Neuroimaging Approaches to the Understanding of Depression and the Identification of Novel Antidepressants

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Summary

There is a significant medical need for new drug therapies to treat major depressive disorder (MDD). However, the poor predictive validity of preclinical methods available to detect the potential efficacy of novel compounds and a lack of common endpoints between preclinical and clinical measures have proved to be major limitations in drug development for MDD. Neuroimaging studies have provided important insights...
into our understanding of MDD. Many imaging methods, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), can be applied in animal species used for preclinical research, in addition to being widely used in clinical studies. Consequently, neuroimaging approaches are becoming increasingly valuable for drug discovery and development, and the potential translation of preclinical promise to clinical therapeutic benefit.

Neuroimaging methods that have been routinely used to study MDD include MRI, fMRI, magnetic resonance spectroscopy (MRS), PET, electroencephalography, and, more recently, magnetoencephalography. In behavioral and cognitive tasks MDD is associated with negative bias, impaired learning and memory, cognition, blunted reward responsiveness, hypersensitivity to punishment, and impaired social cognition. Imaging studies using MRI, fMRI, MRS, and PET have identified a number of brain regions that are functionally, neurochemically, and structurally abnormal in MDD and which are implicated in mediating these cognitive deficits. Potential biomarkers for MDD that have been identified using different imaging methods include hyperactive amygdala, corticobranch dysfunction, hyperactive subgenual cingulate, and frontal asymmetry.

The prevalent hypothesis during the past 30 years of drug discovery and development for MDD has been the monoamine hypothesis. However, the discovery of the rapid-onset antidepressant properties of the N-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, together with new imaging approaches to drug discovery have directed interest toward the brain glutamate system as a promising target for new treatments for MDD.

1.0. INTRODUCTION

Major depressive disorder (MDD) is a leading cause of disability and produces a greater decrement in health than other common chronic diseases, such as angina, arthritis, asthma, and diabetes. MDD is the most common psychiatric disorder worldwide and is associated with a high level of disability and impairment in the quality of life. Current pharmacological therapies target the brain monoamine systems, but approximately only one-third of patients achieve remission with the first medication prescribed and the slow clinical onset of their antidepressant action means that the detection of response or nonresponse requires at least 4—6 weeks of drug treatment. In addition, many patients suffer from significant side effects and may suffer a relapse during long-term treatment. Hence, there is a significant need for new therapies to treat MDD, but their emergence has been limited at least in part by the poor predictive validity of the preclinical methods available to detect the potential efficacy of novel compounds prior to expensive large-scale clinical trials in patients. The lack of common endpoints between preclinical and clinical measures has also proven to be a major limitation in drug development for MDD.

Experimental or translational medicine models in humans, particularly those that incorporate neuroimaging, may have the potential to overcome some of these difficulties by both increasing our understanding of brain function in MDD and their use in compound efficacy screening to identify improved drug therapies.

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2.0. IMAGING TECHNIQUES

Neuroimaging tools currently being used to understand the pathophysiological mechanisms of MDD can be classified into structural, functional, and chemical methods. Structural imaging methods include the use of structural magnetic resonance imaging (sMRI) and diffusion tensor imaging (DTI). Functional imaging approaches include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), perfusion imaging using arterial spin labeling (ASL) methods, electroencephalography (EEG), and magnetoencephalography (MEG). Chemical imaging methods include magnetic resonance spectroscopy (MRS). A brief introduction to these methods will be provided in this section.

2.1. MRI

In 1971, Damadian showed that the nuclear magnetic relaxation times of tissues and tumors differed, thus motivating scientists to consider nuclear magnetic resonance (NMR) for the detection of disease. A few years later, in 1977, Damadian developed field-focusing NMR and was the first to perform a full human body scan to diagnose cancer. It took almost 5 h to collect a single image. NMR was later renamed MRI because the term nuclear was off-putting for patients. Paul Lauterbur and Peter Mansfield were awarded the Nobel Prize in Physiology or Medicine in 2003 for their discoveries concerning MRI.

An MRI machine uses a powerful magnetic field to align the magnetization of atomic nuclei (mainly hydrogen) in the body and radio frequency fields to alter the alignment of this magnetization systematically. This causes the nuclei to produce a rotating magnetic field detectable by the scanner and this information is recorded to construct an image of the scanned area of the body. Magnetic field gradients cause nuclei at different locations to rotate at different speeds. By using gradients in three different directions three-dimensional (3D) volumes can be obtained. One advantage of an MRI scan is that it is harmless to the patient. It uses strong magnetic fields and nonionizing radiation in the radio frequency range, unlike computed tomography scans and traditional X-rays, which both use ionizing radiation.

sMRI scans are usually $T_1$-weighted scans that differentiate fat from water (water being darker and fat brighter), and this provides information on (ab)normal anatomy. sMRI can be important for detecting the affected structure in any psychiatric disorder, as volumes of specific brain regions can be calculated and gray matter abnormalities determined.

2.2. fMRI

Functional magnetic resonance imaging is an MRI procedure that measures brain activity by detecting changes in blood flow. Since the 1980s, it has been known that changes in blood

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iiFor further detailed discussion of neuroimaging modalities, please refer to Wise in Chapter 1, Neuroimaging Modalities: Description, Comparisons, Strengths, and Weaknesses; Brown in Chapter 2, Magnetic Resonance Imaging as a Tool for Modeling Drug Treatment of CNS Disorders: Strengths and Weaknesses; Novak and Einstein in Chapter 4, Structural Magnetic Resonance Imaging as a Biomarker for the Diagnosis, Progression, and Treatment of Alzheimer Disease; and Schmidt et al. in Chapter 5, Positron Emission Tomography in Alzheimer Disease: Diagnosis and Use as Biomarker Endpoints, in this volume.
flow and oxygenation in the brain (collectively known as hemodynamics) are closely linked to neural activity. When neurons become active at rest or during a task, local blood flow to those brain regions increases and oxygen-rich (oxygenated) blood displaces oxygen-depleted (deoxygenated) blood around 2 s later. This rises to a peak over 4–6 s, before falling back to the original level (and typically undershooting slightly). Oxygen is carried by the hemoglobin molecule in red blood cells. Deoxygenated hemoglobin (dHb) is more magnetic (paramagnetic) than oxygenated hemoglobin (Hb), which is virtually nonmagnetic (diamagnetic). This difference leads to an improved magnetic resonance (MR) signal, since the nonmagnetic blood causes less interference with the magnetic MR signal. This improvement can be mapped to infer which brain regions are active at a particular time.

The seminal fMRI work was first carried out in rodents. Subsequently, in 1992, three groups independently obtained results in humans with the blood oxygenation level dependent (BOLD) mechanism, setting off a flood of fMRI publications that have been appearing in scientific journals ever since. Research over the last decade has established that BOLD contrast depends not only on blood oxygenation but also on cerebral blood flow (CBF) and volume (CBV), a complex response controlled by several parameters. Logothetis and colleagues showed that the BOLD response is more closely related to local synaptic activity than the spiking of single or multiple neurons and that changes in the local field potentials are more closely related to the evolution of the BOLD signal than to changes in the spiking activity of single or multiple neurons.

Given that brain-based endophenotypes may hold relatively greater promise as predictors of disease manifestation and progression, owing to the closer association between such measures and the genetic and environmental causes of psychiatric illness than observable behavior, functional brain imaging is a powerful tool for evaluating potential markers of disease vulnerability.

2.3. PET

PET is a nuclear medicine imaging technique that produces a 3D image of functional processes in the body, by detecting pairs of γ rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. Three-dimensional images of tracer concentrations within the body are then constructed by computer analysis. If the biologically active molecule chosen for PET is fluorodeoxyglucose (FDG), an analogue of glucose, the concentrations of tracer imaged indicates tissue metabolic activity, in terms of regional glucose uptake. This method has been used to measure CBF to the brain regions in MDD.

Radiotracers that are specific ligands for receptor subtypes that have been implicated in MDD have also been developed for PET, such as $^{11}$C-McN 5652 and $^{11}$C-DASB [3-amino-4-(2-dimethylaminomethylphenylsulfonyl)benzonitrile] for serotonin transporters and $^{11}$C-WAY-100635 for serotonin 5-HT$_{1A}$ receptors.

2.4. Electroencephalography

Since the first human electroencephalogram was recorded in 1924 and reported in 1929 by Hans Berger, the German physiologist and psychiatrist, EEG has been used extensively
in the clinical diagnosis of epilepsy and sleep disorders. It has become popular over decades in psychiatric research, specifically into depression. EEG is the recording of spontaneous electrical activity over a short period of time, usually 20–40 min, from multiple electrodes placed on the scalp. EEG measures voltage fluctuations resulting from ionic current flows within the neurons of the brain. As the electrical potentials generated by single neurons are too small to be detected by EEG, EEG activity refers to the summation of the synchronous activity of thousands or millions of neurons that have similar spatial orientations. Voltage fields fall off with the square of distance, and therefore activity from deep sources is more difficult to detect than currents near the skull. EEG measures neuronal electrical activity directly, while other methods record changes in blood flow [e.g. single photon emission computed tomography (SPECT) and fMRI] or metabolic activity [PET and near-infrared spectroscopy (NIRS)], which are indirect measures of the brain’s electrical activity. EEG has higher temporal resolution (milliseconds), but significantly lower spatial resolution, compared to fMRI.

Normal rhythmic EEG activity is divided into bands by frequency. The relative distribution of frequency bands varies with age, and is influenced by the level of alertness, medication, and brain pathology. Beta waves (frequency range, 12–30 Hz) are associated with normal waking consciousness and low beta waves are usually observed with active, busy, or anxious thinking and active concentration; alpha waves (8–12 Hz) emerge during relaxation and when the eyes are closed, and attenuate with eye opening or mental exertion; theta waves (4–7 Hz) are present in drowsiness or meditation; and delta waves (up to 4 Hz) are present in healthy adults during slow-wave sleep. EEG signals are also described in terms of the power of the electrical signal. EEG results are sometimes reported in terms of absolute and relative power. Absolute power is the amount of power in an EEG frequency band at a given electrode, measured in microvolts squared ($\mu V^2$). Relative power is the percentage of power contained in a frequency band in relation to the total power across the entire spectrum. EEG asymmetries represent the differences in EEG activity between the left and right hemispheres.

Initially, most EEG recordings reported were resting state EEGs (spontaneous potentials). A more recently reported version of spontaneous EEG is quantitative electroencephalography (or QEEG), which involves computerized spectral analysis of EEG signals, thus providing information that cannot be extracted through visual inspection of EEG recordings alone.

Simultaneous EEG-fMRI procedures are becoming popular in the study of psychiatric disorders. Their advantage is that high temporal resolution data can be recorded at the same time as high spatial resolution data, thereby enabling the identification of common neuronal generators by removing possible intersession biases and allowing the study of spontaneous brain activity. For example, it has been shown that spontaneous fluctuations of EEG alpha power in a resting state covaried with fluctuations of the BOLD resting state signal. Using single trial amplitude of different event-related potential (ERP) components as predictors of BOLD changes, it is possible to identify corresponding brain regions using fMRI at a timescale close to that of EEG.

2.5. Magnetoencephalography

MEG is a noninvasive method of recording neural activity. It is a neurophysiological technique that records the magnetic sources generated from simultaneous firing of groups of
pyramidal cells. MEG measures neuronal activity directly and thus records real-time activity with millisecond resolution.\textsuperscript{19}

In 1968, Cohen made the first recordings of neural activity with a single magnetometer.\textsuperscript{20} With the introduction of superconducting quantum interference devices (SQUIDs), the sensitivity of MEG has been greatly increased. MEG uses several hundred sensors, making it possible to record the magnetic output with high temporal (< 1 ms) and spatial (1–5 mm) resolution, thus making it an invaluable tool in neurophysiological research.

One of the most exciting areas of research using MEG is the analysis of temporal correlations or coherence. MEG data provide an opportunity to examine the synchrony or coherence of neural oscillations at different frequency bands (alpha, beta, gamma, and theta) within a particular brain region or across regions.\textsuperscript{19} This can be achieved during rest or by evoking a response through the presentation of stimuli at specific time periods. Many psychiatric disorders have been shown to have abnormal synchronicity. MEG signals are thought to arise from postsynaptic current flow in apical dendrites and are proposed to correspond closely to BOLD signals.\textsuperscript{21}

2.6. MRS

MRS is the only currently available imaging technique that allows real-time in vivo quantification of brain metabolites in localized brain regions. Among the several nuclei assessed in MRS examinations, proton ($^1$H-)MRS is the most commonly used in investigations of the neurochemical basis of MDD.\textsuperscript{22} This is due to the high natural abundance of hydrogen protons and their high absolute sensitivity to magnetic manipulation. The types of metabolites that are commonly studied in MDD include choline-containing compounds, creatine, myo-inositol, N-acetylaspartate (NAA), $\gamma$-aminobutyric acid (GABA), and glutamate.

2.6.1. N-Acetylaspartate

NAA is the most prominent resonance (peak), with its major resonance occurring at 2.02 parts per million (ppm). NAA is considered a marker for neuronal and axonal integrity, and is associated with formation and maintenance of myelin. A decrease in NAA levels is a sign of neuronal loss or damage. A gradual and progressive increase in NAA is seen during brain development and maturation in infancy.\textsuperscript{23}

2.6.2. Choline

Choline (Cho) is seen as a peak at 3.2 ppm and is an essential precursor of the neurotransmitter acetylcholine.\textsuperscript{23} It represents the sum of choline-containing compounds such as glycerophosphocholine, phosphatidylcholine, and phosphocholine. Choline therefore represents the constituents of the cell membrane and is a marker for membrane turnover.\textsuperscript{24} The Cho peak has received considerable attention in MDD on the basis of theories of cholinergic hyperactivity in depression.\textsuperscript{25}

2.6.3. Creatine

Creatine (Cr), including phosphocreatine (PCr), is displayed at 3.0 ppm and is a marker for brain energy metabolism. It is stable and commonly used as an internal standard. However, variations in Cr levels do occur, as in the gradual loss of Cr, together with other major metabolites, in tissue death or necrosis.\textsuperscript{23}
2.6.4. Myo-Inositol

Myo-inositol (mI) is a sugar involved in the regulation of neuronal osmolarity. A mI signal at 3.56 ppm represents predominantly myo-inositol with minor contributions (<5%) from glycine and inositol-1-phosphate.23

2.6.5. Glutamine and Glutamate

Glutamine (Gln) and glutamate (Glu) are detectable as multiple resonances between 2.2 and 2.4 ppm when using a short echo time (TE). The identification of glutamate using MRS is technically challenging, as the Glu spectrum overlaps with a number of other neurochemicals, primarily glutamine. Stronger magnetic fields and advanced imaging techniques can enable isolation of the glutamate signal; however, early MRS studies reported combined Glu and Gln peaks as glutamix (Glx).26 The first clinical report of MRS being used to examine Glx in depression was of a cancer patient who had recurrent suicidal ideation and depressive symptoms with chemotherapy. In this individual, Glx was reduced in cerebral white matter.27

2.6.6. GABA

GABA is a ubiquitous inhibitory neurotransmitter found almost exclusively in the central nervous system (CNS), with concentrations at least 1000 times greater than that of monoamines. It regulates neuronal excitability through inhibitory feedback loops and controls muscle tone peripherally. Extensive clinical and preclinical investigations indicate that the amygdala, hippocampus, hypothalamus, midbrain, prefrontal cortex (PFC), and tectum are rich in GABAergic neurons.28 GABAergic interneurons selectively attenuate the firing of other neurons in the cortex through cortical inhibition. Cortical inhibition has several important physiological functions including learning, memory, and sensory gating. Sensory gating is the inhibition that reduces aberrant neuronal firing, filters spurious information, and improves the signal-to-noise ratio. Levesque and colleagues proposed that this latter function most likely extends to the regulation of mood and cognition in depression.29 For example, MDD is characterized by excessive negative thinking that is perceived as intrusive and out of one’s control. Dysfunction of cortical inhibition may result in inadequate filtering of ruminative thoughts over time and thereby contribute to the onset or perpetuation of MDD.29

2.7. Arterial Spin Labeling

ASL is a noninvasive procedure for quantifying CBF by measuring perfusion, in which arterial blood is magnetically labeled as an endogenous perfusion tracer. ASL is based on the subtraction of two consecutively acquired images. The first image is usually acquired after inversion of the arterial blood magnetization upstream of the region of interest (ROI). The second image is acquired without any manipulation of the arterial magnetization, and the subtraction of both images provides information about the degree of perfusion. While measurements of perfusion are of direct diagnostic value in vascular disorders, perfusion measurements also serve as biomarkers for a broader range of physiological and pathophysiological functions.
Published comparisons between ASL and PET in healthy volunteers demonstrate a close correlation both at rest\textsuperscript{30} and with task-related activation.\textsuperscript{31} Only a few studies have used ASL to study depression.\textsuperscript{32–35}

ASL can be combined with any imaging sequence and theoretically provides a flow image that is completely independent of scanning parameters. ASL perfusion MRI can be used to localize task activation in a manner similar to BOLD fMRI.\textsuperscript{36} Indeed, ASL-based contrast derived from inversion recovery imaging of task activation was included in one of the earliest reports of fMRI in the human brain.\textsuperscript{12} ASL perfusion MRI can also be used as a measure of brain function at rest, independent of any sensorimotor or cognitive task, and can reveal regional changes in brain function associated with development, behavioral states, or genetic traits.\textsuperscript{35} ASL measures a purely biological parameter, and may therefore be particularly valuable for multicenter studies examining brain function on a variety of scanner platforms or longitudinally.

2.8. Diffusion Tensor Imaging

DTI is a technique that quantifies the degree of diffusion (free or Brownian motion) of the water molecules in the brain. This motion encounters different barriers in the body (cell membranes, fibers, macromolecules, and proteins), which vary according to the location (intracellular or extracellular) and certain pathological modifications (abscess, intracellular edema, or tumors). Diffusion data provide indirect information about the structure surrounding the water molecules. Myelinated fibers restrict the diffusion of water to the axis of the fiber bundle, resulting in visualization of white matter tracts. Beyond conventional MRI, DTI can provide additional information on axonal integrity and bundle coherence, thus estimating the structural efficiency of neural pathways.\textsuperscript{e.g.37} The metrics commonly used in DTI studies include fractional anisotropy (FA) and mean diffusivity (MD). High axonal integrity and resultant limitation of water diffusion in white matter is associated with high FA values and low MD values, as in neurons with axons running parallel with concentric layers of the myelin sheath. Lowest FA values are found during free diffusion where water molecules displace freely in all directions.\textsuperscript{38} Using these coefficients, direction of a particular diffusion can be calculated, thereby tracing the neuronal trajectory.

On the other hand, diffusion weighted (DW)-MRI aims at highlighting the differences in water molecule mobility, irrespective of their direction of displacement. Apparent diffusion coefficient (ADC), derived from DW-MRI is commonly used as an assessment of injury and axonal integrity.\textsuperscript{37}

3.0. CHARACTERIZATION OF DISEASE STATE AND PROGRESSION

3.1. Structural Changes

3.1.1. Volumetric Measurements

In early studies, neuroanatomical abnormalities using T1 images were measured using standard ROI methods, requiring investigators manually to identify a priori ROIs to quantify a volume of interest for group comparisons. More recently, voxel-based morphometry
(VBM), a whole brain semiautomatic technique, has been developed. VBM is used to perform voxel-wise comparison of the local concentration of gray matter between groups. This technique overcomes the important drawback of regional bias of standard ROI methods. Results from studies using both methods are described below.

3.1.1.1. TEMPORAL LOBES

Studies of total temporal volume changes in MDD have produced inconsistent results. One study reported smaller left temporal volumes in MDD patients, but no other studies reported any significant changes in total temporal volume (collapsed across hemispheres) and/or any laterality effect in unipolar MDD compared with healthy controls. However, the patient sample studied in a later study by Vythilingam and colleagues had the longest illness duration compared to the other studies. In this regard, it is possible that left-lateralized temporal lobe changes may reflect either progression of the disorder over time or a distinct pathophysiological process that affects risk or relapse.

3.1.1.2. HIPPOCAMPUS

The hippocampus is the most extensively studied region in MDD, and the resulting findings, albeit not homogeneous, seem to suggest that hippocampal volume reductions are associated with MDD. While reduced hippocampal volume differences have been the most frequent finding, some groups have reported no difference between patients and controls and tendencies toward volumetric enlargements have also been reported. Many studies have reported smaller hippocampal volumes in patients suffering multiple depressive episodes rather than in patients in remission or experiencing their first episode. This could suggest that volumetric reduction of the hippocampus may be associated with repeated depressive episodes. However, the extent of volume reduction in the hippocampus has been shown not to be influenced by the severity and/or length of illness.

Using VBM analysis, two studies reported decreased gray matter in the hippocampus, although two other studies reported no differences.

3.1.1.3. AMYGDALA

It has been reported that amygdala size may also vary in relation to illness duration, while age at onset of illness does not seem to have a major effect. Indeed, while unipolar patients earlier in the course of illness tend to have increased amygdala volume, depressed patients with a longer illness duration and a greater number of MDD episodes tend to show volumetric reductions. This effect is specifically observed in female patients.

In contrast, a meta-analysis of 23 VBM studies reported decreased gray matter volume in the amygdala in first-episode patients compared with chronic patients and controls.

3.1.1.4. FRONTAL LOBES

There are consistent reports of a volumetric reduction of the entire frontal lobe and/or the orbitofrontal cortex (OFC) in more severe MDD patients, but not in less severely ill patients. Interestingly, Lacerda and colleagues reported an inverse correlation between age and left lateral OFC volume in unipolar patients but not in healthy controls, suggesting that MDD duration may progressively affect the volume of the left lateral OFC. Other
studies have reported nonsignificant correlations between clinical variables and the volumes of frontal structures. However, studies using VBM methods have reported gray matter reductions in the OFC in medication-free MDD patients.

3.1.1.5. ANTERIOR CINGULATE CORTEX

Several volumetric studies have investigated whether depression may be related to alterations in the anterior cingulate cortex (ACC), particularly the subgenual cingulate cortex (sgACC), but have reported contradictory findings. This may be due to both the different clinical and demographic features of the patient samples and the different methods used to classify ACC subregions.

No volumetric sgACC alterations have been found in less severely depressed remitted patients, an exception being a study by Monkul and coworkers. In contrast, a decreased volume of the cingulate gyrus (excluding the subgenual area) has been found in currently ill patients, compared with remitted patients and healthy controls, although this change did not appear to correlate with any clinical variable.

In contrast, gray matter reduction in the rostral cingulate cortex in patients with MDD relative to healthy controls has been reported in a number of studies and longer illness duration has been associated with greater gray matter reduction. Gray matter reduction in the OFC and sgACC was reported to correlate with recent stressful life events in controls, suggesting that increasing cumulative exposure to adverse life events is associated with smaller gray matter volume in key prefrontal and limbic regions involved in stress, emotion, reward regulation, and impulse control.

3.1.1.6. BASAL GANGLIA

Studies using an ROI approach have generally failed to detect changes in basal ganglia volume. Volumetric studies have reported inconsistent results, with some studies reporting smaller caudate volumes in depressed patients and others reporting no differences.

However, reductions in gray matter in the caudate and putamen were reported in patients with treatment-resistant depression compared to recovered patients and controls. These studies suggest that the caudate nucleus and putamen may be impaired in more severe subtypes of depression.

3.1.1.7. THALAMUS

Gray matter reductions have been reported in the thalamus.

3.1.1.8. SUMMARY OF VOLUMETRIC RESULTS

A family history of mood disorder has been hypothesized to play a critical role in volumetric changes of the ACC, particularly the sgACC, although the structural findings of familial unipolar patients are contradictory. There are considerable variations in terms of the demographic characteristics of patients, the imaging protocols used, and analysis and clinical factors in the published studies, which could explain the variability in findings. However, in summary, significant volumetric alterations have been reported in the amygdala, basal ganglia, hippocampus, OFC, sgACC, and thalamus in MDD.
3.1.2. White Matter Abnormalities

It has been hypothesized that microstructural changes in the white matter of frontal-subcortical circuits leads to a disconnection syndrome between frontal and subcortical regions.\textsuperscript{84–86} Abnormalities in the white matter of subjects with affective disorders are usually measured using sMRI (white matter hyperintensities) in elderly patients and DTI in younger populations. White matter hyperintensities have been reported in older MDD patients.\textsuperscript{87–90} White matter abnormalities have been associated with poor treatment outcomes,\textsuperscript{91} physical disability,\textsuperscript{92} and cognitive impairment.\textsuperscript{89,93,94}

A number of studies have reported lower FA values in the medial frontal gyrus,\textsuperscript{84,95,96} parietal and occipitotemporal gyrus,\textsuperscript{95} inferior parietal portion of the superior longitudinal fasciculus,\textsuperscript{97,98} anterior limb of the internal capsule,\textsuperscript{98,99} uncinate fasciculus,\textsuperscript{100} cingulate gyri,\textsuperscript{96,99–101} dorsolateral prefrontal cortex (DLPFC),\textsuperscript{101,102} right hippocampal gyrus,\textsuperscript{89} and left striatum.\textsuperscript{103}

Studies of family history report contradictory results. One study reported increased FA values in the splenium of the cingulate cortex,\textsuperscript{104} whereas another study reported decreased FA values in the same region\textsuperscript{105} in first-degree relatives of MDD patients.

Within the DLPFC circuit, the anterior thalamic radiation connects the PFC to the thalamus through the anterior limb of the internal capsule. The uncinate fasciculus provides connections between the frontal and temporal lobes. The findings summarized above provide support for the theory that white matter abnormalities may play a role in a disconnection syndrome between frontal and subcortical regions and may contribute as a risk factor for affective disorders.

3.2. Functional Changes

3.2.1. Depressed Mood and Negative Bias

The role of negative biases in information processing in the etiology and maintenance of depressive disorders has long been hypothesized.\textsuperscript{106} Early theories suggested that these biases affect all aspects of information processing, with depressed patients showing enhanced attention, interpretation, and memory for all negative emotional material.\textsuperscript{106} More recent evidence indicates that cognitive processes are not uniformly biased in depression and that the distinctions between implicit and explicit aspects of performance, attentional engagement and disengagement, and perceptual and conceptual levels of processing are relevant.\textsuperscript{107}

The lack of consistent evidence for biased attentional processing in depression has led some researchers to hypothesize that depression is characterized by memory but not attentional bias, whereas anxiety is characterized by attentional but not memory bias.\textsuperscript{108–110} It has also been proposed that there is little evidence for subliminal attentional bias in depression, and that an attentional bias in depression is typically only found when the material is self-referent and is presented for long (> 1000 ms) durations, which may reflect difficulty in disengaging attention from negative emotional information.\textsuperscript{109,111} Measures of facial expression recognition have typically revealed a bias toward labeling ambiguous facial expressions as negative and/or the perception of positive cues of happiness being reduced in patients with depression.\textsuperscript{112} However, there is evidence to support consistently enhanced selective
memory for negative material particularly seen in explicit memory paradigms.\textsuperscript{113,114} For example, if patients with MDD are asked to recall positive and negative self-descriptors encoded in a classification task, they show a tendency to remember negative rather than positive words.

fMRI studies provide converging evidence for the role of limbic circuitry in negative affective processing biases in MDD.\textsuperscript{115} Thus, for example, in implicit face processing paradigms, depression is associated with exaggerated responses in the amygdala, ventral striatum, and insula to negative (sad or fearful) expressions of emotion,\textsuperscript{116–120} whereas responses to happy facial expressions in the amygdala, hippocampus, putamen, and thalamus appear to be reduced.\textsuperscript{121–123} Although these subcortical areas are usually regarded as important in the initial evaluation of emotion, some of the observed effects may underpin attentional bias. The visual mechanisms for increasing attention to salient and important stimuli are thought to be modulated by signals from both amygdala and frontoparietal circuitry.\textsuperscript{124} Siegle and colleagues\textsuperscript{125} found that amygdala responses to negative words were no longer visible after 10 s in healthy controls but persisted in depressed patients for a mean of 25 s. Increased activity of the amygdala is also seen in conjunction with the expectation of a negative stimulus. Medicated MDD patients cued to anticipate the arrival of disgusting pictures displayed greater BOLD activation in some regions, including the dorsal amygdala and sublenticular nucleus, compared with healthy subjects.\textsuperscript{126} In contrast, few studies have reported negative results. One study reported that a group of medicated MDD subjects were found not to differ from healthy control subjects when presented with sad or fearful faces.\textsuperscript{122}

Alternatively, it has been suggested that the negative bias observed in depression reflects impaired top-down cognitive control\textsuperscript{127,128} linked to reduced activity in cortical regions, including anterior cingulate cortex, DLPFC, and rostral ACC (rACC).\textsuperscript{129–132} It has been proposed that such reduced activity of a top-down system allows unrestrained activation in emotional regions of the brain. Hence, the subcortical regions, unchecked by cognitive control, are thought to reinforce cognitive biases, leading to increased awareness of negative stimuli, which in turn perpetuates depression.\textsuperscript{133} In neuroimaging studies, depressed patients show consistent amygdala hyperactivity in response to negative emotional stimuli,\textsuperscript{125} often combined with reduced responses in areas such as the DLPFC involved in the effortful regulation of affective states.\textsuperscript{120,134,135} A similar pattern has been reported in CBF studies using PET.\textsuperscript{136} In contrast, Dichter and colleagues observed increased DLPFC activity when attending to sad faces.\textsuperscript{137} Similar results were reported during an emotion-interference task: depressed patients showed hyperactivity in the amygdala, DLPFC, and dorsal ACC when attending to happy faces.\textsuperscript{138} Dichter and colleagues proposed that relatively greater prefrontal brain activation was required to disengage from the sad images to respond to the target events.\textsuperscript{137}

In contrast, reduced amygdala activation in response to positive stimuli has been demonstrated in a small number of studies.\textsuperscript{122,139} Increased DLPFC activity to positive versus negative words in both a Stroop and an emotional categorization task was observed in dysphoric participants relative to controls.\textsuperscript{120} Reciprocal connections between the prefrontal cortex and limbic structures when processing negative and positive stimuli may underlie the hyposensitivity observed in the amygdala to positive stimuli. Thus, increased control exerted by cortical regions on the response of limbic structures to positive stimuli might result in anhedonia, a core symptom of depression. On the other hand, decreased control exerted by
cortical regions on the response of limbic structures to negative stimuli may be a cause of the negative bias observed in depression.

Hence, coordinated responses to affective stimuli may provide a neural assay of emotional bias or the salience level of affective stimuli, and help us to understand the neural mechanisms underlying behavioral biases. Such biases may be driven by enhanced negative evaluation within limbic areas coupled with deficient higher-order emotional modulation of cognitive processes within areas such as the prefrontal cortex.\textsuperscript{140}

There is growing evidence that such biases may be present outside episodes of major depression and could represent trait vulnerability markers. For example, there have been reports of increased negative versus positive emotional processing in healthy volunteers at a high risk of developing depression\textsuperscript{141–143} or with a history of major depression.\textsuperscript{144} Although some biases appear to be resolved or absent in high-risk volunteers outside a depressive episode,\textsuperscript{145} they can be triggered following an induction of negative mood.\textsuperscript{146} Thus, rather than being a simple symptom of depression, processing biases may be latent vulnerability mechanisms that can be readily triggered or exaggerated by decreases in mood or lowered serotonin function.

3.2.2. Anhedonia and Hypersensitivity to Negative Feedback

MDD has been associated with a hyposensitive or blunted response to reward (anhedonia) and hypersensitivity to punishment.\textsuperscript{147}

3.2.2.1. HYPERSENSITIVITY TO PUNISHMENT

The hypothesis predicted by models of learned helplessness,\textsuperscript{148} i.e. that patients with MDD manifest an abnormal response to negative feedback, is consistent with findings that depressed patients respond catastrophically to error feedback on memory or planning tasks. Elliott and colleagues demonstrated that a depressed group was not simply worse at planning than controls, regardless of difficulty, as both groups solved the same number of problems. However, if MDD patients made an error on a trial, their performance deteriorated rapidly, which was termed a catastrophic response to perceived failure.\textsuperscript{106} This deficit was shown to correlate with severity of depression\textsuperscript{149} and to be specific to depression.\textsuperscript{150} It has been shown that similar deficits are also evident in remitted depressed patients,\textsuperscript{151} suggesting that abnormal reactions to negative feedback may extend to individuals with increased risk of depression, even in the absence of symptoms. A catastrophic response to negative feedback may be due to perceived failure triggering further failure-related thoughts, thereby interfering with subsequent performance.\textsuperscript{106} Thus, patients with MDD could be hypersensitive to punishment. Alternatively, depressed patients may be hyposensitive to punishment by failing to use negative feedback to improve performance.\textsuperscript{150,152} Holmes and Pizzagalli\textsuperscript{153} found that participants with high scores on the Beck Depression Inventory were significantly less likely to adjust their responses after errors than participants with low scores on the inventory. Such a failure in posterror performance adjustment could reflect underlying deficits in motivation or performance monitoring, or a generally blunted response to reinforcement rather than hypersensitivity to punishment.

Murphy and colleagues\textsuperscript{154} found that MDD patients performed as well as controls after accurate negative feedback, but were more sensitive to misleading negative feedback. This
may be interpreted as a tendency to exaggerate the importance of uncertain or misleading information that could lead to a perceived lack of control. In turn, this could bias future actions and cause a cycle of learned helplessness. In summary, depression seems to be characterized by maladaptive responses to negative feedback by various mechanisms, including failure to adapt, reduced sensitivity to punishment, and/or learned helplessness.

3.2.2.2. HYPOSENSITIVITY TO REWARD

Anhedonia, the inability to experience pleasure, is one of the core symptoms of depression. Willner postulated that a functional impairment of the mesolimbic dopamine (DA) pathway underlies the MDD symptoms of anhedonia and loss of motivation. This hypothesis is consistent with findings showing that euphoria is correlated with amphetamine-induced DA release in the human ventral striatum and that CBF differences between depressed patients and controls have been identified in regions innervated by the mesolimbic DA pathway, including the ACC, amygdala, striatum, and prefrontal cortex.

Using a dopaminergic probe consisting of the oral administration of d-amphetamine Tremblay and colleagues showed that the severity of depression was highly correlated with the rewarding effects of d-amphetamine in a group of unmedicated depressed patients, and that MDD subjects with severe symptoms reported significantly greater rewarding effects than controls. These results provide evidence for hypersensitivity of the brain reward system in MDD that could be related to a DA hypofunction.

McFarland and Klein reported that currently depressed subjects, but not remitted depressed subjects, have a diminished responsiveness to anticipated reward but not to anticipated punishment. An fMRI study investigating the neural responses to monetary incentives reported that unmedicated MDD patients and controls did not differ in their behavioral responses or in nucleus accumbens activation. However, MDD patients showed increased activity of the ACC in anticipation of increasing monetary gains, whereas the ACC was activated in anticipation of increasing losses in controls. Knutson and colleagues interpret these results as supporting the presence of increased conflict during anticipation of gains and a reduced ability to discriminate gain from nongain outcomes in MDD patients. Similarly, Forbes and colleagues reported reduced striatal activation in depressed adolescents during reward anticipation and outcome. In addition, using a monetary incentive delay task, Pizzagalli and coworkers reported reduced putamen activation during reward anticipation and reduced nucleus accumbens and caudate activation during receipt of reward in unmedicated MDD patients.

Smoksi and colleagues investigated whether patients with MDD demonstrated hyporesponsivity in striatal brain regions and/or hyperresponsivity in cortical brain regions involved in conflict monitoring using a Wheel of Fortune task designed to probe responses during reward selection, reward anticipation, and reward feedback. The MDD group was characterized by reduced activation of striatal regions during reward selection, anticipation, and feedback; by hyperresponsivity in OFC during reward selection; and by decreased activation of the middle frontal gyrus and the ACC during reward selection and anticipation.

Epstein and colleagues observed decreased activity in the ventral striatum in depressed patients in response to positive stimuli compared with controls. Similarly,
Surguladze and colleagues found a linear response to expressions of increasing happiness in the right putamen in healthy controls but not in depressed patients. However, a linear response to expressions of increasing sadness in the left putamen was observed in depressed patients.

It is well established that DA neurons code a specific phasic (short duration) reward-learning signal, described by temporal difference theory. A study by Kumar and colleagues examined whether patients with MDD have blunted temporal difference reward-learning signals and if the extent of alteration in temporal difference signals in major depression correlates with illness severity ratings. Their results showed that long-term-medicated MDD patients exhibit a reduced reaction to reward-learning signals in the ventral striatum and ACC and the magnitude of the abnormal signals in MDD correlates with illness severity ratings.

Preliminary results from an fMRI study of appetitive conditioning showed that dysfunctional learning in both appetitive and aversive learning conditions is associated with a pattern of dysfunction in the ACC, amygdala, lateral OFC, and striatum in unmedicated MDD patients.

Finally, a study of participants who had recovered from MDD, and therefore were no longer influenced by mood state or current medication usage, revealed reduced activation to pleasant taste and picture stimuli in reward areas such as ventral striatum compared to healthy controls. These findings indicate that blunted ventral striatal responses during reward are state-independent and may represent a potential endophenotype of MDD.

### 3.2.3. Impaired Learning and Memory

In addition to negative bias in attention and memory, patients with MDD are reported to have impaired working memory. Some authors suggest that working memory impairments in MDD are due to persistent deficits in selective attention. In contrast, deficits in long-term storage and retrieval of declarative memory in MDD have been reported to be influenced by the number of depressive episodes, hippocampal volume reduction, hypercortisolism, and stress, suggesting that these symptoms are a consequence rather than an etiological factor in depression. Rose and colleagues showed that cognitive load increased rACC activity in MDD using an n-back working memory task. This hyperactivity was proposed to be a possible trait marker as it has been reported to persist after clinical recovery.

### 3.2.4. Impaired Executive Function

Impairments in executive function in depressed subjects refer to abnormalities in cognitive behaviors that control and integrate neural activities, including selection strategies, planning, and monitoring performance. These impairments are not specific to MDD and usually recover to normal levels during remission. However, response speed has been found to be unrelated to concurrent depressive symptoms and to remain impaired in recovered depressed patients who are off medication (state independence). Specifically, inspection time, a measure of the speed of information processing that does not require a speeded motor response, has been found to be slower in subjects with unipolar major depression than in age-, IQ-, and sex-matched controls, independent of current mood.
3.2.5. Impaired Social Cognition

Social cognition refers to the ability to interpret and predict the behavior of others in terms of their beliefs and intentions, and to interact in complex social environments.iii Social cognition encompasses facial perception, emotional information processing (including both perception of emotional information in the environment and regulation of mood), theory of mind (understanding others’ beliefs and intentions), self-reference, and working memory.177 Brain regions that are involved in social cognition include the ACC, cingulate, amygdala, fusiform gyrus, OFC, and PFC.177 These same brain regions are reported to be functionally and/or structurally abnormal in MDD patients.178 A significant clinical feature of MDD is often a profound impairment in social functioning. Patients have been reported to exhibit reduced ‘social competence’,179 fewer social interactions,180 reduced awareness of others’ emotions,181 and to have reduced reward value associated with social interaction.182 These negative interpersonal experiences appear to cause depressed individuals to isolate themselves, thus perhaps perpetuating their depressive state.183

3.2.5.1. FACIAL EMOTION PROCESSING

Given their ubiquitous nature, the ability to recognize facial expressions is crucial for intact interpersonal functioning.184 Studies that have examined facial emotion processing in acutely depressed patients have reported a generalized emotion recognition deficit185–194 and impaired recognition of happy facial expressions, relative to matched controls.190,192,195–200 Enhanced recognition of sad facial expressions has also been consistently reported in acutely depressed patients.201–204 Other studies have reported evidence of a negative bias during facial expression recognition and detection tasks200,205–211 including a tendency to identify neutral faces as sad in patients with moderate to severe depressive symptoms, compared with healthy controls.212,213 This bias is accompanied by selective attention to negatively valenced faces depicting sadness122,146,195,211 and anger.214 Overall, these studies point toward a processing bias involving enhanced attention to and recognition of negatively valenced faces during active states of depression that may be accompanied by a tendency to mislabel positively valenced faces as sad and to misjudge (i.e. amplify) the degree of negative emotion conveyed in facial expressions.

Evidence from fMRI studies suggests that patients with acute MDD demonstrate increased activation in the amygdala, OFCs, and ventral striatum to masked118,215,216 and unmasked117,119,217–220 displays of negatively valenced faces (e.g. expressions of disgust, fear, or sadness). However, conflicting findings exist,119,219 which may stem from the use of different emotional processing paradigms and from differences in the clinical status of patients in terms of comorbidity, depression severity, illness burden, and medication.

The functional connectivity between prefrontal and subcortical regions in patients with MDD has been examined. Specifically, during negative facial processing tests (implicit and explicit) consisting of angry and sad facial expressions, the dorsal anterior cingulate and

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iiiFor further discussion of impaired social cognition in psychiatric disorders, please refer to Westphal et al. in Chapter 8, Functional Magnetic Resonance Imaging as a Biomarker for the Diagnosis, Progression, and Treatment of Autistic Spectrum Disorders, in this volume.
the precuneus, a region implicated in self-related mental representations, have been shown to have reduced connectivity with the OFC in unmedicated patients with MDD. Decreased connectivity between the dorsal anterior cingulate and OFC may contribute to dysfunction in the cognitive control of emotional processing. In addition, decreased connectivity between the precuneus and the OFC may contribute to the disturbances in self-referential processes that occur in MDD patients. Further, functional connectivity between the OFC and the DLPFC was increased in patients compared with controls during negative facial processing and this may give rise to the negative processing bias inherent in the disorder. Similarly, it has been reported that a chronic and recurrent course of illness is associated with reduced functional connectivity between the amygdala and DLPFC while passively viewing angry and sad faces and is associated with illness severity, indicating that MDD patients with reduced connectivity between these regions have a more pervasive and severe course of illness. In addition, disruptions in functional coupling between the amygdala and subgenual cingulate, a region also implicated in assessing the salience of emotion and regulation of emotions, have been reported during facial processing tasks.

Studies have also examined the relationship between patterns of neural activation in response to emotional facial expressions and mood state. For example, depression severity has been shown to negatively correlate with the extent of activation in the fusiform gyrus, ACC, and amygdala. Similarly, subgenual cingulate and visual cortical responses to sad but not happy facial stimuli correlated with changes in symptoms during antidepressant therapy. However, a significant number of other studies failed to find a significant association between the level of depression and neural activity in response to facial emotions. It is possible that limited sample sizes and the inclusion of patients with varying levels of depression may contribute to these contradictory findings.

3.2.5.2. THEORY OF MIND

Theoretical models propose that the theory of mind draws on both cognitive (e.g. understanding another’s perspective) and affective (e.g. an emotional response to the feelings of others) processing resources.

Neuroimaging and behavioral studies of theory of mind implicate a core network of neural regions that include cognitive (e.g. DLPFC), affective (e.g. mPFC and anterior paracingulate cortex), and memory systems (e.g. posterior cingulate and temporal poles). Moreover, neuroimaging evidence also implicates the posterior superior temporal sulcus, which is involved in biological motion perception including socially relevant directional cues such as the eye gaze of others, and the adjacent temporoparietal junction, which is involved in the attribution of beliefs to others and is thought to be critical for theory of mind ability.

Research into impaired theory of mind in patients with mood disorders is starting to gain attention after findings that impaired theory of mind ability may be associated with a poor clinical and functional outcome in these patients. A small number of studies conducted in actively depressed patients suggest that patients show impairments on theory of mind tasks that involve decoding mental states from available information, such as facial expressions, tone of voice, gestures, and reasoning (but for contradictory findings, see ), and tasks that involve reasoning about mental states by combining contextual information and prior knowledge of an individual or situation to understand behavior.
To date, studies examining the neural correlates of theory of mind processing in patients with mood disorders have been confined to investigations of patients with bipolar disorder. As brain regions involved in theory of mind are reported to be structurally and/or functionally abnormal in depression, it appears likely that impairments in theory of mind ability may be apparent in MDD patients.

3.3. Resting State Abnormalities

“What does the brain do, when not engaging in a task and what does the brain do at “rest”? These are two of the questions that have intrigued neuroscientists since Marcus Raichle first coined the term default mode in relation to resting state brain function.

The concept of a default mode network arises from an emerging body of evidence demonstrating a consistent pattern of deactivation across a network of brain regions, including the precuneus/posterior cingulate, medial PFC, and medial, lateral, and inferior parietal cortex, that occurs during the initiation of task-related activity. Although deactivated during task performance, this network is active in the resting brain with a high degree of functional connectivity between regions. This network was termed the default mode of brain activity to denote a state in which an individual is awake and alert, but not actively involved in an attention-demanding goal-oriented task. The more demanding the task, the stronger is the deactivation of the default mode network. Interestingly, brain energy utilization has been shown to be only slightly greater in the active than the resting brain.

The issue of understanding how different brain regions are connected functionally at rest and during a task has become a vital question in neuroscience. Different neuroimaging modalities (EEG, fMRI, and PET) can be used to assess neural activity at rest and to compare healthy individuals and patients with MDD.

3.3.1. fMRI

The resting state of the brain has been widely investigated using fMRI. Functional connectivity refers to the temporal correlation between fluctuations in the BOLD signal of discrete anatomical regions. More generally, functional connectivity between two brain regions is considered in terms of the temporal coherence or correlation between the oscillatory firing rates of neuronal assemblies. Assessment of functional connectivity can be achieved through a number of methods, two of which, the ROI seed-based correlation approach and independent component analysis (ICA), are most commonly used. The ROI approach uses regression or correlation analyses to examine temporal coherence between a selected voxel and the time-series of all other voxels in the brain. Unlike seed-based ROI approaches, ICA is a model-free approach and is not bounded by a priori predictions. ICA decomposes data into maximally independent components (temporal or spatial), representing the characteristic time and spatial signatures of the sources underlying the recorded mixed signals.

Anand and colleagues investigated differences in corticolimbic activity and connectivity between depressed patients and healthy controls. Depressed patients had increased activation of cortical and limbic regions. Decreased connectivity was observed between the rACC and amygdala, the rACC and dorsomedial thalamus, and between the rACC, precuneus, and caudate in depressed patients compared to healthy subjects. A further study...
reported that the DLPFC was decoupled from the hippocampus, rACC, and sgACC, which may indicate reduced connectivity.\textsuperscript{246} Interestingly, connectivity between the rACC and sgACC was reported to increase in response to deep brain stimulation.\textsuperscript{240} In another study, the connectivity patterns of three seed regions, the DLPFC, precuneus, and rACC, were reported to converge in the region including the sgACC with some extension into an area of dorsomedial prefrontal cortex (DMPFC). The authors described this region as the ‘dorsal nexus’ and reported increased connectivity between the dorsal nexus and the rostral and posterior cingulate, and ventral medial and dorsolateral PFC in MDD patients compared with controls.\textsuperscript{247} Increased connectivity was also reported between the DLPFC, medial OFC, and rACC,\textsuperscript{248} and between the rACC and thalamus.\textsuperscript{249} Increased connectivity between the hippocampus and the rACC leading to the mPFC was interpreted as increased excitation within limbic/paralimbic regions, whereas decreased connectivity to and from the DLPFC was interpreted as increased neural inhibition in the lateral PFC.\textsuperscript{250} Increased resting state connectivity between the rACC and left anterior insula was found to be predicted by the concentration of glutamate in rACC.\textsuperscript{249} Greicius and colleagues reported that the subgenual cingulate disproportionately contributed to the connectivity of the default mode network in MDD patients, with increases in connectivity associated with depression refractoriness, or the duration of the current depressive episode.\textsuperscript{240} There was also increased connectivity in the thalamus during rest. It was proposed that increased connectivity in affective regions may detrimentally affect connectivity in regions associated with cognitive processing such as the dorsal ACC.\textsuperscript{240}

### 3.3.2. Electroencephalography

Both currently depressed patients and patients with lifelong depression were reported to have decreased frontal activity and increased frontal alpha power, measured by quantitative EEG.\textsuperscript{251–253} This suggests that frontal asymmetry is an endophenotype for depression. It has also been reported that increased cognitive vulnerability to depression was associated with a reduction in left frontal activity. After 3 years, both cognitive vulnerability and frontal asymmetry predicted the onset of the first episode of depression.\textsuperscript{254}

The hypothesis of a default asymmetric mode of depressed patients is based mainly upon the finding of a relative decrease in neural activity. One possible way to investigate this hypo-activation in the left frontal cortex is to measure the correlation between signals of brain activity collected from different cortical regions\textsuperscript{255} using partial directed coherence (PDC) analysis.\textsuperscript{256} This method is of particular interest because of its ability to distinguish direct and indirect causal influences regardless of any common extraneous influences or sources.\textsuperscript{257} PDC analysis therefore offers an opportunity to analyze quantitatively and compare the functional connectivity in the brain of depressed patients. Using this approach, Sun and colleagues\textsuperscript{255} have reported that depression is characterized by a hemispheric asymmetry syndrome.

### 3.3.3. Perfusion Arterial Spin Labeling

Few studies have investigated perfusion abnormalities in depression using ASL. One study reported significant hyperperfusion in the subgenual cingulate in chronic and treatment-resistant MDD patients.\textsuperscript{35} In addition, Clark and colleagues\textsuperscript{33} reported that an increase in baseline perfusion in the sgACC predicted treatment response to partial sleep deprivation.
and was reduced after treatment. In addition, a study on late-life depression reported an increase in normalized white matter CBF.258

### 3.3.4. PET

Hypermetabolism has been reported in the sgACC in MDD and this was shown to correlate with illness severity.259 However, there is some evidence for the reverse relationship.83 Using FDG-PET, Suwa and colleagues reported that, in patients with drug treatment-resistant depression and bipolar disorder, hypometabolism in the superior frontal gyrus and hypermetabolism in the inferior temporal gyri, compared to controls, predicted response to electroconvulsive therapy (ECT).260

### 3.3.5. Receptor Binding

#### 3.3.5.1. SEROTONIN 5-HT\textsubscript{1A} RECEPTOR SIGNALING ABNORMALITIES IN MAJOR DEPRESSIVE DISORDER

Decreased 5-HT\textsubscript{1A} receptor binding has been consistently reported in multiple brain areas of patients with MDD.261,262 The 5-HT\textsubscript{1A} receptor is a G protein-coupled receptor concentrated in regions that receive serotonergic input from the raphe nuclei such as the frontal cortex, amygdala, hippocampus, and hypothalamus.263,264 The 5-HT\textsubscript{1A} receptor serves predominantly as an autoreceptor controlling serotonin release and synthesis in the raphe nuclei, thus reducing serotonergic transmission to its projection areas,265 and as a postsynaptic receptor in the frontal and limbic projection regions.266

PET data are largely suggestive of reduced 5-HT\textsubscript{1A} receptor binding in MDD.267 In a \textsuperscript{11}C-WAY-100635 (a selective 5-HT\textsubscript{1A} receptor antagonist ligand) PET study, reduced 5-HT\textsubscript{1A} receptor binding in the medial temporal cortex, hippocampus, and midbrain raphe was found in depressed bipolar and MDD patients with familial forms of illness,261 and in unmedicated recurrent depressed patients compared with healthy controls.268 In an independent study using \textsuperscript{11}C-WAY-100635, Sargent and colleagues262 reported a widespread reduction (frontal, temporal, and limbic cortices) in 5-HT\textsubscript{1A} receptor binding in both medicated and unmedicated individuals with MDD. In contrast, Bhagwagar and colleagues reported decreased receptor binding in cortical regions, but not in the raphe nuclei, in recovered depressed males.269 Hirvonen and colleagues\textsuperscript{270} replicated this finding in drug-naive individuals with MDD. Reduced 5-HT\textsubscript{1A} receptor binding in the dorsal raphe nucleus of elderly depressed subjects\textsuperscript{271} and in the sgACC, pgACC, and lateral orbital and mesial temporal cortices of postpartum MDD subjects\textsuperscript{228} has also been reported. Animal and postmortem studies are consistent with the human PET literature.85 Thus, it has been proposed that reduced 5-HT\textsubscript{1A} receptor binding might represent trait vulnerability for depression. However, the lack of effect of selective serotonin reuptake inhibitor (SSRI) treatment and hydrocortisone challenge on 5-HT\textsubscript{1A} receptors in recovered patients with MDD suggests state independence of this abnormality.269,272

It should also be noted that the 5-HT\textsubscript{1A} PET literature is not entirely consistent, and Parsey and colleagues\textsuperscript{273} reported that 5-HT\textsubscript{1A} receptor binding was increased across all regions in antidepressant-naive MDD patients. Therefore, it appears that the choice of reference region and outcome measure can produce different 5-HT\textsubscript{1A} receptor binding results in MDD, and this issue requires further work to be resolved.
3.3.5.2. CHANGES IN 5-HTT BINDING

The 5-HT transporter (5-HTT) contributes to the regulation of serotonergic neurotransmission through the reuptake of 5-HT in the synaptic cleft. An inverse relationship exists between 5-HTT binding and extracellular 5-HT levels. A study using the 5-HTT radioligand $^{11}$C-(+)-McN5652 reported a 23% increase in thalamic 5-HTT binding in medication-free MDD subjects compared with controls. In contrast, Parsey and colleagues reported decreased 5-HTT binding in the amygdala and midbrain but no change in other regions of the brain using the same radioligand. It was later reported that lower 5-HTT binding in the ACC, amygdala, and midbrain predicted the absence of remission at 1 year. Similarly, Reimold and colleagues reported reduced 5-HTT binding in the thalami (but not other regions such as the amygdala and midbrain) of patients with MDD, and a negative correlation between 5-HTT availability in the amygdala and thalamus and depression and anxiety scores. In contrast, although no overall intergroup difference in 5-HTT binding was detected, scores on the Dysfunctional Attitude Scale were found to positively correlate with increased 5-HTT binding in the anterior cingulate, putamen, and thalamus. This finding is consistent with the data of Cannon and colleagues, who showed that depressed, unmedicated MDD patients had increased 5-HTT binding in the thalamus (24% increase), periaqueductal gray matter (PAG; 22%), insula (15%), and striatum (12%) relative to healthy subjects. Furthermore, the depression-associated personality trait, neuroticism, is reportedly associated with higher thalamic 5-HTT binding, and clinically depressed patients with Parkinson’s disease also show increased 5-HTT binding in the PFC compared with healthy controls.

3.3.5.3. DOPAMINE

Cannon and colleagues reported that D1 dopamine receptor binding was reduced in the caudate of depressed patients and that this difference correlated with disease duration and anhedonia rating. This difference was more evident in the NAc and putamen, regions that play a role in reinforcement learning, which can be profoundly affected in MDD. Similarly, Dougherty and colleagues reported that D1 receptor hypofunction in the striatum distinguished depressed patients from controls. There are inconsistencies in the literature for D2 receptors, with PET studies showing higher, lower, or unchanged striatal D2 receptor density in MDD compared with controls.

3.4. Biochemical Alterations in Major Depressive Disorder Changes Detected Through $^1$H-MRS

3.4.1. N-Acetylaspartate

NAA levels in the caudate, PFC, and ACC were reported to be reduced in MDD patients, compared with healthy controls. Portella and colleagues reported that levels of NAA in the ventromedial prefrontal cortex (VMPFC) were reduced only in patients with chronic and recurrent depression and that normal levels were seen in treatment-naive patients. It has been proposed that NAA levels correlate with the age of onset of MDD and the severity and duration of illness. Treatment-resistant patients had decreased NAA levels in the thalamus and ACC and normal levels in amygdala, hippocampus, and basal ganglia. In contrast, no significant differences in NAA levels between
patients with MDD and controls were reported in the basal ganglia, PFC, DLPFC, ACC, putamen, and thalamus. A meta-analysis of MRS studies reported that MDD patients had similar NAA values to those of controls in both the basal ganglia and frontal lobe structures.

No significant differences in NAA levels in pediatric MDD patients were reported in the DLPFC, caudate, putamen, ACC, occipital cortex (OCC), OFC, amygdala, and thalamus. Consistent with this, a meta-analysis reported no significant alteration in NAA levels in pediatric MDD patients.

### 3.4.2. Choline Compounds

Higher values of Cho have been reported in the basal ganglia of MDD patients, although one study reported significantly lower values in the basal ganglia that increased with fluoxetine (Prozac) treatment. Another two studies reported that choline levels in the basal ganglia decreased with successful treatment with fluoxetine. A meta-analysis performed using the results from these three studies showed no significant decrease in choline levels with antidepressant treatment in the basal ganglia. No significant alterations were found in the OCC, ACC, DLPFC, or the amygdala. Treatment-naive patients had increased levels of choline in the hippocampus that correlated with past burden of illness. However, chronic patients had increased levels in the VMPFC that correlated with duration of illness.

Pediatric MDD studies have reported increased choline levels in the caudate, DLPFC, and OFC. One study reported no change in the DLPFC in pediatric patients and in another study reduced choline levels were reported in the amygdala in pediatric patients. A meta-analysis performed over three studies indicated similar Cho values in the frontal lobe structures of pediatric patients as in controls.

### 3.4.3. Myo-Inositol

In cerebrospinal fluid (CSF), markedly reduced levels of myo-inositol have been reported in depressed patients with unipolar or bipolar affective disorder. Under double-blind conditions, the intake of myo-inositol has been reported to lead to an improvement in depression.

Reduced myo-inositol levels in the PFC and normal levels in the basal ganglia and ACC were reported in MDD patients. Treatment-naive patients showed an increase in myo-inositol levels in the hippocampus, whereas recovered depressed patients showed an increase in the ACC myo-inositol levels. One study reported that pediatric patients had an increase in myo-inositol levels in the DLPFC. In contrast, Mirza and colleagues reported no significant alterations in myo-inositol levels in the ACC in MDD patients.

### 3.4.4. GABA

Studies dating back to the early 1980s have demonstrated abnormally low levels of GABA in the CSF and plasma of depressed patients. More recent findings have augmented this body of evidence by demonstrating specific neurophysiological effects that are likely to be related to GABAergic changes in the brains of individuals suffering with MDD.

Sanacora and colleagues reported that GABA levels in the occipital cortex (OCC) were reduced in MDD patients. This finding was later replicated in another large sample of MDD patients. A similar pattern of results in the OCC was reported in recovered depressed
patients. This suggests that reduced GABA may be a trait marker of susceptibility to affective disorders as opposed to a biochemical marker of active illness. Occipital cortex and ACC GABA levels were quantified in treatment-resistant patients and healthy controls, and it was observed that treatment-resistant patients had the lowest GABA levels in the OCC, which increased with successful ECT treatment. Similar results were reported after 2 months of treatment with SSRI antidepressants.

In addition, a reduction in GABA levels in the PFC was reported in MDD whereas PFC GABA was increased with antidepressant treatment in cocaine-dependent subjects. Reductions in both occipital and prefrontal cortex GABA were greatest in patients with treatment-resistant depression or melancholic major depression, suggesting that GABAergic abnormalities differ between MDD subgroups.

One study provided evidence for GABAergic deficits in MDD by showing a reduced density of GABAergic interneurons in various cortical regions of patients with MDD. Another study reported that the elevated resting state activity in various cortical and subcortical regions observed in MDD might be due to these GABA reductions. Both GABA_A and GABA_B receptors may be dysfunctional in MDD, as animal models have consistently shown decreased GABA_A/B receptor expression and sensitivity in metabolically hyperactive cortical and subcortical structures.

### 3.4.5. Glutamate

As glutamate is difficult to measure by MRS, the Glu:Gln ratio or combined Glu and Gln peaks, termed Glx, has been measured and reported, particularly in early studies (see Section 2.6.5). Glx levels were found to be decreased in MDD across various regions including DMPFC, VMPFC, ACC, hippocampus, amygdala, and left DLPFC. Moreover, Glx levels in the ACC, amygdala, and DLPFC normalized after successful ECT treatment in treatment-resistant depression. In addition, reduced glutamate levels in the VMPFC and ACC were only observed in chronic and recurrent depression and correlated with illness duration. In contrast, Milne and colleagues and Price and coworkers found no difference in glutamate levels in the hippocampus and in the ACC and OCC, respectively. In the ACC, one study found a decrease in Glx levels but no change in the glutamate levels, except for in severely depressed patients, thus suggesting a reduction in glutamine in MDD. Walter and colleagues reported patients with increased anhedonia had reduced glutamine but normal glutamate levels. In the hippocampus, both Glx and glutamine signals were reduced and Sanacora and colleagues reported elevated glutamate levels in the OCC, with no abnormalities in glutamine. In remitted patients, Hasler and coworkers reported no significant abnormality in Glx levels in the DMPFC and VMPFC and Bhagwagar and coworkers reported increased Glx levels in the OCC compared to healthy controls. In addition, elevated serum, plasma, and CSF levels of glutamate have been reported in MDD.

### 4.0. CHARACTERIZATION OF THERAPEUTIC MANIPULATIONS

#### 4.1. Pharmacological Studies

Many studies have investigated whether pharmacological or other therapies can reverse the impairments observed in MDD and thereby potentially identify biomarkers for treatment...
response. An increasing number of studies have examined the actions of antidepressants on the responses of healthy controls during specific tasks and attempted to determine whether the observed effects can be translated to a clinical population.

### 4.1.1. Negative Bias

Using fMRI, Fales and colleagues\(^\text{134}\) demonstrated that MDD patients showed hypoactivity in the right DLPFC and increased activation in the amygdala when performing cognitive tasks that required participants to ignore negatively valenced distracters. After 8 weeks of SSRI antidepressant treatment, patients showed significantly increased DLPFC activity to unattended fear-related stimuli and no longer differed from controls in either DLPFC or amygdala activity during an emotional interference task.\(^\text{227}\) In addition, antidepressants have been reported to reduce amygdala responsiveness to negative stimuli when presented outside conscious awareness.\(^\text{118}\) Harmer and colleagues have reported that both acute and chronic antidepressant treatment reverse negative biases in healthy controls, dysphoric participants, and patients with MDD.\(^\text{107,120}\)

Similarly, using PET Mayberg and colleagues reported that following recovery from depression (after 6 weeks of treatment with the SSRI fluoxetine), the reversal pattern involving the same regions was observed, with limbic metabolic decreases and neocortical increases. A significant inverse correlation between subgenual cingulate and right dorsolateral prefrontal activity was also demonstrated in both conditions.\(^\text{136}\) Resting hypoactivation in DLPFC has long been a recognized concomitant of depression, and this resting hypoactivity has been observed to increase toward normal levels with antidepressant treatment. Enhanced task-related activation of DLPFC following antidepressant treatment has also been reported.\(^\text{136,334}\)

A decreased correlation between activity in the broader ACC and the amygdala at rest and during exposure to neutral, negatively valenced, and positively valenced pictures has been reported in MDD.\(^\text{245}\) After 6 weeks of treatment with the SSRI sertraline (Zoloft), the same MDD sample displayed an increase in ACC-limbic connectivity in the resting state and during exposure to neutral and positive, but not to negative, pictures.\(^\text{245}\) In contrast, reduced functional coupling of the medial and ventral PFC with the amygdala observed in MDD during exposure to sad faces was ameliorated by 8 weeks of treatment with fluoxetine.\(^\text{224}\)

Finally, studies using repetitive transcranial magnetic stimulation (rTMS) indicated that high-frequency rTMS inhibited negative bias in depressed individuals.\(^\text{335,336}\)

### 4.1.2. Social Cognition

Antidepressant therapy may normalize patterns of neuronal responding to affective facial stimuli. For example, a study by Fu and colleagues\(^\text{121}\) examined the response to positive stimuli in patients with MDD compared with matched controls and found reduced activation in the basal ganglia, hippocampus, and extrastriatal regions among acutely ill patients with MDD; this pattern was attenuated following treatment with fluoxetine. Similarly, Keedwell and colleagues\(^\text{226}\) found that severely depressed patients showed increased visual cortex responses to sad faces and reduced visual cortex responses to happy faces in the early stages of antidepressant treatment. Following continued antidepressant therapy and clinical improvement, these patterns were reversed. Similarly, Victor and colleagues found that exaggerated amygdala responses to masked sad faces and reduced amygdala activity to masked
happy faces were reversed following 4 weeks treatment with sertraline. Moreover, in keeping with previous findings demonstrating subgenual cingulate activity as a marker of treatment response, further analysis of data from the study by Keedwell and colleagues showed that increased activity in the right visual cortex and subgenual cingulate to sad but not happy facial expressions in the first few weeks of treatment were predictive of a greater clinical recovery. In contrast, enhanced responses to happy and sad stimuli in the ventrolateral prefrontal cortex were associated with a poor clinical outcome. These findings indicate that the negative bias toward sad faces improves and a positive bias toward happy faces emerges with antidepressant treatment. Similarly, administration of erythropoietin, a potential candidate treatment for psychiatric disorders that exerts neurotrophic and neurorestorative effects, reduced neural responses in the amygdala and hippocampus to fearful compared with happy faces.

A study by Lisiecka and colleagues examined the connectivity of the OFC, a key region in the emotion regulation circuit, to other brain areas in patients with MDD. Lisiecka and colleagues found that during a facial emotion identification task, responders to the antidepressants mirtazapine (Remeron) and venlafaxine (Effexor) were characterized by increased functional coupling between the OFC and motor areas that was evident at baseline. The magnitude of response to antidepressant treatment also positively correlated with functional coupling between the left OFC and the caudate and thalamus. In contrast, increased connectivity between the OFC and the cerebellum was associated with nonresponse to antidepressant treatment.

Taken together, these results suggest that conventional antidepressants and novel treatments may dampen hyperactive responses to negative stimuli and enhance the salience of positive stimuli and that these changes may precede and predict changes in mood measured by clinical rating scales.

4.2. PET

It has been proposed that enhanced 5-HT transmission in MDD can compensate for abnormalities in the density and sensitivity of certain 5-HT receptor subtypes, and this hypothesis is supported by evidence from postmortem, neuroimaging, and pharmacological challenge studies of depression. For example, in PET studies reduced 5-HT1A receptor binding in MDD has been reported by Drevets and colleagues, and this is reversed by chronic treatment with an antidepressant. Furthermore, half of the remitted patients who were unmedicated or treated with SSRIs have been reported to experience depressive relapse after tryptophan depletion.

4.3. Glutamate

The glutamatergic system was first implicated in mood disorders when D-cycloserine, a partial agonist at the N-methyl-D-aspartate (NMDA) receptor glycine site and an antagonist at higher doses, showed antidepressant-like properties. Several other medications with glutamatergic activity have subsequently been studied for their antidepressant properties. One drug of particular interest is ketamine (Ketanest), a noncompetitive
NMDA antagonist, which has been shown to have antidepressant effects after a single intravenous infusion in a number of double-blind, placebo-controlled studies. A study by Deakin and colleagues examined the cognitive effects of a novel low-trapping NMDA channel blocker, AZD6765, compared with ketamine in a pharmacological MRI study in untreated MDD. Both AZD6765 and ketamine increased sgACC activity and these changes correlated with improvement in depression ratings 24 h and 7 days postinfusion.

Elevated serum and plasma glutamate levels were significantly reduced after antidepressant treatment. Reduced Glx levels in the DLPFC and ACC have also been shown to normalize after successful ECT therapy in patients with treatment-resistant depression. Responders to rTMS in one trial showed lower baseline glutamate concentrations in the left DLPFC that increased in a dose-dependent fashion after exposure to therapy. Therapeutic sleep deprivation also increased Glx and glutamine in the same brain area in male responders with MDD and in responders with melancholic depression.

5.0. USE OF NEUROIMAGING IN BIOMARKER IDENTIFICATION AND EARLY DRUG DISCOVERY

Neuroimaging has utility at several levels in the drug discovery and development process:

(1) In characterizing preclinical models;

(2) In early clinical studies to show that target engagement by a novel compound induces the biological change(s) predicted to give clinical benefit;

(3) In clinical trials to demonstrate proof of concept (PoC) or, in other words, that engaging a particular target is linked to a meaningful change in a clinical endpoint and thereby demonstrating the effectiveness of the compound being tested.

Neuroimaging provides a valuable opportunity to image healthy and disordered brain structure and function in vivo. As such, it can help to identify biomarkers for drug development, measure drug efficacy and potentially predict treatment response. A biomarker is defined as a response that can be objectively measured and evaluated as an indicator of normal or abnormal biological processes, or as an indicator of pharmacological responses to a therapeutic intervention. The National Institutes of Health Biomarkers and Surrogate Endpoint Working Group has defined three levels of biomarkers: Type 0 are used to track the natural course of a disease; Type 1 can be used to examine the effects of intervention together with the known mechanism of action of a test compound but without a strict relationship to clinical outcome; and surrogate endpoint Type 2 biomarkers are predictive of clinical outcome. At present, most imaging methods in psychiatry do not meet biomarker status. Some, however, may be considered as emerging biomarkers or prebiomarkers because they enable the identification of therapy-relevant characteristics of a disease.

In general, neuroimaging has potential utility in a number of the steps required to determine the properties of a candidate compound including the comparison of its pharmacokinetic and pharmacodynamic properties.
5.1. Role of Various Neuroimaging Modalities in Drug Development for Depression

5.1.1. PET

PET has been successfully used to explore a number of neurotransmitter systems, in particular the serotonin and DA systems for which specific radioligands have been developed. For example, presynaptic DA synthesis and storage have been studied with $^{18}$F-fluorodopa; postsynaptic D$_1$ and D$_5$ receptor binding has been studied with $^{11}$C-NNC 112; and striatal postsynaptic D$_2$, D$_3$, and D$_4$ receptor binding has been measured with $^{11}$C-raclopride.$^{352}$ The most commonly used PET tracers for studying 5-HT function include $^{11}$C-WAY-100635 (5-HT$_{1A}$)$^{353}$ and $^{11}$C-MCN5652 (5-HTT)$^{354}$.

PET has been used for a variety of applications in drug development, for instance, by using established or newly developed PET radiotracers to characterize a particular target. In addition, PET has been used to determine the degree of target engagement needed to exert therapeutic effects. In addition, PET can be used to study the effect of a novel compound on an enzyme or a second messenger system.$^{350}$

Two major approaches have been used in PET drug development for depression:

(1) To radiolabel a novel compound;
(2) To use a tracer ligand to estimate the target occupancy of a novel compound.

If a novel compound is radiolabeled, important characteristics can be determined, such as brain distribution, washout characteristics, and whether the compound is a substrate for blood–brain barrier pumps. Depending on the nature of the radiolabel, studies can be carried out both in experimental animals and in humans with potentially less stringent requirements for GMP (i.e. good manufacturing practice) material and preclinical safety data due to the use of microdosing.$^{355}$ When studying dosing for antidepressants, SSRIs have been shown to occupy $\geq 80\%$ of the serotonin transporter binding sites (SERTs) at clinically used doses; within this class of drugs, occupancy appears to be independent of the specific SSRI examined. However, the tricyclic antidepressant (TCA) clomipramine has been reported to occupy 80% of the SERT at doses as low as 10 mg, at a plasma concentration of 1.42 ng/mL.$^{356}$ However, clinically used doses of clomipramine are 50–150 mg/day and therapeutic plasma concentrations range between 175 and 450 ng/mL.$^{357}$ This apparent discrepancy raises some obvious questions: For example, is SERT blockade not the only mechanism by which clomipramine (and other TCAs) act? Alternatively, is the noradrenaline (norepinephrine) transporter also responsible for the therapeutic action of clomipramine (and of other TCAs), at least in part? Further, it appears likely that TCAs act differently from SSRIs due to their broad pharmacological actions at many different molecular targets.$^{351}$

In other studies using PET, abnormal serotonin receptor distributions in MDD have been discovered that may help to develop new drugs that target specific receptor subtypes. For instance, 5-HT$_{1A}$ receptors are reportedly downregulated in the raphe nuclei, medial temporal lobe, and mPFC in depression.$^{267}$ In addition, serotonin transporter binding is altered in MDD.$^{85}$

5.1.2. fMRI

A functional approach such as fMRI provides a systems neuroscience evaluation of the circuitry that may underlie the behavioral effects of a drug, independent of its specific
biochemical mechanism of action. Many CNS drugs have multiple mechanisms of action and can vary in efficacy across CNS targets with which they interact. fMRI monitors the combined or integrated effect of these interactions across multiple systems and thereby reflects activity of the neural circuitry that drives behavior. fMRI can serve as a bridge between preclinical and subsequent clinical testing and evaluation. Both awake animals and humans can be assessed using fMRI during rest and the performance of tasks, thereby providing information about neural circuit activity in response to specific, reproducible and well-characterized stimuli that can serve as a fingerprint of specific function. While fMRI is much more widely used than PET for the study of cognition, to date it has not been used as extensively as PET in drug development. However, fMRI is becoming increasingly used to identify and translate biomarkers from preclinical to clinical studies and vice versa (translation and reverse translation) in the characterization of novel compounds. fMRI studies can be useful in drug development in the following areas:

1. Relating molecular targets to behavior;
2. Enrichment of study populations with treatment responders;
3. Differentiation of strong placebo responders;
4. Identification of pharmacodynamic markers;
5. Identification of potentially more sensitive measures of treatment response.

For example, hyperactivity of the default mode network has been reported in MDD and this has been proposed to be a valuable biomarker for the illness.

5.1.3. Electroencephalography

In terms of predictors and biomarkers, EEG has obvious advantages as it is widely available and has a relatively low cost (compared to neuroimaging). A number of pretreatment EEG parameters have been shown to differentiate responders and nonresponders and to predict treatment response to antidepressants.

5.1.3.1. Electroencephalography Alpha Band Activity

Ulrich and colleagues reported differences between MDD patient responders and nonresponders after 4 weeks of treatment with TCAs. Responders showed left lateralization of alpha power at baseline and decreases in alpha power from baseline to week 4. In a follow-up study, early changes in alpha band EEG after the first TCA dose were associated with treatment response at 3 weeks. Similarly, Knott and colleagues showed that imipramine responders had increased alpha power compared to nonresponders at baseline, although this did not reach significance. A similar result was observed in paroxetine responders compared to nonresponders.

In a study by Bruder and colleagues, EEG alpha asymmetry between brain hemispheres recorded at baseline was shown to differentiate SSRI antidepressant treatment responders and nonresponders. Nonresponders showed greater activation (less alpha) over the right hemisphere, but responders did not. This result has been replicated by the same group.

5.1.3.2. Electroencephalography Theta Activity

Changes in frontal EEG measures in the theta band have been interpreted as reflecting altered activity in the anterior cingulate regions implicated in emotional regulation.
is the same area that Mayberg and colleagues proposed to be associated with predicting treatment response.259

Alterations in theta activity in MDD have been shown in association with treatment with a range of antidepressants363,364 and with ECT368 although findings are inconsistent. One study reported that lower theta baseline activity predicted response to imipramine and another study reported that greater theta activity differentiated paroxetine responders from nonresponders.364 In another study, frontal theta band relative power at baseline and at week 1 was a significant predictor of treatment response. Baseline relative theta power was lower in treatment responders, predicting treatment response at 8 weeks with an accuracy of 63%. After 1 week of treatment, relative theta power predicted treatment response with 60% accuracy.369

5.1.3.3. ANTIDEPRESSANT TREATMENT RESPONSE INDEX

The antidepressant treatment response index (ATR) is a nonlinear combination of three features: relative combined theta and alpha power (3–12 Hz), plus alpha power in two different alpha bands (8.5–12 Hz and 9–11.5 Hz). The ATR index is defined as a probability score ranging from 0 (low probability of response to treatment) to 100 (high probability of response).17

An initial study reported that the ATR index predicted treatment response with an accuracy of 70%.369 A large multicenter study (BRITE-MD) then tested this hypothesis on 220 patients treated with escitalopram (Cipralex) or bupropion (Wellbutrin). All patients started treatment with escitalopram and 1 week later continued with escitalopram, switched to bupropion, or were augmented with bupropion.370,371 Overall, ATR predicted both remission and response with 70% accuracy. The other important question addressed by BRITE-MD was whether participants who are unresponsive to an initial antidepressant treatment should be switched to a different agent or whether they would also respond poorly to other treatments. ATR was useful for predicting differential responses to either escitalopram or bupropion monotherapy. Subjects with high ATR values were more than 2.4 times as likely to respond to escitalopram as those with low ATR values.370 Subjects with ATR values below the threshold who were switched to bupropion were 1.9 times as likely to respond to bupropion alone than those who remained on escitalopram treatment. It is possible that if these results are replicated they could help to guide treatment decision making, i.e. continuing or changing an antidepressant treatment after only 1 week rather than after the standard 4–6 weeks.17

5.1.3.4. THETA QUANTITATIVE ELECTROENCEPHALOGRAPHY CORDANCE

Cordance is a measure that combines EEG absolute and relative power according to a specific formula.372 It has been claimed that a decrease in prefrontal theta cordance at 1 week after starting medication was a significant predictor of antidepressant response79,373–375 with overall accuracy ranging from 72% to 88%.17

5.1.3.5. EVENT-RELATED POTENTIALS

ERPs measure voltage changes on the scalp surface that correspond to cortical or brain stem activity in response to sensory stimuli (e.g. sound or light). P300, the wave recorded 300 ms after the presentation of an auditory stimulus, is interpreted as an ERP index of early
attention switching. Bruder and colleagues recorded P300 waves during dichotic listening tests and showed that treatment response in patients with MDD was associated with higher amplitude of the P300 wave only at occipital electrodes. Another study reported that elderly MDD nonresponders had a longer P300 latency at baseline.

Another ERP tested in MDD is the loudness dependence of the auditory evoked potential (LDAEP), which describes how one ERP component (N1/P2), generated in the auditory cortex, changes with increasing loudness of the auditory stimulus. The LDAEP is believed to correspond to the magnitude of serotonergic neurotransmission, particularly in the primary auditory cortex. It has been suggested that LDAEP may be a differential marker of response for antidepressant drugs with serotonergic versus nonserotonergic mechanisms of action. Stronger LDAEP slopes at baseline are reported to predict a response to citalopram (Celexa) and paroxetine (Paxil), whereas responders to reboxetine (Edronax) and bupropion are reported to have weak LDAEP slopes.

5.1.4. Biomarkers from MRS

5.1.4.1. N-ACETYLASPARTATE

After successful ECT and/or antidepressant treatment, normal levels of NAA in the ACC, basal ganglia, amygdala, and thalamus were reported. Interestingly, lower pretreatment NAA levels in the ACC and hippocampus were associated with a greater treatment response to ECT and antidepressants, and this may in turn predict clinical outcome.

5.1.4.2. GLUTAMIX

Reduced Glx levels in the ACC, amygdala, and DLPFC in MDD patients were normalized after successful ECT treatment in treatment-resistant depression. In addition, reduced glutamate levels in the VMPFC and ACC were only observed in chronic and recurrent depression and correlated with illness duration.

5.1.4.3. GABA

It has been reported that patients with treatment-resistant depression had lowest GABA levels in the OCC, which increased with successful ECT treatment. Similar results were reported after 2 months of SSRI treatment. Increased GABA levels after multiple ECT sessions have also been reported in animal models and this is consistent with the established anticonvulsant effects of ECT. Moreover, Bajbouj and colleagues examined changes in cortical inhibitory measures in patients after 10 sessions of right unilateral ECT. After the final session of ECT, the mean cortical silent period increased significantly compared to baseline, suggesting that the GABAergic system is enhanced with multiple ECT treatments. Treatment with low-frequency (1 Hz or less) rTMS is known to increase cortical inhibition and GABAergic functioning. Other work examining the inhibitory effects of various rTMS frequencies indicated that both low- (1 Hz) and high- (10 or 20 Hz) frequency stimulation increased the duration of the cortical silent period in healthy subjects, indicating potentiation of GABA\textsubscript{A} functioning. It has been proposed that this may be partly due to presynaptic GABA\textsubscript{B} receptor inhibition of GABA release. This suggests that the therapeutic effects of rTMS may be partially mediated through enhancement of GABAergic...
inhibitory neurotransmission. Finally, a reduction in GABA levels in the PFC has also been reported in MDD.323

5.2. Identification of Specific Regional Biomarkers in the Brain Using FMRI, PET, and Electroencephalography

5.2.1. Amygdala

fMRI has been used to identify biomarkers in the study of depression and to improve the chances of success in the development of novel treatments. The SSRI antidepressant citalopram reduced amygdala activation in response to fearful faces in healthy volunteers.388 The amygdala response to fearful stimuli has been proposed as a potential biomarker for antidepressant effects.389 Indeed, given sufficient evidence from fMRI studies, hyperactivity in the amygdala to negative stimuli in MDD patients could be translated into a valuable biomarker, as successful antidepressant treatment has been shown to decrease this response.118,134 Interestingly, a study comparing a novel low-trapping NMDA channel blocker, AZD6765, with ketamine in untreated MDD reported that both drugs reduce amygdala responses to fear and sadness in an emotional faces task 24 h postinfusion.346 Antidepressants have also been found to normalize anomalies in resting activity in the amygdala.158 Furthermore, it has been reported that greater amygdala activation to emotional facial expressions in MDD patients at baseline predicts symptom reduction 8 months later.391 The associations between elevated amygdala activity, depressive symptoms, plasma cortisol,392 and rapid eye movement sleep393 support the plausibility of this potential biomarker for MDD.

5.2.2. Hypoactive Prefrontal Cortex

Corticolimbic dysfunction with hyperactive limbic and hypoactive prefrontal regions has been repeatedly reported in MDD patients, and can be reversed by antidepressant treatment.134 This has been replicated in a study using PET.136 Resting hypoactivation in DLPFC has long been a recognized concomitant of depression and this resting hypoactivity appears to return toward normal levels with antidepressant treatment. Enhanced task-related activation of DLPFC has also been reported following antidepressant treatment.136,334

5.2.3. Subgenual Cingulate Cortex

Imaging studies that assessed sgACC activity have indicated increased resting glucose metabolism or BOLD activity in the sgACC. In addition, Greicius and colleagues240 conducted a resting state connectivity analysis of MDD patients and suggested that the altered pattern of resting state connectivity in MDD is driven primarily by elevated activity of the sgACC. In line with these data, sgACC metabolism and CBF were reported to be higher in the depressed, unmedicated phase versus the remitted phase of MDD patients. Elevated sgACC BOLD activity has also been observed in MDD patients performing the stop-signal task394 and an emotional interference task.134 Consistent with observations that experimentally induced sadness increases blood flow to the sgACC, the severity of depressive symptoms in MDD was found to correlate with glucose metabolism in this region. Moreover, various treatment paradigms, including antidepressant treatment,227,395 ECT,338 and deep brain stimulation of the sgACC,396 result
in decreased activity of the sgACC. In addition, fMRI studies have suggested that baseline hyperactivity in this region predicts treatment response in acutely depressed patients.\(^{259,397}\)

Pizzagalli and colleagues\(^{398}\) reported that resting rACC activity in the theta EEG band correlates with treatment response after 4 months on nortriptyline (Aventyl) measured using the Beck Depression Inventory. Using the same low-resolution electromagnetic tomography analysis (LORETA; a 3D EEG source localization method) technique, Mulert and colleagues reported that in a group of 20 MDD patients treated with citalopram or reboxetine, treatment response was associated with increased pretreatment resting theta activity in the rACC.\(^{383}\) Pretreatment EEG LORETA revealed higher resting theta activity (current density) in the rACC and OFC in responders to medication (fluoxetine or venlafaxine) in separate studies. Responders to placebo did not differ from nonresponders on this metric. These EEG LORETA results add to a large body of neuroimaging evidence correlating pretreatment increased rACC activity with treatment response.\(^{259,397}\) In addition, the LORETA results\(^{383,397,399}\) suggest that the link between increased resting rACC theta activity and treatment response may generalize across antidepressant drug classes.

A study using MEG reported that healthy controls showed a decrease in neuromagnetic activity in rACC across repeated exposures to fearful faces, whereas MDD patients showed an increased activity in the rACC.\(^{400}\) This increase correlated with an antidepressant response to ketamine, suggesting that it may be a possible biomarker. In addition, during an n-back task\(^{401}\) decreased rACC activity was shown to correlate with the ketamine response. Taken together, high rACC in response to emotional faces but low rACC activity to cognitive demand appears to predict treatment response. In addition, subjects with lower source coherence between rACC and amygdala were most likely to respond to ketamine.

6.0. BEHAVIORAL CORRELATES AND USE OF NEUROIMAGING BIOMARKERS IN MODELS OF DEPRESSION

6.1. Theories of Human Major Depressive Disorder

6.1.1. Monoamine Hypothesis

The monoamine hypothesis is that depression is caused by underactivity of brain monoamine neurotransmitters such as DA, noradrenaline (norepinephrine), and serotonin. In the 1950s monoamine oxidase inhibitors (MAOIs) and TCAs were serendipitously discovered to be effective in the treatment of depression.\(^{402-404}\) These findings and other supporting evidence prompted Schildkraut to propose the ‘Catecholamine Hypothesis of Affective Disorders.’\(^{405}\) Schildkraut proposed that ‘the biological basis of depression is a deficiency of brain catecholamine and serotonin systems and that ameliorating this neuronal deficiency with an antidepressant would restore normal function in patients with MDD.’ The monoamine hypothesis has been a major focus of research in depression for over 30 years and has led to the development of new classes of antidepressant drugs, such as SSRIs, selective noradrenergic reuptake inhibitors (SNRIs), and selective and reversible MAOIs.\(^{404}\)
According to the monoamine hypothesis, the therapeutic action of antidepressants is mediated by one of two mechanisms:

1. Enhancement of monoaminergic neurotransmission by increased synaptic levels of DA, noradrenaline, and serotonin;
2. Specific agonist effects on serotonin, DA, or noradrenaline receptors.

The monoamine systems in the brain have complex interactions with other neurotransmitter systems. Furthermore, there appears to be a mismatch in the timing of the effects of antidepressants on brain monoamines and their therapeutic actions. Thus, antidepressant drugs increase synaptic levels of monoamines within 24 h but their therapeutic effects are not evident until at least 4–6 weeks of drug treatment. Similarly, a significant proportion of patients with MDD are resistant to monoaminergic antidepressant therapies. Therefore, the current prevalent view is that the monoamine hypothesis may only partially explain MDD and the response to antidepressant drugs. Nevertheless, monoamine depletion has been useful as a model to investigate MDD and antidepressant mechanisms, and a number of such approaches are considered below.

6.1.1. TRYPTOPHAN DEPLETION

Evidence from biochemical challenge, imaging, and postmortem studies has associated MDD with reduced function of central serotonergic systems. Tryptophan depletion has been a useful approach to investigate the relationship between serotonergic function and depression. This model assesses mood changes in response to serotonin depletion, achieved by consumption of an imbalanced amino acid mixture consisting of all essential amino acids except for the dietary 5-HT precursor, tryptophan. The transient reduction in plasma tryptophan concentrations and brain 5-HT synthesis and concentrations, resulting from this dietary manipulation induces symptoms of depression in remitted depressed patients who are either off medication or being treated with antidepressants. In addition, tryptophan depletion also reverses the effects of light therapy in patients with seasonal affective disorder.

Symptoms induced by tryptophan depletion show a relatively high specificity for MDD and seem to be heritable. Thus, in remitted depressed patients polymorphism in the promoter (also known as the 5-HTT-linked polymorphic region; 5-HTTLPR) of the long (l) allele of the sodium-dependent serotonin transporter gene (SLC6A4 or 5HTT) predicted response to tryptophan depletion, while in healthy women the short (s) allele of this functional polymorphism and a positive family history of depression represented additive risk factors for tryptophan depletion-induced symptoms of depression. In addition, healthy subjects with a family history of depression were shown to experience depressed mood symptoms following tryptophan depletion, and this effect was smaller than in remitted depressed patients but distinct from subjects without familial risk who showed no mood changes following tryptophan depletion.

In vulnerable individuals, acute depletion of tryptophan induces mood-congruent memory bias and impairs memory consolidation. Similarly, in healthy volunteers acute depletion of tryptophan alters reward-related behaviors and significantly impairs the recognition of fearful facial expressions in females, but not in males. Severe acute serotonin depletion leads to biological changes associated with MDD, including enhanced
noradrenaline (norepinephrine) transporter mRNA levels and reduced serotonin transporter mRNA levels,\textsuperscript{428} an increased number of mineralocorticoid receptor binding sites,\textsuperscript{429} and altered \textit{BDNF} (\textit{brain-derived neurotrophic factor}) gene expression in the dentate gyrus.\textsuperscript{430} These changes are comparable to the mood and biological changes that occur in MDD.

Tryptophan depletion is associated with increased regional cerebral metabolic rates for glucose (rCMRGlu) in the OFC, ACC and ventral striatum. Abnormal CBF and glucose metabolic rate in these areas, as well as in the amygdala and hippocampus, have also been described in medicated patients with recurrent MDD during tryptophan depletion and in patients with MDD during spontaneous episodes of MDD. Although there is a growing consensus that this corticostriatolimbic circuit is involved in MDD, not all regions are reported in all studies, and there is considerable variability in the direction of CBF and rCMRGlu changes.\textsuperscript{85}

\subsection*{6.1.1.2. CATECHOLAMINE DEPLETION}

MDD has been associated with noradrenergic and dopaminergic dysfunction (see Section 6.1.1 above). Catecholaminergic dysfunction has been implicated in the pathophysiology of depression by studies of neurotransmitter synthesis and storage, which show that reduction of catecholamine stores exacerbates depressive symptoms.\textsuperscript{431}

Lowered catecholamine brain function can be investigated experimentally in two ways: blockade of catecholamine synthesis by administration of alpha-methyl-\textit{para}-tyrosine (AMPT) or dietary restriction of the immediate precursors phenylalanine and tyrosine: i.e. acute phenylalanine/tyrosine depletion (APTD).

\subsubsection*{6.1.1.2.1. AMPT DEPLETION}

Mood responses to AMPT depletion in healthy subjects are usually not significant.\textsuperscript{432} The presence of depressive symptoms induced by catecholamine depletion in unmedicated remitted patients with MDD suggests state independence of this biological marker.\textsuperscript{433} The depressive symptoms evoked by catecholamine depletion are often similar to those experienced by patients during a depressive episode, suggesting clinical plausibility.\textsuperscript{170} However, catecholamine depletion failed to exacerbate depression in untreated, symptomatic depressed patients prior to initiation of antidepressant therapy.\textsuperscript{434} This finding may be due to brain catecholamine function being maximally dysfunctional in symptomatic depressed patients (a ceiling effect).\textsuperscript{435} Catecholamine depletion reversed the therapeutic effects of antidepressants in treated depressed patients, particularly the effects of catecholamine reuptake inhibitors.\textsuperscript{434} Catecholamine depletion also reversed the effects of light therapy in patients with seasonal affective disorder.\textsuperscript{419}

The return of depressive symptoms following catecholamine depletion has been associated with decreased brain metabolism in the OFC and DLPFC. Similarly, increased resting metabolism in the prefrontal cortex and limbic areas has been found to increase vulnerability to catecholamine depletion-induced exacerbation of depressive symptoms.\textsuperscript{436}

AMPT impaired attention, but not psychomotor speed, in a D\textsubscript{2} ligand-binding PET study, using \textsuperscript{11}C-raclopride in healthy volunteers.\textsuperscript{437} Impaired attention induced by AMPT was associated with increased raclopride binding.\textsuperscript{437} This study has the limitation that it did not include a placebo condition, but nevertheless suggests that the effects of AMPT on cognitive performance may be associated with lowered DA function.\textsuperscript{438} Interestingly, decreased performance on memory and attention tasks relative to placebo was reported when AMPT
was followed by 40 h sleep deprivation, but there were no significant effects after AMPT or sleep deprivation alone.439

6.1.1.2.2. ACUTE PHENYLALANINE/TYROSINE DEPLETION In healthy individuals, APTD (like AMPT) does not induce depressive symptoms. A meta-analysis of APTD studies found that self-report ratings of depressed mood are unaffected by APTD, except when it is followed by a public speaking task.438

APTD reduces the psychostimulant effects of amphetamine (indicated by self-report and cognitive tests).440,441 In addition, cognitive processes are affected by APTD, and it has been suggested that APTD specifically interferes with spatial short-term and working memory but has no effect on sustained attention or other memory processes.442,443 However, it has also been reported that APTD impaired the retrieval of words from long-term memory, whereas attention and memory for abstract figures were unchanged.444

Nathan and colleagues compared the cognitive effects of acute tyrosine depletion and APTD in a double-blind, placebo-controlled crossover study in healthy volunteers. Acute tyrosine depletion selectively impaired memory consolidation, whereas APTD selectively impaired working memory performance.445 A pilot PET study found that APTD did not induce changes in D2 receptor binding.446 Prolactin levels are increased after APTD,441,443 which is indicative of reduced central DA receptor function. In contrast, levels of melatonin and IL-6 (interleukin-6) were unaffected by APTD.447,448 Finally, in a study of euthymic subjects with a history of major depression, APTD attenuated DA function, reflected by increased plasma prolactin levels, and decreased spatial memory performance.449 However, ratings of depression were unaffected, suggesting that disruption of dopaminergic function by APTD (unlike disruption of serotonergic function by tryptophan depletion) does not induce a lowering of mood in individuals who are vulnerable to depression.

6.1.2. Glutamate Hypothesis

A considerable body of evidence suggests that brain glutamate systems may be involved in the pathophysiology of MDD and in the mechanism of action of antidepressants.408,450–455 Although almost all current antidepressant drugs (e.g. TCAs, SSRIs and SNRIs) have their major action(s) on brain monoaminergic neurotransmitter mechanisms (see above), their delayed onset of action (generally at least 4–6 weeks is required for significant symptom relief) suggests that other processes are involved in the mediation of their therapeutic effects.408,455 Furthermore, a significant proportion of MDD patients do not achieve remission following treatment with standard monoaminergic therapies and are termed treatment resistant. Thus, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, conducted in a large adult outpatient treatment-seeking sample with MDD (n = 3671), found that only 36.8% of patients achieved remission following an optimized trial of the SSRI citalopram for up to 12 weeks.456,457 Remission in half of the patients often required 6 months of treatment and two antidepressant trials.456,457

The role of glutamate in synaptic plasticity and adaptive processes in the brain coupled with the discovery of the rapid-onset antidepressant effects of the noncompetitive NMDA antagonist, ketamine, have prompted a renewed interest in the glutamate theory of depression and the development of novel glutamate antagonists as therapeutic agents.345,408,451–453,455,458,459 Indeed, it has been suggested that a paradigm shift from
a monoamine hypothesis of depression to a neuroplasticity hypothesis focused on glutamate may represent a substantial advancement in the working hypothesis that drives research for new drugs and therapies for MDD. In particular, it has been proposed that glutamate approaches may help to address the two most challenging problems with current antidepressant therapies, namely slow onset of action and treatment resistance. Encouragingly for the glutamate hypothesis, antidepressant effects have now been reported in clinical studies with both ionotropic and metabotropic glutamate antagonists and further exploratory and large-scale clinical trials are underway.

In mechanistic studies of the glutamate hypothesis, Salvadore and colleagues used $^1$H-MRS to investigate whether prefrontal levels of amino acid neurotransmitters predict the antidepressant response to a single intravenous infusion of ketamine in MDD patients. Correlation analyses were conducted to determine whether pretreatment with GABA or glutamate, or the Glx:Glu ratio predicted change in depression symptoms after ketamine administration. The pretreatment Glx:Glu ratio in the dorsomedial and dorsal anterolateral PFC negatively correlated with improvement in depressive symptoms suggesting an association between a lower Glx:Glu ratio and a greater improvement in response to ketamine treatment.

The term glutamate-based depression (GBD) has been proposed by McCarthy and colleagues. GBD is defined as a chronic depressive illness associated with environmental stress and diseases associated with altered glutamate neurotransmission. It has been proposed that glutamate-induced hyperactivation of NMDA receptors in the sgACC (Brodmann area 25) plays an important role in the etiology of depression and may be responsible for the high incidence of comorbid depression associated with diseases with glutamate etiology. Supporting evidence for this hypothesis is the finding that a range of antidepressant treatments, including SSRIs, ketamine, ECT, and deep brain stimulation, have a dampening effect on sgACC activity over time courses that are consistent with their therapeutic effects. In addition, a study showed that both the novel NMDA antagonist AZD6765 and ketamine increased sgACC activity and these changes correlated with improvement in depression ratings 24 h and 7 days postinfusion.

6.1.3. Neurotropic Theories

6.1.3.1. CYTOKINE HYPOTHESIS

In 1927, Wagner-Jauregg won the Nobel Prize for the seminal observation that activation of the immune system by an infectious agent (i.e. malaria inoculation) can affect psychiatric functioning. He concluded that cytokines have an important signaling role and can serve as mediators between the immune system and the CNS. Maes and colleagues investigated plasma concentration and in vitro production of several cytokines, including IL-6 and IL-1, and concluded that an increase in proinflammatory cytokines in patients with MDD appears to correlate with the severity of illness and measures of hypothalamic-pituitary-adrenal (HPA) hyperactivity. Cytokines have been reported to elicit depression, for example IFN-α (interferon α)-induced depression in cancer patients was comparable to that of patients with MDD. Interestingly, IFN-induced depression was associated with increased psychomotor retardation, weight loss and significantly less severe feelings of guilt, suggesting that cytokines may preferentially target brain mechanisms that mediate psychomotor responses, such as the basal ganglia. A meta-analysis of studies between 1967 and
2008 revealed that depression is often associated with an increase in proinflammatory cytokines [IL-1β, IL-6, TNF-α (tumor necrosis factor α), and IFN-γ].

Cytokines and other immune molecules can impact mood and cognition in part through the modulation of neuronal circuits and functioning. Plasticity is critical for mood, cognition, development, and behavior throughout the lifespan. Cytokines and other immune factors play a key role in modulating early brain development as well as neuronal plasticity. Indeed, prolonged exposure to proinflammatory cytokines can impair neuronal plasticity, thereby contributing to cognition and mood disorders.

The brain regions with the highest concentrations of proinflammatory cytokines (specifically IL-1β, IL-6, and TNF-α) include the cortex, hippocampus, and hypothalamus, areas that are critical for antidepressant responses and cognitive function. Cytokines can contribute to HPA axis hyperactivity and also modulate 5-HT and DA systems, which may subsequently lead to mood changes and the emergence of symptoms of depression.

Eisenberger and colleagues measured neural responses to social exclusion during a Cyber Ball passing game in healthy volunteers after acute administration of placebo or endotoxin. It was reported that an observed increase in IL-6 correlated with subjectively scored depressed mood. Interestingly, changes in brain regions that are involved in mediating responses to pain, such as the anterior and posterior insula, and regions associated with changes in mood, such as the DMPFC, MPFC, and precuneus, were reported to correlate with IL-6 levels in the endotoxin-treated group. Harrison and colleagues reported that inflammation caused by administration of typhoid vaccine modulated neural activity in brain regions representing internal bodily state. Another study using a face perception task showed that the functional connectivity between the sgACC and MPFC, NAc, amygdala, and superior temporal sulcus, negatively correlated with IL-6 levels caused by administration of typhoid vaccine in healthy controls. Mood level was shown to decrease with IL-6 levels. These changes might underpin the marked decrease in social behavior associated with acute sickness, possibly reflecting an internal self orientation of attentional focus. No studies have been performed with depressed patients, but increased proinflammatory levels associated with depression might relate to social impairments that are reported in MDD.

6.1.3.2. BDNF HYPOTHESIS

An increasing body of evidence indicates that alterations of BDNF expression in limbic brain regions may have a critical role in the pathophysiology and/or treatment of MDD. BDNF is expressed abundantly in adult limbic brain structures and there are reports from preclinical studies that stress reduces BDNF-mediated signaling in the hippocampus, whereas chronic treatment with antidepressants increases BDNF-mediated signaling. A study reported the unexpected finding that peripheral administration of BDNF produces antidepressant-like effects in cellular and behavioral models. Taken together, these data provide support for the BDNF hypothesis of depression, although conflicting findings exist (see below).

Clinical postmortem studies have detected decreased BDNF and TrkB (neurotrophic tyrosine kinase receptor type 2) expression in the hippocampus of suicide victims and increased levels in patients treated with antidepressants before death. Furthermore, serum BDNF in depressed patients is abnormally low, but can be restored following pharmacological treatment. In addition, Dias and colleagues reported an increase in BDNF following chronic ECT, tranylcypromine
(Parnate), and desipramine (Norpramin) treatment. In contrast, following administration of fluoxetine in rats, both downregulation of BDNF expression in the hippocampus or no effect on exon-specific BDNF transcript levels have been reported. These conflicting findings may be due to species differences or could be specific to SSRIs.

Interestingly, a clinical study measured pre- and posttreatment serum BDNF levels in patients with treatment-resistant depression treated with ECT or rTMS, and results suggested that ECT and rTMS may not exert their clinical effects by altering serum BDNF levels in patients with treatment-resistant depression.

The human BDNF gene is complex, comprising eight exons that provide multiple transcripts. Therefore, it has been proposed that differential regulation of BDNF transcripts by stress and antidepressant treatments may result in contrasting functional effects.

Finally, a meta-analysis has indicated that serum BDNF levels are differentially regulated by stress and antidepressants in MDD patients, suggesting that serum BDNF could be a useful biomarker for MDD and antidepressant efficacy, although further validation studies are required.

6.1.3.3. HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

A consistent, characteristic feature of MDD reported in many studies is hyperactivity of the HPA axis. Severe depression is associated with hypersecretion of cortisol and with pituitary and adrenal gland enlargement. HPA abnormalities in MDD include increased secretion of cortisol, elevated basal CSF corticotrophin-releasing hormone levels, and increased size and activity of the pituitary and adrenal glands. Abnormal cortisol responses in MDD patients were reported to be independent of depressive state, suggesting that this is a state-independent marker. Depression-like alterations of PFC functions, such as inhibitory control, attentional regulation, and planning, following cortisol administration and the bidirectional associations between amygdala activity and cortisol levels suggest clinical plausibility of cortisol-related endophenotypes for MDD.

6.1.4. Neurodevelopmental Theories—Genetic Polymorphisms

Mental illness tends to run in families, strongly implicating genetic causation, and genetic studies enable a better understanding of candidate genetic factors and functional pathways that may underlie the pathophysiology of MDD. Genetic approaches combined with neuroimaging methods can be useful tools in identifying brain changes that may be modulated by underlying genetic factors, and are integral to neural function, including neuronal organization, neuronal signaling, and interneuronal communication.

For further discussion regarding the use of neuroimaging to study the genetic basis of neuropsychiatric disorders in human subjects and in animal models please refer to Tost et al. in Chapter 6, Rethinking the Contribution of Neuroimaging in Translation in Schizophrenia; Steckler and Salvadore in Chapter 7, Neuroimaging as a Translational Tool in Animal and Human Models of Schizophrenia; Westphal et al. in Chapter 8, Functional Magnetic Resonance Imaging as a Biomarker for the Diagnosis, Progression, and Treatment of Autistic Spectrum Disorders; Badura et al. in Chapter 9, Translational Neuroimaging for Drug Discovery and Development in Autism Spectrum Disorders: Guidance from Clinical Imaging and Preclinical Research; Schmidt et al. in Chapter 5, Positron Emission Tomography in Alzheimer Disease: Diagnosis and Use as Biomarker Endpoints; and Novak and Einstein in Chapter 4, Structural Magnetic Resonance Imaging as a Biomarker for the Diagnosis, Progression, and Treatment of Alzheimer Disease, in this volume.
Studies on the biological basis of depression have found stronger associations between specific biological dysfunctions and certain components of major depression than with the presence or absence of MDD, as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. Symptoms such as anhedonia, cognitive deficits, lowered mood, psychomotor retardation and rumination have been associated with specific focal abnormalities in CBF. It has been proposed that recurrence of depressive episodes has a high genetic liability, while a high temporal stability of the phenotype is favorable for genetic studies.

### 6.1.4.1. AMYGDALA HYPERACTIVITY

Significant differences have been reported between the s/s and l/l genotypes of the SLC6A4 (5HTT) gene promoter in the amygdala response to fearful faces in the absence of behavioral differences. The s allele was associated with increased amygdala activation in response to negatively valenced faces or decreased amygdala activation to neutral stimuli. Similar results have been reported using dot probe and emotionally valenced pictures and public speaking as stimuli. In addition, the s allele has been associated with elevated baseline amygdala activity and reduced amygdala volume in healthy subjects. A PET study reported that during tryptophan depletion, MDD carriers of the s allele showed reduced glucose metabolism of the left amygdala compared with l/l homozygotes. The impact of the SLC6A4 genotype on amygdala function has also been reported in studies with stressed rhesus monkeys and patients with MDD. However, a meta-analysis suggests that these effects are only marginally significant.

The 5-HT1A receptor, encoded by the HTR1A gene, plays a critical role in serotonergic signaling and has been implicated in MDD. It was reported that the G allele of a functional single nucleotide polymorphism (SNP; rs6295) was associated with greater amygdala reactivity in response to emotionally valenced faces in a MDD sample and to threat-related stimuli and the level of trait anxiety in healthy individuals.

The tryptophan hydroxylase 2 (TPH2) gene is another candidate for modulation of amygdala function. TPH2 is involved in the synthesis of neuronal 5-HT. Brown and colleagues reported that the T allele of rs4570625 was associated with a greater amygdala response to angry or fearful faces while Canli and colleagues found that the effect of the rs4570625 variant on amygdala function extended to both positively and negatively valenced stimuli in healthy controls. In a subsequent study by Canli and colleagues, an additive effect of TPH2 and SLC6A4 polymorphisms was reported on amygdala reactivity that was most robust for sad or fearful faces: carriers of the t and s alleles displayed a 0.24% greater BOLD response in the amygdala than subjects who did not possess either a t or an s allele. These data derive further support from a PET study, which showed that the TPH2 G allele predicted a placebo-induced improvement in social anxiety that was associated with a reduction in amygdala activity. In contrast, Lee and Ham reported that individuals homozygous for the G allele of rs4570625 showed higher levels of amygdala activity in response to sad (but not angry) faces than their counterparts who did not carry the G allele.

Polymorphisms of the BDNF, COMT (catechol-o-methyltransferase), and MAOA (monoamine oxidase A) genes have also been associated with differing degrees of amygdala reactivity in healthy controls and different patient groups.
6.1.4.2. ANHEDONIA

Anhedonia has been proposed to be a specific symptom of depression as, in schizophrenia, anhedonia has been related to the depressive syndrome rather than the deficit syndrome of the disorder. Neurotropic factors, including CREB (cyclic AMP-responsive element-binding protein), BDNF, and the transcription factor fosB (or delta-FosB), may represent molecular mechanisms involved in long-term alterations of the brain reward system. Preliminary evidence for a potential heritability includes a functional polymorphism of the COMT gene that has been associated with individual variation in the response of the brain to dopaminergic challenge. Epidemiological research provides clues for state independence, heritability, and familial association of dysfunctions of the brain reward system as endophenotypes for MDD.

6.1.4.3. SUBGENUAL ALTERATIONS

The serotonin transporter (5-HTTR) has been associated with elevated sgACC activity in unmedicated MDD. It has been reported that the s allele was associated with reduced left middle frontal gyrus, pregenual and sgACC volumes. The reported association between reduced volume of BA9 and the short 5-HTTLPR allele is interesting because glial cell loss and a reduction in neuronal size postmortem has also been observed in this region in MDD.

6.1.4.4. CORTICOLIMBIC DYSFUNCTION

A heuristic model of MDD is a loss of top-down PFC control over limbic regions, such as the amygdala, leading to emotional, behavioral, cognitive, and endocrine changes characteristic of the disorder. The genetic basis of this abnormal PFC-limbic functional coupling is in the early stage of investigation. It has been reported the s allele of the 5-HTTLPR polymorphism was associated with reduced functional coupling between the supragenual ACC and the amygdala, but increased functional coupling between the VMPFC and the amygdala in healthy controls exposed to threatening faces. Additionally, the degree of functional coupling between the perigenual ACC and the amygdala predicted approximately 30% of the variance in scores on the harm avoidance subscale of the Temperament and Personality Questionnaire. The greater VMPFC—amygdala coupling observed in the 5-HTTLPR s allele carriers replicated a report of a similar effect in healthy volunteers shown aversive pictures. Similarly, Dannlowski and colleagues reported that the inverse functional correlation between dorsal anterior cingulate cortex (dACC) and amygdala activity observed in their healthy control sample was attenuated in carriers of the high-activity MAOA promoter polymorphism alleles (3.5R or 4R). Further, MDD cases with high-activity MAOA variants showed the weakest amygdala—dACC coupling and the most severe course of illness.

6.1.4.5. SEROTONIN RECEPTORS

6.1.4.5.1. 5-HT1A RECEPTOR The first evidence for a functional genetic association of a 5-HT receptor polymorphism with MDD was reported by Lemonde and colleagues. Their results suggested a molecular mechanism by which the single nucleotide C(-1019)G polymorphism may regulate HTR1A gene (which encodes 5-HT1A receptors) expression in vivo by impairment of repression of the HTR1A promoter in presynaptic raphe neurons leading to reduced serotonergic neurotransmission and potentially predisposing individuals
to MDD. Interestingly, the G(-1019) allele depresses presynaptic HTR1A transcription, but may have the opposite effect of reducing NUDR (Nuclear deformed epidermal autoregulatory factor 1 homologue)-enhanced HTR1A transcription in postsynaptic cells in regions such as the hippocampus and septum. The net effect of these changes would be a reduction in serotonergic neurotransmission.\textsuperscript{527} In patients with MDD the homozygous G(-1019) allele was enriched twofold versus controls.\textsuperscript{527} These data have been independently replicated in another MDD sample,\textsuperscript{528} as well as in elderly patients who became depressed after suffering hip fractures\textsuperscript{529} and hepatitis C patients with IFN-induced depression.\textsuperscript{530} In a subsequent study by Lemonde and colleagues, patients with MDD with the homozygous G(-1019) genotype were reported to be approximately twice as likely to be nonresponders to an antidepressant as those with the C(-1019)C genotype.\textsuperscript{531}

6.1.4.5.2. 5-HTT \ Frokjaer and colleagues\textsuperscript{532} found that healthy individuals, who were at high risk of developing MDD by virtue of having a twin with the disorder, displayed reduced 5-HTT binding in the DLPFC and, to a lesser extent, the ACC. Nevertheless, given the nature of this study, it is unclear whether the reduction in 5-HTT binding is indicative of a genetic vulnerability to MDD or whether it reflects an adaptive compensation for impaired serotonergic function.

7.0. RECIPROCAL NATURE OF NEUROIMAGING RESULTS IN ANIMAL AND HUMAN MODELS OF DEPRESSION

In drug discovery research and development for MDD, using methods that can be translated from preclinical to clinical platforms and \textit{vice versa} facilitates the early identification of promising compounds to advance to late-stage clinical trials. Translational neuroimaging can provide qualitative and quantitative information on brain morphology and function in preclinical models, healthy participants and patients with MDD.\textsuperscript{v}

Using different neuroimaging tools, potential biomarkers for depression have been discovered, the most promising of which have been identified using several independent modalities. For example, hyperactivity of the sgACC in MDD and its reduction after antidepressant, ECT, or rTMS treatment has been reported using EEG, fMRI, MEG, MEG and PET methods.

7.1. Advances in Developing Drugs for Depression Through the Use of Neuroimaging

Neuroimaging techniques have advanced rapidly and are playing an increasingly important role in understanding abnormal brain structure and function in MDD. Neuroimaging

\textsuperscript{v}For further discussion regarding the use of small animal imaging in bridging studies between preclinical and clinical studies during CNS drug discovery and development, please refer to Ferris et al. in Chapter 3, Small Animal Imaging as a Tool For Modeling CNS Disorders: Strengths and Weaknesses; Badura et al. in Chapter 9, Translational Neuroimaging for Drug Discovery and Development in Autism Spectrum Disorders: Guidance from Clinical Imaging and Preclinical Research; Steckler and Salvadore in Chapter 7, Neuroimaging as a Translational Tool in Animal and Human Models of Schizophrenia; and Schwarz et al. in Chapter 11, Translational Neuroimaging: Substance Abuse Disorders, in this volume.
approaches, in particular fMRI, are also being increasingly used in early drug development from preclinical studies to Phase I and Phase IIa clinical trials. Thus, fMRI can serve as a bridge between preclinical and clinical testing and evaluation.\textsuperscript{359} Responses in animals and humans can be assessed using fMRI during rest and while performing a wide range of tasks, thereby providing information about neural circuits that are activated in response to specific, reproducible, and well-characterized stimuli that can serve as \textit{fingerprints} of specific functions.\textsuperscript{358} A disadvantage of the fMRI approach is that the data generated are indirect and qualitative rather than quantitative. Even though the BOLD response is recorded close to neuronal activity (local field potentials), BOLD is a result of a combination of various events including CBF, CBV and oxygen metabolism. However, new approaches are being developed and tested in MRI to measure CBF. For example, ASL magnetically labels the blood, thereby creating a noninvasive endogenous contrast agent.\textsuperscript{533} ASL can be used to produce a quantitative baseline measurement of CBF or images can be acquired over a period of time to measure changes in CBF.

In comparison to fMRI, PET has the advantage of providing quantitative rather than qualitative data. However, PET also has limitations as it is an expensive method, requires exposure to radiation, and the, often challenging, development of a selective high-affinity radioligand.

EEG and MEG measure neuronal activity with superior temporal resolution compared to PET and fMRI. Indeed, as current MEG systems use several hundred sensors; good spatial resolution can be obtained using this technique.

Thus, each neuroimaging tool has advantages and disadvantages but if combined can provide complimentary measurement of neuronal information related to both healthy and disordered function. Therefore, multimodal imaging is likely to be increasingly used in future as, by combining different modalities, it may be possible to define common neuronal generators that will increase our understanding of MDD and thereby help to identify improved therapies for this disorder.

\section*{8.0. SUMMARY AND FUTURE PROSPECTS}

MDD is a common and disabling disorder that is poorly treated by currently prescribed drug therapies. Many patients with MDD do not respond to available antidepressant drugs and following a number of drug treatment cycle failures are termed treatment resistant. Patients that do respond to drug therapy generally experience significant side effects and a delay of 4–6 weeks before a therapeutic benefit is observed. Indeed, often multiple 4–6-week treatment cycles with different drugs are required to identify an effective therapy. Hence, there is a significant medical need for new drug therapies to treat MDD. However, the poor predictive validity of the preclinical methods available to detect the potential efficacy of novel compounds and a lack of common endpoints between preclinical and clinical measures have proven to be major limitations in drug development for MDD. Thus, preclinical and early clinical studies with novel putative antidepressants have often identified promising trends that have not been confirmed by the results of subsequent large Phase III studies. Unfortunately, placebo-controlled trials are difficult to conduct in patients with the type and degree of depression that most requires pharmacological intervention and high placebo response rates can confound detection of positive treatment effects.
Neuroimaging studies are providing important insights into our understanding of the neuroanatomical and neurochemical substrates of MDD. Many imaging methods such as fMRI and PET can be applied in animal species used for preclinical research in addition to being widely used in clinical studies. Consequently, neuroimaging approaches are becoming increasingly valuable for drug discovery and development and the potential translation of preclinical promise to clinical therapeutic benefit.

Neuroimaging methods that have been routinely used to study MDD include MRI, fMRI, MRS, PET, EEG, and more recently MEG. Each method has advantages and disadvantages; for example PET can provide quantitative measurement of neurotransmitter receptor occupancy but requires the development of a high-affinity, selective radiotracer, which is often very challenging. In contrast, BOLD fMRI signals can be recorded at rest (described as the default mode network) and in response to a wide range of stimuli and drugs, but the data generated are indirect and qualitative. Therefore, increasing numbers of clinical development programs are using different methods in parallel (e.g. fMRI and PET) or multimodal imaging approaches, such as the simultaneous acquisition of EEG and fMRI data.

A considerable body of evidence indicates that MDD is associated with blunted reward responsiveness, hypersensitivity to punishment, impaired learning and memory, impaired social cognition, and negative bias. These deficits were identified by extensive studies of patients with MDD in behavioral and cognitive tasks. Imaging studies using MRI, fMRI, and PET have identified a number of brain regions that are functionally and structurally abnormal in MDD and which are implicated in mediating these cognitive deficits. These brain regions include the ACC, amygdala, basal ganglia, hippocampus, OFC, PFC, sgACC, and thalamus. Similarly, in MRS and PET studies, MDD patients have been reported to have reduced levels of neurotransmitter metabolites including GABA, glutamate, and NAA, and alterations in the density and/or affinity of neurotransmitter receptors and transporters, including a number of serotonin receptor subtypes. Some of these deficits have been shown to reverse and/or normalize after successful antidepressant, ECT, and/or TMS treatment.

It is increasingly recognized that the introduction of imaging biomarkers is a potentially significant step forward for drug development in MDD. Such studies bridge the gap between animal and human studies and have the potential to accelerate clinical trials by providing rapid Go/No-Go decisions. Potential biomarkers for MDD that have been identified using different imaging methods (such as EEG, fMRI, and PET) include corticolimbic dysfunction, frontal asymmetry hyperactive amygdala, and hyperactive sgACC. These and other biomarkers (see above) have been used to investigate the neural substrates of MDD and to assess the potential efficacy of novel compounds in early-phase clinical trials.

Since the 1970s, the prevalent hypothesis of drug discovery and development for MDD has been the monoamine hypothesis. This hypothesis arose from the serendipitous discovery of the antidepressant properties of MAOIs and tricyclics, which subsequently led to the development of first-line antidepressant therapies such as SSRIs and SNRIs. As described above, these drug classes have significant limitations in terms of side effects, nonresponders, and a latency of 4–6 weeks before the onset of their therapeutic action. However, the discovery of the rapid-onset antidepressant properties of the NMDA receptor antagonist ketamine together with new imaging approaches to drug discovery have directed interest toward the brain glutamate system as a promising target for new treatments for MDD.
Indeed, it has been suggested that glutamate-induced hyperactivation of NMDA receptors in the sgACC area (BA25) plays an important role in the etiology of depression and may be responsible for the high incidence of comorbid depression observed in diseases with glutamate etiology. This is an exciting example of convergent approaches to drug discovery and development, in which neuroimaging results combined with a novel therapeutic discovery has generated a new working hypothesis that has the potential to drive research for new drug therapies for MDD.

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