Molecular Imaging for Depressive Disorders

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ABSTRACT

SUMMARY: Molecular imaging is the visualization, characterization, and measurement of biologic processes at the molecular and cellular levels in humans and other living systems. Molecular imaging techniques such as MR spectroscopy and PET have been used to explore the molecular pathophysiology of depression and assess treatment responses. MR spectroscopy is a noninvasive technique that assesses the levels of biochemical metabolites in the brain, while PET uses radioligands injected in the bloodstream that have high binding affinity for target molecules. MR spectroscopy findings suggest a role for glutamate/glutamine and gamma-aminobutyric acid in depression. PET has generally failed to find a correlation between radioligand binding potential and depression severity or treatment response, though it may offer promise in distinguishing responders and nonresponders to treatment. A major challenge for both modalities is that depression is a heterogeneous, multifactorial disorder, while MR spectroscopy and PET are limited to examining a few metabolites or a single radioligand at a time. This difference makes a comprehensive evaluation of neurochemical changes in the brain difficult.

ABBREVIATIONS: GABA = gamma-aminobutyric acid; Gln = glutamine; Glu = glutamate; 5-HT = serotonin; MDD = major depressive disorder; PCr = phosphocreatinine

The standard definition of molecular imaging, as provided by the Molecular Imaging Center for Excellence Standard Definitions Task Force, is the visualization, characterization, and measurement of biologic processes at the molecular and cellular levels in humans and other living systems.[1] In this way, molecular imaging may offer further insights into the molecular pathology of various diseases in animal models and human subjects, aid in the development of molecular-targeted drugs, and potentially individualize established therapy regimens in clinical practice.

Molecular imaging requires probes that can bind to the target molecule with high affinity. Such probes may be radiopharmaceuticals or paramagnetic or fluorescent materials; small molecules such as receptor ligands or enzyme substrates; or higher molecular-weight-affinity ligands such as recombinant proteins.[2] Another emerging application in molecular imaging is in the use of reporter genes. These genes can be used to visualize the levels of expression of specific endogenous and exogenous genes, as well as other intracellular biologic phenomena such as signal transduction pathways and protein-protein interactions.[3]

Molecular imaging is performed with tools that enable signals from probes to be visualized and quantified in space and with time and includes techniques such as optical imaging, radiotracer imaging, sonography, MR spectroscopy, and PET. In the present article, we will focus on the use of MR spectroscopy and PET in the examination of depression.

Major Depressive Disorder

Major depressive disorder (MDD) is a great source of morbidity and suffering worldwide. In the United States, the 12-month prevalence is 6.6% and the lifetime prevalence is 16.2%.[4] According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5),[5] the diagnosis of MDD is made when 5 of 9 core symptoms are present for >2 weeks and indicate a change from a previous level of functioning. These core symptoms in MDD are the following: depressed mood; diminished interests; appetite changes leading to weight gain or loss; sleep dysregulation; psychomotor changes; loss of energy; feelings of worthlessness and excessive guilt; diminished concentration; and recurrent thoughts of death. These symptoms should not be attributable to the physiologic effects of a medical condition or substance misuse. The diagnostic criteria for MDD are largely unchanged from the DSM-IV Text Revision,[6] and this similarity allows comparisons of current and future research studies with those that were previ-

ously conducted. Now, a diagnosis of depression is made on the basis of clinical judgment. As of yet, there are no definitive blood/CSF biomarkers or neuroimaging findings that can aid the clinician in making the diagnosis. However, blood, CSF, and urine analyses and brain scans may be ordered by the clinician to exclude a medical condition or substance misuse that may have led to the depressive symptoms.

Molecular Neurobiology of Depression

The neurobiologic basis for depression has not yet been definitively characterized. Existing theories involve alterations of neurotransmitter systems and networks in limbic and cortical brain regions. Early hypotheses involve the monoamines serotonin (5-HT), norepinephrine, and dopamine; more recently, the involvement of glutamate (Glu) and gamma-aminobutyric acid (GABA) has also been proposed. Other neuropeptides involved in the pathophysiology of depression and that may have a role in treatment include corticotrophin-releasing factor, neuropeptide Y, neurokinin/substance P, and galanin.[7]

The accepted early dogma in the pathophysiology of depression involved a reduction in monoamines 5-HT and norepinephrine and, to a lesser extent, dopamine. Early findings that reserpine, an antihypertensive that depletes vesicular monoamine stores, led to depressive symptoms suggested a role of monoamines, especially catecholamines. Drugs that selectively inhibited the reuptake of 5-HT and norepinephrine and hence increased the intrasynaptic levels of these neurotransmitters were shown to be effective antidepressants.[8] However, direct analysis of monoamine levels has been mixed, with some reports of reduced levels of 5-HT and norepinephrine in blood, CSF, and postmortem brain tissue.[9,10] Currently, why, while the reuptake inhibitory effects of antidepressants occur within hours and days, the symptomatic improvement takes weeks remains an unresolved and vexing issue. Hence, other mechanisms are likely in play.[11]

In recent years, the focus has shifted away from monoamines to the amino acid neurotransmitters Glu and GABA.[12] GABA is the major inhibitory neurotransmitter in the brain and counterbalances Glu, the major excitatory neurotransmitter. The balance of Glu and GABA is essential for normal brain function. GABA deficiency has been proposed as a model for anxiety and depression. In a series of studies, plasma GABA levels were lower in subjects with depression.[13] Patients show a cortical GABA deficit, which is reversed by chronic selective serotonin reuptake inhibitor treatment.[14] Moreover a partial GABA(A) receptor deficit is causal for depression-like behavior in animals and is reversed by chronic antidepressant treatment.[15] Other evidence comes from postmortem studies that show a loss of GABAergic interneurons in the dorsal lateral prefrontal cortex of subjects with depression[16] and a decrease in the glutamic acid decarboxylase.[17] As with GABA, there is some evidence that the glutaminergic system, especially abnormalities of Glu and N-methyl-D-aspartate receptors, contributes to the pathophysiology of depression.[18-20] N-methyl-D-aspartate receptor antagonists have demonstrated antidepressant-like activity in preclinical and clinical studies. It has been postulated that glutamatergic receptor modulation may facilitate neuronal stem cell enhancement (neurogenesis) and release of neurotransmitters associated with treatment response.

From an anatomic point of view, the prefrontal cortex and limbic structures, such as the anterior cingulate cortex, have been implicated in the exaggerated response of a sense of guilt and despair to negative emotions. On the other hand, alterations in the hypothalamus and parts of the brain stem have been proposed as responsible for neurovegetative symptoms (eg, sleep and appetite dysfunction and fatigue).[21] This may constitute the interface with the hypothalamus-pituitary axis. Hypothalamus-pituitary axis hyperactivity is also associated with depression, and this is manifested by increased plasma concentrations of cortisol and nonsuppression of adrenocorticotropic hormone and cortisol in the classic dexamethasone suppression test.

Impact of Oxidative Stress in Depression

Oxidative stress occurs when redox homeostasis is tipped toward an excess of free radicals, either due to their overproduction or deficiencies in antioxidant defenses.[22] While oxygen is essential for aerobic functioning, excessive amounts of its free radical metabolic by-products are toxic. In high concentrations, free radicals can lead to the damage of cellular proteins, lipids, carbohydrates, and nucleic acids, which consequently may result in apoptosis and cell death.[23]

A growing body of research supports the involvement of oxidative stress in the pathophysiology of depression. A recent meta-analysis observed a significant association between depression and oxidative stress across studies.[24] Markers of oxidative stress have been found to be higher in patients with major depression relative to controls,[25,26] including in a postmortem study.[27] Evidence for the impact of antidepressant treatment on oxidative stress, however, has been mixed, with some studies finding a decrease in oxidative stress after antidepressant treatment[28] and others finding no such change.[26,29]

Neuroimaging Application for Depression Research

Neuroimaging is not routinely used for clinical diagnosis of depressive disorders. However, it may be used for excluding a neurologic lesion (eg, stroke, tumor, or atrophy that may physiologically contribute to mood dysregulation). Nevertheless, neuroimaging tools have been used to study the underlying biology and brain circuits that are relevant to the onset of depression, course of disease, and response to treatment. Volumetric studies have shown gray matter loss and volumetric reductions in subregions of the prefrontal cortex, medial temporal lobe, amygdala, and hippocampus across all age ranges.[30,31] Hippocampal volume loss may be due to hyperactivity of the hypothalamus-pituitary axis, which leads to increased circulating glucocorticoids.[32] Functional imaging, which is not covered in this review, has been used to show the activation in the corticolimbic mood-regulating circuit.[33]

Role of Molecular Imaging for Depression

The living brains of humans can now be studied at a molecular level by neuroimaging tools such as MR spectroscopy and PET. While not used for clinical diagnosis, molecular imaging has a role to play in characterizing the molecular pathophysiology of the disease, studying putative new treatments in proof-of-concept trials, and assessing treatment response. Molecular imaging meth-

Table 1: Summary of metabolites/compounds detected by each type of MRS and findings

Type of MRS	Metabolites/Compounds Detected	Summary of Findings
^{19}F MRS	Fluorine 19 nucleus Fluorinated drugs (including SSRIs)	Used to study pharmacokinetics of fluoxetine and fluvoxamine[31]
^{31}P MRS	Phosphomonoesters Phosphodiesters Inorganic phosphate Phosphocreatine α-, β-, and γ-nucleoside triphosphate	Membrane phospholipid and energy metabolism abnormalities in frontal and temporal lobes of patients with bipolar disorder[36] Lower phosphomonoester levels for patients with euthymic bipolar disorder[37] Increased phosphomonoester and decreased ATP levels in frontal lobes of unipolar depressed patients[38]
Proton MRS	Glx GABA NAA Cr+PCr Cho mIns	See Table 2

Note:—APT indicates adenosine triphosphate; SSRI, selective serotonin reuptake inhibitor.

ods were initially used to test the hypothesis of decreasing monoaminergic function, in particular serotonin, in depression. Given the rapid advances in imaging technologies and radiotracer chemistry, any review will likely be supplanted shortly after publication. Hence, we will simply summarize the findings to date.

MR Spectroscopy

MR spectroscopy is a noninvasive technique that can provide a quantitative measure of biochemical concentration in the living brain. It has high spatial resolution and requires neither radioactive tracers nor ionizing radiation. Different molecules have unique MR spectra, which can be quantified by taking the area under the signal curve and measuring it against the curve of a standard metabolite. Unlike MR imaging, which measures the signal from protons in water molecules (typically at concentrations of approximately 35 mol/L[34]) to produce images, MR spectroscopy detects the signals from compounds in lower concentrations (typically at concentrations of 0.5–10 mmol/L).[35] These include [1]H (proton), phosphorus 31 (^{31}P), sodium 23, lithium 7,[36] and fluorine 19 (^{19}F).[37] MR spectroscopy has been used to understand the neurobiology of depression and to study its use in predicting treatment response. Table 1 offers a summary of metabolites and compounds detected by each type of MR spectroscopy and their key findings.

Fluorine MR Spectroscopy

[19]F MR spectroscopy has been of interest to researchers since the early days of MR spectroscopy because a large number of psychiatric medications contain the fluorine-19 nucleus, which is naturally abundant and has an MR spectroscopy sensitivity of 83% relative to protons.[38] Fluorinated drugs include selective serotonin reuptake inhibitors such as fluoxetine and fluvoxamine, which are the most commonly prescribed antidepressants in the world today. [19]F MR spectroscopy has been used in understanding the pharmacokinetics in target organs and in correlating brain concentrations of the selective serotonin reuptake inhibitors fluoxetine and fluvoxamine with clinical responses in patients with MDD,[39] as well as in social phobia[40] and in a pediatric population treated for pervasive developmental disorders.[41] Fluorine levels in the brain are very high and are sustained for a long time, reflecting the accumulation of the drug in the brain, which explains the potential long duration of action of the drug. Recently, Wolters et

al[42] explored the possibility of using dual-targeted molecular imaging in the form of hybrid ^{1}H/^{19}F imaging and spectroscopy for various clinical applications. Nevertheless, despite the potential utility of ^{19}F MR spectroscopy for examining the mechanisms of antidepressants, the extant literature is scarce, and this remains an area worth exploring in future research.

Phosphorus MR Spectroscopy

Phosphorus MR spectroscopy can measure phosphate metabolism, providing important information about cellular energetics, membrane metabolism, and pH. The common brain metabolites and molecules measured by ^{31}P MR spectroscopy include phosphomonoesters; phosphodiesters; inorganic phosphate; phosphocreatine; and α-, β-, and γ-nucleoside triphosphate.[43]

[31]P MR spectroscopy has more often been used to examine bipolar disorders, though findings of some studies have been extended to MDD. [31]P MR spectroscopy studies have suggested membrane phospholipid and energy metabolism abnormalities in the frontal and temporal lobes of patients with bipolar disorders,[44] with a meta-analysis finding significantly lower phosphomonoester levels in patients with euthymic bipolar disorders than in healthy controls and patients with depression having significantly higher phosphomonoester levels than those with euthymic bipolar disorders.[45] This may suggest trait- and possible state-dependent abnormalities of membrane phospholipid metabolism, which may indicate dysfunction in brain-signal transduction systems in bipolar and depressive disorders.

When one focuses on MDD alone, significantly increased phosphomonoester and decreased adenosine triphosphate levels have also been found in the frontal lobes of patients with unipolar depression compared with healthy controls.[46] In a recent study on late-life depression, glycerophosphoethanolamine was found to be elevated in the white matter of subjects with depression, suggesting an enhanced degeneration of cell membranes in these subjects relative to healthy elderly.[47] An important limitation of ^{31}P MR spectroscopy, however, is the relatively low sensitivity of the method—approximately 5% of that of proton MR spectroscopy.[48]

Proton MR Spectroscopy

The proton is one of the most commonly studied nuclei in MR spectroscopy due to its high sensitivity and abundance in most metabo-

lites. In addition, proton spectroscopy is easier to perform and provides much higher signal-to-noise ratios than either sodium or phosphorus. Proton MR spectroscopy can be performed within 10–15 minutes by using clinical MR imaging systems with standard radiofrequency coils developed for the acquisition of diagnostic MR images; in contrast, the standard MR imaging coils cannot be used when nuclei other than ^{1}H are studied, and special coils tuned to the desired nucleus have to be bought or built instead.[35]

Proton MR spectroscopy can reliably detect NAA, Cr+ phosphocreatine (PCr), Cho-containing compounds including phosphocholine and glycerophosphocholine, and myo-inositol in the brain. These brain metabolites measured by [1]H-MR spectroscopy are involved in cellular metabolism, neurotransmission, and cell membrane synthesis, or they serve as a specific marker for neurons and glia. The Cr+PCr level is relatively stable across the brain and is used as an internal concentration reference, with some caveats—for example, Cr+PCr ratios may be distorted in cases of tumor and stroke. At higher field strengths, Glu and glutamine (Gln) and others can be resolved. Gln is the precursor and a product of Glu and then is metabolized to GABA. Glu in synapses is also rapidly removed and converted back to Gln. Glutamine is synthesized in astrocytes from Glu and then in neurons is converted back to Glu. Hence Glu and Gln are closely linked molecules.

NAA functions as an acetyl donor for acetyl coenzyme A and takes part in lipid synthesis, including myelin. Because it is localized only in neurons, it is a putative neuronal marker. Hence a reduction in NAA levels is indicative of neuronal loss and dysfunction. In the case of chronic depression, it may suggest a neurodegenerative process. Figure 1 shows an example of chemical shift imaging output of NAA for MR spectroscopy.

Cho is the precursor of neurotransmitter acetylcholine and membrane lipids, phosphatidylcholine and sphingomyelin,[49] and is a marker for the state of membrane phospholipid metabolism. An elevated Cho signal, therefore, most likely reflects higher membrane turnover and damage to myelin or neurons. The Cho signal measures not just Cho but also the underlying phosphocholine and glycerophosphocholine.

Myo-inositol is traditionally considered a glial marker[50] because it is actively transported into astrocytes[51,52] and functions in osmoregulation in brain glial cells.[53] Higher levels probably reflect gliosis.

Table 2 lists some important properties of and findings for the metabolites of interest described above, while Fig 2 shows proton MR spectroscopy identification for assignments of these compounds.

Comprehensive reviews and a major meta-analysis on MR spectroscopy for MDD and a review of MR spectroscopy for evaluation of treatment efficacy have been conducted by Yildiz-Yesiloglu and Ankerst,[50] Rao et al,[54] and Caverzasi et al,[55] respectively, and interested readers can refer to these articles for more information on the extant literature. In this article, we present key findings and attempt to draw conclusions from the data available.

Despite the wide diversity of proton MR spectroscopy methods applied, brain regions studied, and molecules, there appears to be some evidence of a correlation between certain metabolite concentrations, in particular Glu, GABA and Cho, and a good treatment response to pharmacotherapy or stimulation techniques.

Metabolites of Interest

Glx. Studies have shown decreased Glx in the anterior cingulate cortex[19,56,57] in patients with depression, and these levels improved with electroconvulsive therapy.[19] Decreased levels of Glx have also been found in the amygdala-hippocampus of patients with depression.[58] In contrast, Sanacora et al[59] found elevated Glu in the occipital cortex. There is evidence, however, that Glu may be differentially involved in depression and bipolar disorder. In a review, Yüksel and Öngür[60] found an overall reduction of Glx in depressive disorders in contrast to elevation in bipolar disorders. This finding may suggest that depressive and manic episodes are characterized by modulation of the Glx ratio in opposite directions.

GABA. Using MR spectroscopy, Sanacora et al[61] found that patients with depression have significantly lower GABA concentrations in the occipital cortex relative to healthy controls. These findings were replicated in a subsequent study by the same authors[59] by using a larger sample size. Patients who were most severely depressed and who had the lowest GABA concentrations showed the largest increase after selective serotonin-reuptake-inhibitor treatment. In contrast to findings of reduced GABA concentrations only in the occipital cortex, Hasler et al[62] reported that GABA, Glu, and Gln were all reduced in the prefrontal cortex of subjects with depression. Bhagwagar et al[63] also reported low GABA in the occipital and anterior cingulate cortices.

NAA or NAA/Cr. A meta-analysis showed no significant differences in NAA values between patients with depression and healthy subjects in both the basal ganglia and frontal lobe structures,[50] though a few studies have found a decreased NAA/Cr ratio in the thalamus[64] and decreased NAA in the caudate[65] in patients with depression. The general lack of significant findings for NAA or NAA/Cr may suggest an increased membrane turnover in MDD without a neurodegenerative outcome.

Cho. Results were mixed, but in the meta-analysis of proton MR spectroscopy, Yildiz-Yesiloglu and Ankerst[50] found that adult patients with MDD had higher Cho/Cr values in the basal ganglia compared with controls. Moreover, the Cho elevation reversed with antidepressant treatment.

Myo-inositol. Most studies reported negative findings in the anterior cingulate cortex and basal ganglia and mixed results for the prefrontal cortex.[54]

Proton MR spectroscopy has also been used to examine depression and the impact of antidepressants in animal models. The chronic mild stress model is well-documented as an animal model of depression.[66] In rats, repeated exposure to mild stressors induced anhedonia—a core symptom of major depressive disorder. The most consistent finding emerging from animal studies is that NAA is significantly reduced in the hippocampi of both rat[67,68] and tree shrew[69] models subjected to chronic stress, with the administration of antidepressants escitalopram and tianeptine reversing these decreases to normal levels in the latter 2 studies, respectively. Similar to results from human depression studies, Hemanth Kumar et al[67] also found a significant decrease of Glu, Gln, and GABA levels in the hippocampus and prefrontal cortex of rats with chronic mild stress. In contrast, Perrine et al[70] found

a significant increase in GABA levels in the anterior cingulate of stressed rats, with the administration of ketamine blunting depression-like behavior and increasing GABA levels in the anterior cingulate. However, the results of these studies are not directly comparable because the GABA levels were measured in different areas of the brain. Other findings that require further support include decreased in vivo concentrations of Cr, pCr, and Cho in tree shrew models[69] and elevated levels of mIns and τ in rats with chronic mild stress.[67]

Hence, we can summarize that from proton MR spectroscopy, several molecules are likely to play a role in MDD. These include Glu, GABA, NAA, and Cho. Specifically looking for treatment response, Caverzasi et al[55] found a correlation between changes in Glu, NAA, and Cho with good treatment response to either pharmacotherapy or stimulation techniques. A study by Renshaw et al[71] also found lower brain purine levels in female patients with depression who responded to treatment with fluoxetine, suggesting that treatment response might be predicted by using proton MR spectroscopy.

PET

PET uses molecules that are radio-labeled with a relatively short-lived positron-emitting nuclide, which is injected into the bloodstream so that the PET scanner can provide a digital record of the time
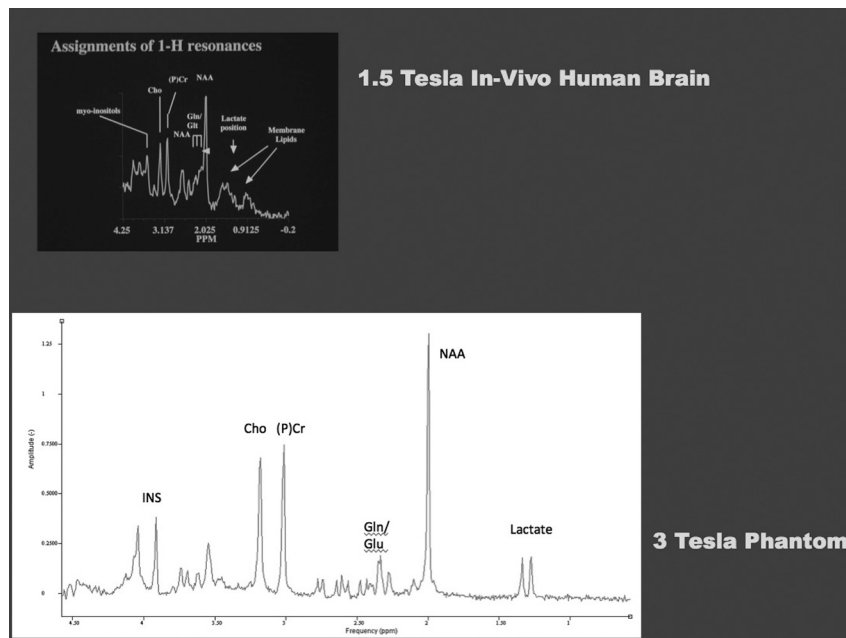

FIG 1. 2D chemical shift imaging of *N*-acetylaspartate. Courtesy of Cecil Charles, Duke University.

Table 2: Summary of metabolites detected by proton MRS and findings

Metabolite of Interest	Key Properties	Findings for Patients with Depression Relative to Controls
Glu/Glutamine	Complex signal Requires difference technique at intermediate field strengths	Decreased Glu/Gln in anterior cingulate[19,56,57] Decreased Glu/Gln in amygdala-hippocampus[58] Decreased Glu/Gln in prefrontal cortex[62] Elevated Glu in occipital cortex[59] Reduction of Glx (mainly Glu and Gln) in depressive disorder but elevation in bipolar disorder[60] Response to treatment: increased levels with ECT[19]
GABA	Complex signal Requires different technique at intermediate field strengths	Decreased in occipital cortex[59,61] Decreased in occipital and anterior cingulate cortex[63] Decreased in prefrontal cortex[62] Response to treatment: patients who were most severely depressed and who had lowest GABA concentrations showed largest increase after SSRI treatment[59]
NAA or NAA/Cr	Only present in neurons Change reflects loss or decreased function of neurons	Decreased NAA/Cr in thalamus[64] Decreased NAA in caudate[65] Meta-analysis: no significant differences in NAA between depressed and healthy subjects in basal ganglia and frontal lobe structures[50]
Cr+PCr	Reflects tissue energetics ~7.8 μmol/g[117]	No significant differences in Cr levels in frontal lobe structures between depressed and healthy subjects[50]
Cho	Concentration of pool: ~2 μmol/g[117] Reflects mobile Cho (TMA) moieties Cho concentration rate limiting step in acetylcholine synthesis Dynamic equilibrium with acetylcholine and phospholipid pathways	Mixed findings Meta-analysis: elevated in basal ganglia[50] Response to treatment: choline elevation reverses with antidepressant treatment[50]
mIns	Diverse role in central nervous system (gliosis, cytoskeleton, cellular signaling)	Generally negative findings in anterior cingulate cortex, basal ganglia Mixed results for prefrontal cortex[54]

Note:—ECT indicates electroconvulsive therapy; SSRI, selective serotonin reuptake inhibitor; TMA, trimethylamine.
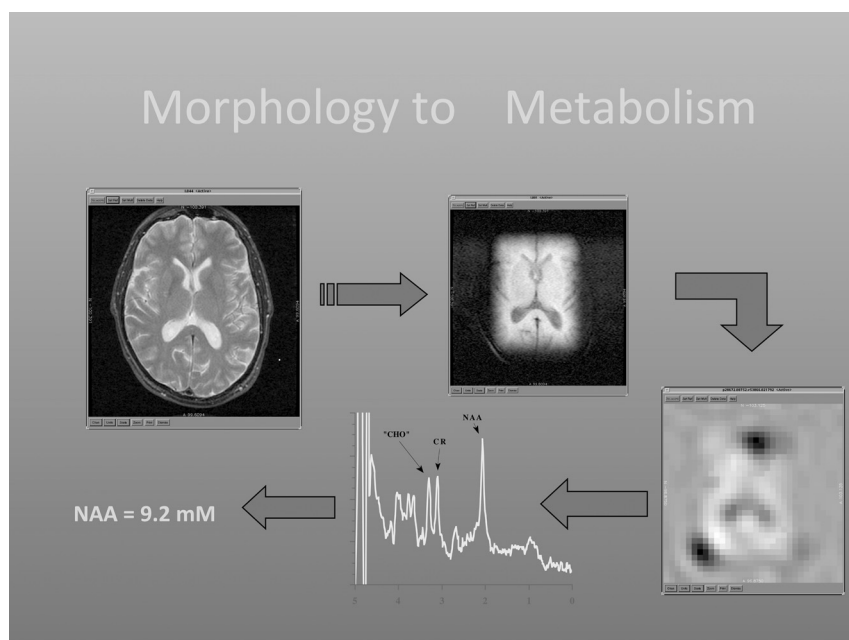
FIG 2. Proton MR spectroscopy identification of assignments of compounds. Courtesy of Cecil Charles, Duke University.

Table 3: Common neurotransmitter systems studied in PET imaging and radioligands used for each component or pathway involved

Neurotransmitter System	Component/ Pathway Involved	Radioligands Used
Serotonin	Synthesis	α-[^{11}C]MTrp
	Transport	[^{11}C]McNeil 5652
		[^{11}C]DASB
	Type 1A receptor	[^{11}C]WAY-100635
		[^{18}F]FCWAY
		[^{18}F]MPPF
	Type 1B receptor	[^{11}C]P943
	Type 2 receptor	[^{18}F]setoperone
		[^{18}F]altanserin
		[^{11}C]MDL 100907
		[^{18}F]FESP
Dopamine	Synthesis	[^{18}F]fluoro-L-DOPA
	D1 receptor	[^{11}C]SCH 23,390
		[^{11}C]NNC 112
	D2/3 receptor	[^{11}C]raclopride
		[^{11}C]FLB 457
	Transporter	[^{11}C]RTI-32
		[^{11}C]CFT
Monoamine oxidase	Monoamine oxidase type A	[^{11}C]harmine
Non-monoaminergic mechanisms	Muscarinic type 2 receptor	[^{18}F]FP-TZTP
	Nicotinic receptor	[^{18}F]FA-85380
	GABA type A receptor	[^{11}C]flumazenil
	Phosphodiesterase type 4	[^{11}C]-(R)-rolipram
	Amyloid and τ proteins	[^{18}F]DDNP
	p-glycoprotein	[^{11}C]verapamil
	Histamine H1 receptor	[^{11}C]doxepin

binding depend on a combination of 3 factors: 1) the number of neuroreceptors in a molecular conformation, 2) the affinity of neuroreceptors for the radioisotope, and 3) concentration of endogenous neurotransmitters near the neuroreceptors.

In seminal articles, Smith and Jakobsen[72,73] and Savitz and Drevets[74] offered comprehensive reviews of the research findings in the field to date for various radioligands, categorized by their target neurotransmitter systems, with serotonin type 1A receptors being the most extensively studied. Table 3 provides an overview of the most common neurotransmitter systems studied with PET imaging and the radioligands that have been used to target each component or pathway. We hereby present a few key findings of these reviews and examine their implications.

In general, PET studies have produced differences between patients with depression and healthy controls across various radioligands and neurotransmitter systems, though contrary evidence has also been found. In serotonin transport, binding potentials are found to be lower in various brain regions of depressed patients with both unipolar or bipolar disorder for both radioligands [^{11}C]McNeil 5652[75-77] and [^{11}C]DASB,[78-81] though some studies were unable to find a reliable difference between depressed and healthy subjects[82,83] for [^{11}C]DASB. For the serotonin type 1A receptor, for which most research has been done by using [^{11}C]WAY-100635, most studies have found that the binding potential is reduced for patients with depression compared with healthy subjects,[84-88] though some studies have found results in the converse direction,[89,90] and a few others have found no difference between depressed and healthy subjects.[91,92] However, none of the results were robust enough to be used for diagnostic purposes.

While other neurotransmitter systems have not been as extensively researched, the literature available also suggested a reduction in binding potentials for patients with depression compared with healthy subjects for the serotonin type 2 receptor by using [^{18}F]altanserin,[93,94] dopamine synthesis by using[^{18}F]fluoro-L-DOPA,[95] dopamine D1 receptor by using [^{11}C]SCH 23,390[96] and [^{11}C]NNC 112,[97] histamine H1 receptor by using [^{11}C]doxepin,[98] and phosphodiesterase type 4 by by using [^{11}C](R)-rolipram.[99] Conflicting findings have emerged for dopamine D2/3 receptors by using [^{11}C]raclopride, with Meyer et

and place of radio-labeled molecules in the brain. The basic molecular tools for PET studies are typically synthetic compounds radio-labeled with positron-emitting nuclides, which enter the brain and bind to target macromolecules. The status of neuroreceptors is typically described in terms of the magnitude with which they bind the PET radioligand, expressed as binding potential or distribution volume. Hence PET estimates of receptor

al[100] finding elevated binding potentials for patients with depression compared with healthy controls and Montgomery et al[101] finding the converse. For monoamine oxidase type A, elevated levels of binding potential have been found for patients with depression compared with healthy subjects by using [^{11}C]harmine.[102,103]

Despite detecting group differences between depressed and healthy subjects, most studies have unfortunately failed to find any correlation between binding potentials and depression severity or clinical conditions across various radioligands and neurotransmitter systems. This failure to find a correlation surfaced for serotonin synthesis by using α-[^{11}C]MTrp[104]; serotonin transport by using [^{11}C]McNeil 5652[75,76]; serotonin type 1A receptor by using [^{11}C]WAY-100635[87,88,90,91,105]; serotonin type 2 receptor by using [^{18}F]setoperone,[106] [^{18}F]altanserin,[93,94] and [^{11}C]MDL 100 907[107]; D1 receptor by using [^{11}C]NNC 112[97]; and D2/3 receptor by using [^{11}C]raclopride.[100,101]

Similarly, studies that have examined the effects of antidepressant or electroconvulsive treatment have likewise failed to find a correlation between binding potentials and depression scores when comparing pre- and posttreatment outcomes. For example, in investigating monoamine oxidase type A, Meyer et al[103] found that the beneficial clinical effects of antidepressant treatment failed to reliably affect regional binding of [^{11}C]harmine in the brain. In investigating the effects of electroconvulsive therapy, no correlation was found between binding potentials and treatment effects for serotonin type 1A receptor by using [^{11}C]WAY-100635,[85,107] for serotonin type 2 receptor by using [^{18}F]setoperone,[108] and for D2/3 receptor by using [^{11}C]FLB 457,[109] despite clinical improvement in most or all patients. This may limit the utility of PET imaging for assessing the clinical efficacy of medications.

Nevertheless, PET imaging may offer some promise in distinguishing responders and nonresponders to treatment. For example, Moses-Kolko et al[110] found that binding of [^{11}C]WAY-100635 for serotonin type 1A receptors in the orbital cortex was higher before treatment in nonresponders compared with responders. Miller et al[111] also found that accumulation of [^{11}C]McNeil 5652 for serotonin transporters was reduced in patients who failed to show remission after 1 year of treatment compared with healthy subjects, in contrast to those who remitted. Again, the findings are not robust enough for clinical use. More research, however, is needed in this area before more definitive conclusions can be drawn.

Another promising area in which PET imaging may be used is in examining the effects of psychotherapeutic treatment approaches as opposed to medication. In a seminal study by Karlsson et al,[112] the pre- and posttreatment binding potentials of [^{11}C]WAY-100635 for serotonin type 1A receptor were compared for patients who underwent psychodynamic therapy and those who received antidepressants. Surprisingly, it was found that only the former group had increased binding potential post-treatment, despite similar improvement in clinical outcomes in both groups. These findings may suggest that changes in binding potential could be related to changes in depressive cognition, as opposed to the current depressive affect as reflected by depression scores. In potential support of this finding, Meyer et al[113] found correlations between the binding potential of [^{18}F]setoperone for serotonin type 2 receptor and the level of dysfunctional attitudes, as well as between the binding potential of [^{11}C]DASB and the magnitude of negative thinking[82] in separate studies, even though binding potentials were not correlated with depression scores in the former and were related to current depressive episodes in the latter.

Some researchers have also explored the use of PET imaging in looking for potential differences between patients with depression who have recovered and healthy subjects, with the aim of examining whether depression involves persistent underlying molecular abnormalities and whether recurrence of illness may be predicted by PET. Findings, however, have been limited and mixed. For example, Bhagwagar et al[114] found no difference in binding potential between recovered patients and healthy subjects for serotonin transporter by using [^{11}C]DASB. In addition, while Bhagwagar et al[105] found that recovered patients had lower binding potentials of [^{11}C]WAY-100635 for serotonin type 1A receptor compared with healthy subjects, Miller et al[89] found that binding potentials were increased for the same radioligand for both remitted and currently subjects with depression compared with healthy subjects. Estimates of binding potential for the latter study, however, depended heavily on the region used as reference tissue, with cerebellar white matter giving higher values for binding potentials in remitted patients compared with healthy subjects and cerebellar gray matter producing results in the converse direction. In investigating serotonin type 2 receptor, Bhagwagar et al[107] found that recovered depressed patients had higher binding potentials of [^{11}C]MDL 100907 in various brain regions compared with healthy subjects; for monoamine oxidase type A, binding potentials were similarly elevated for [^{11}C]harmine in brain regions of both currently depressed and recovered depressed subjects compared with never-depressed subjects, with recurrence of depression associated with elevated binding.[103] To our knowledge, no longitudinal studies have yet been conducted to explore the utility of PET imaging for predicting the recurrence of depression in recovered patients; this may be a promising area for future research.

Given the growing interest in GABA, Klumpers et al[115] showed that bilateral reduction in limbic parahippocampal and right temporal [^{11}C]flumazenil binding found in MDD indicates decreased GABA(A)-benzodiazepine receptor complex affinity and/or number. The inverse relationship between GABA(A) binding in the temporal lobe and hypothalamus-pituitary axis activity suggests that hypothalamus-pituitary axis hyperactivity is partly due to reduced GABAergic inhibition. However, this study was based only on 11 subjects with depression and 9 controls and awaits further replication.

DISCUSSION

Molecular imaging was envisaged to explain and explore the neuromolecular basis of depressive disorders, to study pharmacologic agents, and assess treatment outcomes. In general, the monoamines are studied by using PET, and small molecules are studied by using MR spectroscopy. Despite rapid advances in imaging technology and new radio-labeled compounds and a series of published studies, molecular imaging for MDD is still very much a work in progress.

The findings thus far can be briefly summarized. For MR spectroscopy, the common metabolites studied are NAA, Glx, GABA, Cho, and mIns. Presently, findings for Glx and GABA appear to be the most promising, with most studies finding a decrease in Glx and GABA levels in brain regions of patients with depression relative to healthy controls, which increase in response to treatment. Nevertheless, contrary results have been found. No significant differences have generally been found between depressed and healthy subjects for NAA or NAA/Cr and mIns, though conflict-

ing findings exist, and evidence is mixed for Cho. Despite the limitations in MR spectroscopy studies, Caverzasi et al[55] opined that MR spectroscopy has good potential for enhancing the understanding of the mechanisms underlying MDD treatments and for helping in evaluating treatment efficacy.

For PET, comparison studies between subjects with depression and healthy controls have been shown for several radiolabeled compounds. A few studies have found differences in binding potentials for medication-naïve patients relative to healthy subjects or remitted, depressive patients treated with medication. These results provide some reassurance of the validity of the monoamine hypothesis in depression. However, several studies have failed to find a correlation between binding potential and depression severity or other clinical variables. Moreover, there is no correlation between the beneficial effects of potent antidepressant treatment and PET binding. This severely limits the utility of PET to study pharmacologic effects and treatment outcomes. If this means that the molecular systems probed by current PET ligands are stable, then the discovery of new radioactive ligands along with new scanning protocols will be needed.

The clinical utility of PET is also currently limited. While PET imaging may show differences in depressed-versus-healthy subjects, it is unlikely to be used for diagnostic purposes for the following reasons: First, as mentioned above, the preponderance of evidence has shown that PET imaging is not correlated with depression severity and other clinical variables. Second, although the bulk of studies have found differences between depressed and healthy subjects, all of these differences have been found post hoc and there is, to our knowledge, no study that has examined the specificity and sensitivity of using PET as a potential diagnostic tool. Moreover, the ethical issues involved in injecting a potentially hazardous radioactive substance into patients may preclude its widespread use in clinical diagnosis. There is, nevertheless, some promise in using PET imaging to distinguish between responders and nonresponders to treatment, which may give us more insight into why a certain population responds to certain medications and thus whether certain medications are more suitable for certain populations. In addition, most studies in PET imaging in depression have been performed on serotonergic neurotransmissions, and more research may be needed for dopaminergic, monoamine oxidase, and catecholaminergic transmissions.

Few comparisons can be drawn between the findings of MR spectroscopy and PET because MR spectroscopy and PET have generally been used to examine different compounds. A common compound studied by both, however, is GABA, in which a putative role in the pathophysiology of depression has recently been postulated. Preliminary evidence from both modalities has suggested decreased levels of GABA in subjects with MDD compared with controls, albeit in different brain regions. Decreases in GABA have mainly been reported in the occipital cortex by using MR spectroscopy, while PET found a reduction mainly in the limbic parahippocampal and temporal areas. However, to our knowledge, only a single study has been conducted on GABA with PET on a small sample. Although more studies have been performed using MR spectroscopy on GABA, definitive conclusions about the role of GABA in depression await further research and replication.

If one compared the 2 modalities, a distinct advantage of MR spectroscopy over PET is that MR spectroscopy is noninvasive and requires neither radioactive ligands nor ionizing radiation. On the other hand, MR spectroscopy is, by its very nature, restricted to the study of small metabolites and cannot be used to examine larger compounds that are of most interest in depression, such as serotonin and dopamine.

Limitations and Future Directions

The current state of research in MR spectroscopy and PET imaging shares similar limitations. First, studies for both modalities are generally small, and few are replicated. Second, methodologies of implementation and analysis vary widely across different studies for both modalities, and this variation may contribute to the production of confusing and conflicting results. Uniform or standardized protocols should be established to allow better comparison of studies

Last, one of the major challenges for both modalities is that depression is a clinical diagnosis that has no definitive neurobiologic correlates. It is inherently heterogeneous and multifactorial, involving an array of genetic factors, environmental input, and possible epigenetic interactions, and it involves multiple regions and domains in the brain. Because MR spectroscopy and PET are limited to only examining a single metabolite or radioligand at any single time, respectively, they may be capable only of offering microwindows into the workings of the disorder and may be unable to provide insight into the multitude of interactions that surely underlie the complex nature of the disorder.

In the future, studies involving other technologies (eg, fMRI and tractography) can complement these molecular imaging studies to perhaps provide a more nuanced and comprehensive characterization of the disorder. In addition, while numerous studies have provided evidence for the role of oxidative stress in depression, no researchers have yet used molecular imaging to examine metabolite levels in patients with depression. To our knowledge, only a single study[116] has used proton MR spectroscopy to examine, in vivo, glutathione levels in young people with bipolar disorder, and it found no significant correlations between glutathione (an antioxidant) and clinical scores of depression or mania. The use of molecular imaging for exploring the role of oxidative stress in depression is worth further research.

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