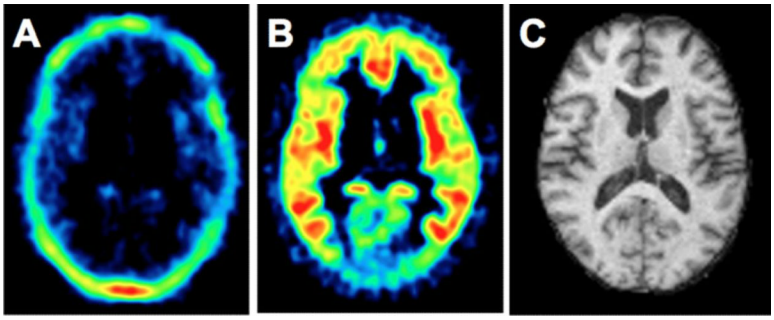

Figure 3. Axial brain PET images in a healthy subject acquired two hours after injection of [^{18}F]FCWAY at baseline and after administration of disulfiram

(**A**) The baseline image shows high uptake in skull of [^{18}F]fluoride ion, the result of defluorination in liver by a cytochrome P450 enzyme (CYP2E1). The [^{18}F]fluoride ion is also taken up by other bones in the body. (**B**) The same subject shown in panel A was imaged on another day after receiving disulfiram (Antabuse®) on the evening prior to the repeat PET scan. The dose of disulfiram (500 mg orally) was the same as that taken on a daily basis to maintain sobriety from alcohol. Disulfiram is a potent inhibitor of CYP2E1 and blocks defluorination in liver. Disulfiram not only blocked almost all uptake of radioactivity in skull but also markedly increased brain uptake of [^{18}F]FCWAY. Thus, disulfiram decreased metabolism (via defluorination) and thereby increased the concentration of parent radioligand in plasma, which then increased brain uptake and delineation of 5-HT$_{1A}$ receptors. These two PET images clearly show the effect of one metabolic path (i.e., defluorination) of [^{18}F]FCWAY. However, these images do not show the result of another metabolic path that generates [^{18}F]*trans*-4-fluorocyclohexanecarboxylic acid (FC) (Fig. 1). The radiometabolite, [^{18}F]FC, has no affinity for 5-HT$_{1A}$ receptors but enters brain and variably contaminates radioactivity that is typically assumed to be only parent radioligand. Although brain activity can be partially corrected for contamination of radiometabolites, the methods are controversial and should, in general, be avoided. That is, rather than correcting the post hoc problems of a radioligand, effort might be better spent on developing a new radioligand that does not generate brain-penetrant radiometabolites. (**C**) MRI for anatomical localization.

Table 1

A summary of PET imaging studies using [*carbonyl*-^{11}C]WAY-100635 in patients with MDD *vs*. healthy subjects. Numbers denote mean ± SD, N, or N/N.

Reference	Healthy subjects			Patients with MDD									
	N	Age	Sex (M/F)	N	Age	Sex (M/F)	Drug-naive	HDRS	Arterial blood	Outcome measure	Cerebellar reference region	Result	Correlation with symptoms
Drevets et al. 1999	8	35.3 ± 13.5	4/4	12	35.8 ±9.7	5/7	0	22 ± 6	No	BP_{ND}	Gray	↓ all regions	No
Sargent et al. 2000	18	36.4 ± 8.3	17/1	15[a]	37.7 ± 13.7	15/0	7	22 ± 5	No	BP_{ND}	Gray	↓ all regions	No
Meltzer et al. 2004	17	70.0 ± 6.7	8/9	17	71.4 ± 5.9	4/13	N.R.	18 ± 3	Yes	BP_P	Gray	↓ raphe	Yes
Parsey et al. 2006, 2010[c]	51	37.4 ± 14.4	29/22	30[b]	40.6 ± 13.1	8/22	23	26 ± 7	Yes	BP_F,BP_{ND}	White, Gray	↑(BP_F) ↓(BP_{ND})	No
Drevets et al. 2007	8	32.4 ± 12.1	4/4	16	32.4 ± 10	6/10	N.R.	18 ± 7	No	BP_{ND}	Gray	↓ all regions	No
Hirvonen et al. 2008	15	32.6 ± 7.7	5/10	21	40.1 ± 9.0	8/13	20	18 ± 3	Yes	BP_P	White	↓ all regions	No
Mickey et al. 2008	14	34 ± 12	9/8	17	38 ± 11	5/9	10	18.9 ± 2.6	No	BP_{ND}	Gray	↔	N.R.

BP, binding potential; HDRS, 17-item Hamilton Depression Rating Scale; N, number; N.R., not reported, wk, week.

↓lower 5-HT$_{1A}$ binding in patients with MDD than in healthy subjects

↑higher 5-HT$_{1A}$ binding in patients with MDD than in healthy subjects

[a]Number of untreated first-episode patients

[b]Number of not recently medicated patients

[c]Reported results are only from the combined 2006–2010 cohort