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Pharmacological Modulation of Prefrontal Cortex in Affective Disorders

Emerging Therapies

Angela C. Roberts and Conor Liston

Abstract

There is an ever-expanding range of pharmacological treatments for psychiatric disorders but our understanding of their efficacy at the level of disorders, symptoms, and especially at the level of individuals is extremely limited. Neuroimaging studies reveal dysregulation in the higher-order cognitive and emotional control networks of the prefrontal and anterior cingulate cortices in patients suffering from affective disorders such as depression and anxiety. Moreover, successful treatment by antidepressants or anxiolytics is often associated with an amelioration of the dysregulation in these control networks. Treatment resistance is a common occurrence across patients, and without a detailed understanding of the neurobiological actions of efficacious pharmacotherapies, we are still far from being able to tailor the specific classes of pharmacotherapies to any one individual. Important insights into how the different prefrontal control networks may be differentially affected by different classes of antidepressants can be revealed by considering the marked heterogeneity in the neurochemical modulation of prefrontal and anterior cingulate cortices. For example, the distribution of receptors, transporters, and neuronal subtypes that are the targets of current antidepressants, including the monoamine, glutamate, GABA and opiate systems, differentially target those prefrontal and anterior cingulate regions involved in reward, affective, salience, executive, and default mode networks. However, while large-scale patient neuroimaging studies have implicated changes in activity within specific regions of prefrontal and anterior cingulate cortex (and associated networks) as mediators, predictors, and/or moderators of antidepressant efficacy, insight into the differential actions of the different classes of antidepressants has not been forthcoming. Experimental studies in animals, on the other hand, are beginning to provide important insights into cellular and molecular plasticity mechanisms within prefrontal cortex that may underlie antidepressant efficacy. Still, a

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major unanswered question is why there is such marked variation in efficacy between individual patients. Future work needs to directly compare the neuroimaging profiles of different classes of antidepressants in patients and take into account efficacy at the level of specific symptoms as well as treatment history. In addition, a greater focus on the comparison of the actions of different classes of antidepressants is needed in animals alongside a comparison of their actions within distinct regions of prefrontal and anterior cingulate cortex. Only then can we begin to identify the factors that may determine the treatment strategy for any given individual.

Introduction

Only 30–40% of individuals diagnosed with an affective disorder, such as anxiety or depression, show remission following first-line treatments, whether they be pharmacological or behavioral-cognitive-focused therapies. Moreover, even when treatment is successful, the underlying mechanism is poorly understood. As a consequence, it is currently not possible to tailor treatment strategies to individuals. Evidence from functional and structural neuroimaging, as well as postmortem studies of affected individuals highlights the marked alterations in the prefrontal cortex (PFC) that accompany these disorders. Perhaps not surprisingly, many of these alterations are reversed following successful treatment, but the neurobiological, neurochemical, and cognitive mechanisms by which this remission is achieved, and whether the effects of the treatment are due to direct or indirect targeting of prefrontal functioning, is still to be determined.

In this review, we consider the evidence that relates pharmacological treatment strategies with the modulation of prefrontal function, particularly in the context of depression. There have been a number of experimental approaches that have implicated the PFC. The most common in humans has been to image brain structure and function of affected individuals, either at the level of individual brain regions or at the level of connectivity patterns and circuit analysis. In some studies, imaging is performed before and after treatment, and posttreatment changes that are related to treatment efficacy are used to provide insight into the efficacious actions or mediators of the drug. Other studies focus on pretreatment and determine whether differences in brain structure and function between individuals can predict subsequent treatment efficacy. Limitations of all these studies include the issue of clinical heterogeneity, which can be offset somewhat by emerging approaches for stratifying patients according to clinical symptom profiles, behavior, or biological measures, although this is rarely achieved. Few studies compare against a placebo control group, and so for the majority of findings, it is not possible to identify drug-selective biomarkers of treatment response (moderators) separate from those of placebo. Even fewer studies have directly compared different antidepressants, such as selective serotonin inhibitors (SSRIs) versus noradrenergic inhibitors, important to tailor treatment strategies effectively. A less common approach is to study the action

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of pharmacological treatments in healthy controls. This, however, has the major limitation that baseline brain function almost certainly differs from that of affected individuals, thereby influencing the actions of any given pharmacological therapy and limiting the translatability of any results to the clinical condition.

In animals, the effects of antidepressants have either been studied in normal healthy controls, with the same caveats as raised above in humans, or alternatively investigated in chronic stress models that recapitulate some—but not all—behavioral features of clinical affective disorders. The latter include prolonged experience with social stressors (e.g., chronic social defeat, social isolation) or physical stressors (e.g., chronic restraint, chronic unpredictable mild stress) or prolonged treatment with the stress hormone, corticosterone. The efficacy of pharmacological agents to relieve these behavioral changes is then established. An additional dimension in animal studies is that the pharmacological agent can be given not only systemically, as in the clinic, but also centrally, targeting specific brain regions to provide insight into their target of action. Furthermore, rapidly developing approaches for recording and manipulating the activity of large populations of neurons in specific circuits and cell types (e.g., two-photon imaging, photometry, optogenetics) are enabling investigators to establish causal mechanisms linking the molecular effects of a given drug with circuit function and behavior. In all these studies, whether clinical or experimental studies in animals, particular insight is gained when treatments are directly compared with one another, including different pharmacological therapies (e.g., SSRIs versus dopamine transporter inhibitors) or different therapeutic approaches, such as pharmacological versus cognitive behavior therapy (CBT).

We begin with a brief summary of the most consistent alterations in PFC structure and function in depression as revealed by neuroimaging. We then consider the neurochemical signatures of prefrontal brain regions before reviewing our current understanding of their sensitivity to the actions of a range of classes of antidepressants. We focus, in particular, on those agents that target the monoamines as well as the more recently discovered rapid-acting antidepressants (RAADs).

Prefrontal Dysregulation in Depression

Neuroimaging tools have become a mainstay of studies aimed at identifying and characterizing pathological correlates of depression and other affective disorders. While a comprehensive review is outside the scope of this chapter, here we highlight major findings from structural and functional MRI studies of depression, which may be useful for contextualizing the findings reviewed in the following sections on pharmacological effects on PFC function. We focus on the most consistently replicated findings in large-scale studies. Three themes emerge from this literature. First, meta-analyses of structural MRI

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studies, such as those conducted by the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium, confirm that cortical thickness is consistently reduced in multiple areas of the PFC (Schmaal et al. 2017, 2020), including the medial orbitofrontal cortex, anterior cingulate cortex (ACC), and areas of the lateral PFC as well as areas outside the PFC, including the anterior insula, posterior cingulate cortex, and hippocampus. Reductions in cortical volume may be related to changes in the density of neurons or glia or stress effects on dendritic arborization, among other mechanisms (Davidson et al. 2002; Krystal and State 2014). Of note, these effects are modest (Cohen's $d = 0.14–0.17$) and highly variable, but also highly reliable and statistically significant in this meta-analysis which involved over 1,700 patients with unipolar depression and over 7,000 healthy controls. Furthermore, these effects are not specific to unipolar major depression: a meta-analysis of voxel-based morphometry studies spanning six diagnostic groups (schizophrenia, bipolar disorder, major depressive disorder, obsessive-compulsive disorder, substance use disorders, and anxiety disorders) identified three areas with gray-matter volume reductions in all six groups: the dorsal ACC and the bilateral insular cortex (Goodkind et al. 2015).

Second, resting-state fMRI have identified a variety of alterations in functional connectivity in depression-related brain networks (Greicius et al. 2007; Sheline et al. 2010; Yan et al. 2019), some of which may be present only in subgroups of patients with this highly heterogeneous diagnosis (Drysdale et al. 2017; Price et al. 2017). One of the most consistent findings involves bidirectional alterations in functional connectivity seeded from dorsomedial prefrontal areas of the default mode network that are modulated by sex and may relate to a propensity for excessive rumination in some patients (Hamilton et al. 2011; Kaiser et al. 2015; Talishinsky et al. 2022; Yan et al. 2019). Another is reductions in functional connectivity between the salience network (with prefrontal nodes in the lateral PFC and ACC) and midline areas of the default mode network and frontoparietal control networks (Kaiser et al. 2015), which may relate to deficits in emotion regulation (Wager et al. 2008).

Third, the task-based fMRI literature is complicated to interpret owing to methodological differences across studies and smaller sample sizes. Overall, they implicate subcallosal anterior cingulate cortex (scACC) hyperactivity in both normal sadness and depression (Mayberg et al. 1999, 2005), excessive coupling between a hyperactive scACC and default mode network areas in rumination (Grimm et al. 2009; Hamilton et al. 2011), and hypoactivity in ACC, OFC, and striatum in anhedonia (Pizzagalli 2014).

Neurochemical Targets for Antidepressants

SSRIs (e.g., escitalopram, fluoxetine, sertraline, paroxetine) are the first-line treatment for anxiety and major depression in adults, but their efficacy in

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inducing remission is dependent on their chronic treatment in the order of 4–6 weeks. While these drugs target the serotonin transporter (5-HTT), they often have other actions. For example, sertraline is a weak dopamine transporter inhibitor, paroxetine is a weak noradrenergic inhibitor, and fluoxetine targets ion channels and has effects via the SNARE (SNAp REceptors) protein complex. If SSRIs are ineffective, then alternatives include combined serotonin and noradrenergic transporter inhibitors (SNRIs), noradrenergic transporter inhibitors, and combined noradrenergic and dopaminergic transporter inhibitors. In addition, there are mixed drugs, such as vortioxetine, an inhibitor of the 5-HTT but also a receptor antagonist at 5-HT3, 5-HT7, and 5-HT1D receptors and an agonist at 5-HT1A and 5-HT1B receptors. Also, trazadone, which besides inhibiting the 5-HTT is an antagonist at 5-HT2A, 2B, 2C, 2D receptors and at histamine and alpha-1 receptors. On the other hand, the relatively recently identified RAADs include ketamine, a dissociative anesthetic that acts as an antagonist at NMDA receptors but also interacts with binding sites for opioid, monoaminergic, cholinergic, nicotinic, and muscarinic receptors (Mion and Villevieille 2013) and psychedelics such as psilocybin which bind in particular to 5-HT-1A, 2A and 2C receptors as well as mTOR (mammalian target of rapamycin) and TrkB (Dodd et al. 2022).

Neurochemical Signatures of Prefrontal and Anterior Cingulate Cortices

Neurochemical parcellation studies of PFC in humans reveal the marked heterogeneity in the modulation of its regions, which can provide important insight into the likely target of different antidepressants (Figure 13.1a). In terms of overall cortical organization, there is an increase in the diversity of neurotransmitter receptor densities from sensory to association areas including PFC. Along the same sensory to association axis there is also an increase in the ratio of excitation to inhibitory receptor density and a gradient change in ionotropic and metabotropic receptors with ionotropic decreasing and metabotropic increasing (Goulas et al. 2021). This characteristic patterning, which was originally based on autoradiographic analysis, has since been corroborated using positron emission tomography (PET) (Hansen et al. 2022). Moreover, receptor pattern similarities between regions are not only greater between pairs of regions that are anatomically connected but also greater between regions within the same, compared to different intrinsic networks (Hansen et al. 2022). Of specific relevance to our discussion below on prefrontal targets for antidepressants, the scACC (particularly caudal regions including area 25) is a hotspot not only for 5-HTT (James et al. 2019; Palomero-Gallagher et al. 2009b) but also 5-HT1A receptors, although the latter are also dense across much of the rest of medial PFC, extending onto the dorsolateral surface. Since area 25 also sends dense projections into the dorsal raphe nucleus (Freedman

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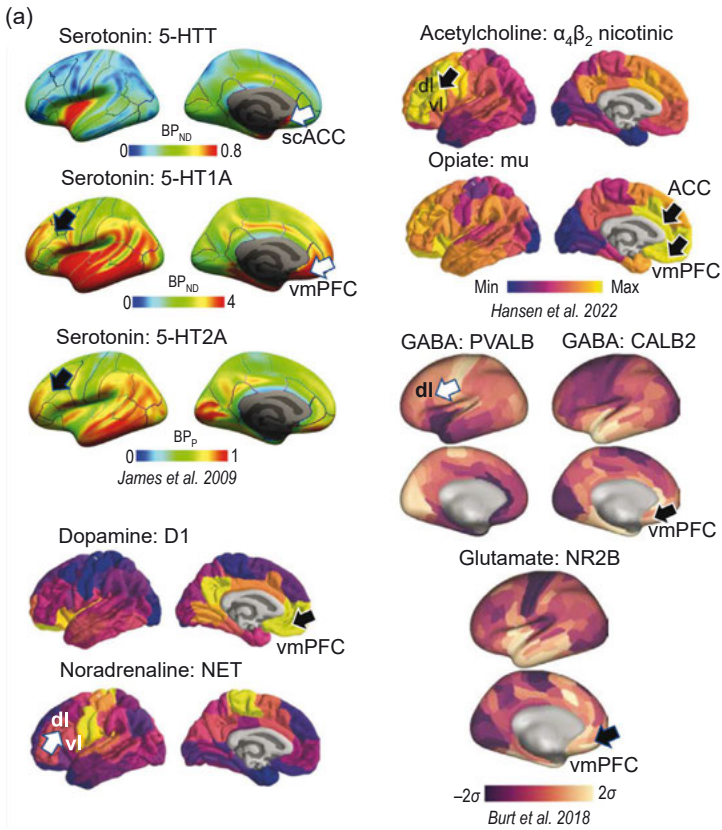

Figure 13.1 Targets of antidepressant actions in the PFC. (a) Distribution of receptors, transporters, and neuronal subtypes across the human brain based on PET (Hansen et al. 2022; James et al. 2019) and postmortem gene expression (Burt et al. 2018). All pictures reproduced with permission under a Creative Commons Attribution 4.0 International License. Arrows (white or black) highlight high densities of different receptors, transporters, and neuronal subtypes in anterior cingulate and prefrontal brain regions (and associated networks) of relevance to the efficacious actions of fast- and slow-acting antidepressants. Note, in particular, high densities of 5-HTT and 5-HT1A receptors in scACC, 5-HT2A receptors in dlPFC and vlPFC, D1 receptors in vmPFC, noradrenaline transporter (NET) in dlPFC and vlPFC, α4β2 acetylcholine nicotinic receptors in dl and vlPFC, mu opiate receptors in vmPFC and ACC, GABAergic pavalbumin (PVALB) and calbindin (CALB2) neurons in vmPFC and NR2B glutamate metabotropic receptors in vmPFC. $\boldsymbol{\sigma}$ – units plotted as standard deviation from the mean.

et al. 2000), which in turn sends serotonergic projections to much of the cortex, it likely has a marked impact on cortical serotonergic transmission more generally (Palomero-Gallagher et al. 2009b). Thus, SSRIs are likely to impact a dysfunctioning subcallosal network while newly discovered psychedelics, the targets of which include 5-HT1A receptors, may have effects that extend into higher-order cognitive networks as well.

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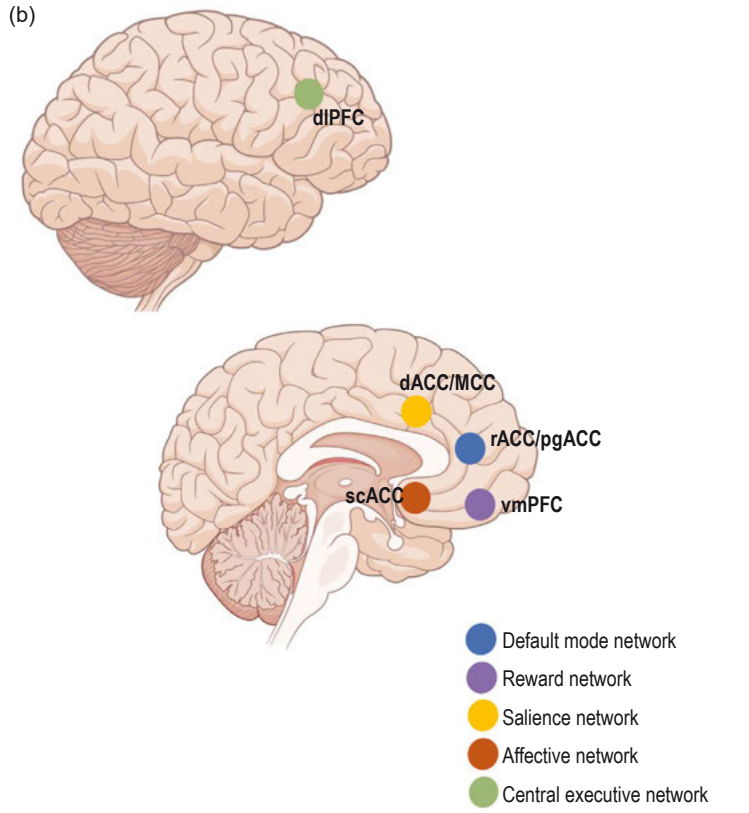

Figure 13.1 *(continued)* (b) Regions across PFC and ACC and their associated resting-state networks implicated in the mediation, prediction, and moderation of antidepressant efficacy.

Dopamine D1 and D2 receptors, alongside the dopamine transporter, also show relatively higher densities in medial PFC, including subcallosal cortex, compared to lateral regions while noradrenergic transporter (NET) and the $\alpha_4\beta_2$ nicotinic receptor show greater densities across dorsolateral and ventrolateral prefrontal regions. Thus, it might be predicted that antidepressants targeting, for example, NET are more likely to influence higher-order cognitive systems within the PFC. On the other hand, while area 25 has a marked abundance of glutamate receptors, including AMPA and NMDA, it has less abundance of metabotropic receptors compared to lateral PFC regions and also less $GABA_A$ and $GABA_B$ receptors compared to both medial and lateral PFC (Palomero-Gallagher et al. 2009b). Indeed, it has been suggested that the antidepressant actions of the RAAD, ketamine, acting as an NMDA antagonist may act early to inhibit an overactive scACC and on a slower timescale to enhance plasticity

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mechanisms in dorsolateral PFC in part via metabotropic receptor pathways (Arnsten et al. 2023; Opler et al. 2016). Ventromedial prefrontal regions, including scACC, also contain high levels of mu opiate receptors with less but still significant expression in OFC and lateral PFC (Hansen et al. 2022). Opiate pathways, as we shall see later, are linked to some of the actions of ketamine. Thus, overall, the distinct neurochemical signatures across regions of PFC and ACC provide a possible substrate for the differential efficacy of distinct classes of antidepressants (see Figure 13.1a).

It should be noted that the above descriptions are largely based on neurochemical receptor distributions in humans. How comparable they are across species, including monkeys and rodents, remains to be fully determined, although in comparison to rodents, macaque and humans have been shown to share a very similar profile of receptors at the regional and laminar level (Zilles and Palomero-Gallagher 2017). Specific examples of differences in the chemical microstructure of cortical circuits between primate and nonprimate species that would impact the efficacy of potential drug treatments include cortical cholinergic suppression (Disney and Robert 2019), of relevance to schizophrenia and Alzheimer disease, and mGluR3 localization in working memory circuit motifs in dorsolateral PFC (Datta and Arnsten 2018), of relevance to a range of psychiatric and neurodevelopmental disorders. For further details, see Izquierdo (this volume).

Monoamine-Targeting Anxiolytics and Antidepressants

Serotonin and Noradrenaline Targeting Therapeutics in Humans

Subcallosal Cingulate Cortex

An early study in which unmedicated patients suffering from major depression disorder (MDD) were imaged before and after treatment using PET (Drevets et al. 2002), a reduction in activity in rostral subcallosal cingulate cortex (scACC) at the level of the genu was revealed following chronic paroxetine (>4 weeks). When compared with CBT in unmedicated MDD patients, remission following 6 weeks treatment with paroxetine (5-HTT inhibitor and weaker NET inhibitor too) was associated with a similar reduction in activity in vlPFC (area 47) to CBT but to have opposite effects in dlPFC (area 9), which in the case of paroxetine were increases. Unique to paroxetine treatment, however, were decreases in the scACC (Goldapple et al. 2004), although the region was more caudal to that shown by Drevets et al. (2002). It was suggested that paroxetine reduced circadian and vegetative systems alongside increasing attentional-cognitive systems. Another study compared CBT with venlafaxine, a mixed serotonin and noradrenergic reuptake inhibitor (Kennedy et al. 2007). There were common decreases in activity in the right and left OFC and left dmPFC while venlafaxine-unique decreases were again seen in the caudal scACC, consistent

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with that seen for paroxetine. In contrast, unique increases in activity were seen in more rostral regions of scACC (area 32) associated with CBT. Comparison of brain activity pretreatment, on the other hand, has shown that increased activity within scACC-perigenual ACC border predicts nonresponse to both CBT and citalopram (Konarski et al. 2009; McGrath et al. 2014). It should be noted that scACC is quite an extensive region along the rostro-caudal axis composed of area 25, 24, and 32 (Öngür and Price 2000; Petrides et al. 2012); thus, alterations in activity within these scACC regions that accompany the treatment response or are predictive of treatment nonresponse may well be functionally distinct. Indeed, given the repeated reports of relationships between scACC, MDD, and its treatment, a more recent study focused on rostral scACC resting-state connectivity, this time comparing 12 weeks randomized treatment with escitalopram (SSRI) or duloxetine (SNRI) with CBT in treatment naïve patients (Dunlop et al. 2017). Again, the focus was on PFC activity pretreatment and the three regions that showed significant connectivity with rostral scACC; namely, vlPFC, vmPFC, and dorsal midbrain. Negative summed functional connectivity scores across all three regions were associated with remitters to medication and treatment failure to CBT, while positive summed scores were associated with remitters to CBT and treatment failure to medication.

In all the studies considered so far, there was no accompanying placebo group. Nevertheless, regions were identified that selectively predicted antidepressant outcomes compared to CBT or vice versa. Thus, placebo effects were unlikely to underlie these differential effects if it is assumed that placebo contributes relatively equally to both treatment strategies. In addition, Dunlop et al. (2017) also highlighted the importance of taking into account the current state of the patient at the time of treatment as a patient's brain state may be very different depending on whether they are treatment naïve or treatment experienced/resistant.

In summary, there is evidence for reductions in activity within caudal scACC (area 25) to accompany the treatment response to SSRI/SNRIs. It should be noted, however, that the associated increases in this region with MDD tend to be located more rostrally at the level of the genu. In addition, positive and negative connectivity, respectively, within this more rostral scACC region with other brain regions differentially predicts a treatment response to SSRI/SNRI and CBT.

Dorsal Anterior Cingulate Cortex

The structure and function of scACC are not, however, the only predictors of treatment outcome with SSRIs and SNRIs. The dorsal anterior cingulate cortex (dACC) has also been implicated. In this case, a task-based rather than resting-state fMRI study showed that increased positive connectivity from dACC to the amygdala (as opposed to negative connectivity), when viewing fearful versus happy facial expressions, was associated with the nonresponse

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to escitalopram, six weeks later (Vai et al. 2016). Reduced positive connectivity from the amygdala to the ACC and to the vlPFC was also reported in the same study. In contrast, changes in activity within dACC after just 1 week of SSRI treatment is predictive of a 6-week therapeutic response. Specifically, the hyperactivity of dACC to fearful facial expressions compared to happy facial expressions seen in depressed patients was reduced one week after treatment with the SSRI, escitalopram (Godlewska et al. 2016). More recently, a slightly more rostral region of ACC, around the level of the genu, showing increased activity to masked sad versus happy facial expressions at baseline, predicted later treatment response to escitalopram (Godlewska et al. 2018). A leave-one-out analysis suggested that activity in ACC was able to predict response status at the level of individuals. Davidson et al. (2003) also implicated dACC in the treatment response to venlafaxine, an SNRI, although here it had appeared that lower activation in this region to negative stimuli, pretreatment, was a predictor of success. Thus far, none of these studies were placebo controlled so the effects were only predictive of treatment responsiveness in general. Where dACC activity did differentiate, in this case, sertraline from placebo, it was under conditions of emotion conflict: the greater downregulation in activity a patient displayed in dACC (along with anterior insula and frontal pole), the better the outcome on sertraline (Fonzo et al. 2019).

Pregenual Anterior Cingulate Cortex

Even more rostrally in ACC, pregenual ACC is also implicated in SSRI treatment prediction (including escitalopram, sertraline, and SNRI, venlafaxine), with no significant differences between treatments. Specifically, intact functional connectivity between rostral anterior and posterior cingulate cortex (a major component of the default mode network) predicted an effective treatment response (Goldstein-Piekarski et al. 2018). This was shown to be independent of any other treatment response predictors such as comorbid anxiety, early life trauma, cognitive impairment, and body mass index. Indeed, structural changes in rostral ACC have been repeatedly identified as predictors of treatment response with SSRIs, including fluoxetine (Chen et al. 2007) and escitalopram (Gunning et al. 2009). Where such a relationship was not found with sertraline, increases in volume within the first week of treatment were significantly correlated with improvement at eight weeks (Bartlett et al. 2018). Functionally, rACC theta has been correlated with antidepressant response in two large trials using either rsMRI with the SSRI, sertraline (Pizzagalli et al. 2018) or EEG with three different medications: escitalopram, sertraline, or venlafaxine, a SNRI (Arns et al. 2015). However, converse results have been reported and the effects are not restricted to SSRIs but also placebo effects (Pizzagalli et al. 2018; Sikora et al. 2016). Thus, its utility for informing treatment selection appears limited. Moreover, greater consideration should be given as to whether the patients are relatively treatment resistant or not.

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Dorsolateral Prefrontal Cortex

Outside of the ACC, dlPFC activity has been reported to be predictive of remission following SSRI and SNRI treatment. For example, greater activation within dlPFC (but not exclusive to this region) was reported in MDD patients compared to controls when performing correct rejections in a go/no-go task involving inhibition, activity that predicted posttreatment improvement in depressive symptoms with escitalopram (Langenecker et al. 2007). In addition, medication-free outpatients with MDD, who displayed remission in the iSPOT-D cohort, showed dlPFC activation during inhibitory no-go responses in a go/no-go task, similar to that seen in controls, whereas non-remitters showed hypoactivity (Gyurak et al. 2016). Of note, inferior parietal activation differentiated SSRI versus SNRI remitters: following SSRI treatment, remitters showed normal activation whereas non-remitters showed hypoactivation. The opposite was true for SNRI remission. This suggests that remission following SSRI and SNRI treatment is dependent on intact dlPFC functional activity. Consistent with this, greater dlPFC functional activation during working memory performance at pretreatment in a subset of patients in the iSPOT-D cohort predicted the extent of the antidepressant response (sertraline, escitalopram and venlafaxine) but only in patients without childhood maltreatment (Miller et al. 2015). Conversely, a volumetric study identified a cluster in the caudal sector of the left middle frontal gyrus that below a certain volume predicted a subset of non-remitters to sertraline, venlafaxine, or escitalopram (Korgaonkar et al. 2015). If reduced volume is taken to reflect reduced functioning then this result is still consistent with the hypothesis that intact functioning of dlPFC is necessary for successful treatment. The predictive value of dlPFC, however, has recently been brought into question in a large placebo-controlled trial. In the EMBARC study with over 100 patients in each group, improvements in the depression score for patients treated with sertraline occurred regardless of the connectivity values in a dlPFC resting-state network (derived from a focused dlPFC seed region), although high connectivity values did predict improvements following placebo and low connectivity differentiated sertraline from placebo (Chin Fatt et al. 2021). These results could be interpreted to suggest that positive treatment outcome for sertraline at high dlPFC connectivity reflected a placebo response, whereas the true impact of sertraline was only seen in those patients with low dlPFC connectivity. It should be noted, however, that the model chosen to describe the dlPFC relationship with placebo and sertraline was also dependent on activity being low within rostral scACC and high in nucleus accumbens and amygdala. Nevertheless, the overall result appears contrary to those studies described above, showing that greater dlPFC activity was predictive of an antidepressant treatment response. Still, the majority of these other studies measured task-based functional activity in dlPFC rather than resting state, which may have

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contributed to the contrasting effects. Importantly, those other studies were without placebo controls and so placebo effects may underlie the positive treatment outcomes. Indeed, additional support for dlPFC activity predicting the placebo response comes from another measure of brain activity within the same EMBARC patient population; namely, arterial spin labeling, rather than BOLD, which revealed that increased dlPFC perfusion only predicted a placebo and not a sertraline treatment response (Cooper et al. 2019). Thus, intact dlPFC activity is a likely prerequisite for placebo-induced improvements and is hypothesized to reflect active cognitive appraisal mechanisms contributing to the impact that expectation of mood enhancement can have on mood state (Zilcha-Mano et al. 2019).

Dopamine-Targeting Therapeutics

Although most monoaminergic antidepressants and anxiolytics target serotonin or noradrenaline signaling, at least two important drugs target dopamine as well. First, as noted above, bupropion is a noradrenaline-dopamine reuptake inhibitor and is among the most commonly prescribed drugs that target dopaminergic signaling in depression. Its antidepressant effects, however, are thought to be driven primarily by effects on noradrenergic signaling, due in part to the fact that its effects on dopamine reuptake are modest compared to its effects on noradrenaline. Very few studies to date have examined bupropion effects on PFC function in depression. In one such study, involving ten patients with unipolar depression scanned before and after an 8-week course of treatment, bupropion was found to reduce fMRI responses to negative emotional visual stimuli in the right OFC, left dmPFC, right vmPFC, right ACC, and right inferior frontal cortex (Robertson et al. 2007). Second, pramipexole is a relatively selective D2 receptor agonist, which is not indicated as a monotherapy for depression or anxiety but is frequently used as an augmentation strategy, especially for patients with pronounced anhedonia. Again, very few studies have examined pramipexole effects on PFC in depression, but those that have indicate that pramipexole may modulate prefrontal activity in the context of reward processing. For example, Whitton et al. (2020) found that in patients with depression, reward learning was slowed, with modestly blunted reward prediction error signals and modestly increased amphetamine-induced dopamine release as indexed by PET. Pramipexole improved depressive symptoms, including hedonic function, but had no direct effect on reward learning in the lab. Baseline reward learning, D2 receptor availability, and amphetamine-induced dopamine release did, however, predict greater improvements. As noted above, in both of these studies, there was no placebo control arm, so it is unclear whether changes in activity were related to bupropion or pramipexole treatment versus nonspecific improvements in mood.

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Summary

Many regions across the prefrontal, orbitofrontal, and anterior cingulate regions have been implicated in monoamine-targeting antidepressant treatment responses in patients with MDD. In many cases, whether the brain changes that accompany or predict successful treatment are due to the antidepressant itself cannot be determined since a placebo control group has been lacking. Where placebo controls have been studied, it is evident that there is considerable overlap in the prefrontal circuitry predicting a placebo response and that predicting an antidepressant response. In some cases, the same brain region is implicated in both, differing only in the direction of the relationship. For example, while high levels of dlPFC activity predict a placebo response, low levels are more likely to predict a response to SSRIs compared to placebo (Chin Fatt et al. 2021), especially when levels in rostral subcallosal cingulate are also low. Activity in ACC is also variably associated with antidepressant response. Activity in pregenual regions is associated with placebo and so does not appear selective for antidepressants (Pizzagalli et al. 2018; Sikora et al. 2016), while at least one study shows differential task-based activity in dACC related to sertraline and not placebo (Fonzo et al. 2019). Finally, right inferior orbital frontal gyrus is selective for sertraline over placebo (Cooper et al. 2019).

Even less well established are differences between the varied monoamine-targeting antidepressants within PFC and ACC. This is somewhat surprising since the pattern of innervation of the monoamines differs markedly across the distinct regions of PFC (see above). One study compared sertraline, bupropion, and placebo but the only selective predictors for buproprion (a noradrenergic and dopaminergic uptake inhibitor) that were located in the PFC were higher anticipatory activity in the superior frontal gyrus and higher reward expectancy activation in the orbitofrontal cortex, both of which predicted less improvement with buproprion (Nguyen et al. 2022). Moreover, the caveat here was that patients who were moved on to buproprion failed to show a response to sertraline, so not only were numbers considerably lower but the cohorts distinct and thus comparison made difficult. When comparing SSRIs and SNRIs, little in the way of differences has been noted although opposing alterations in inferior parietal cortex did differentiate remitters from non-remitters between the two (Gyurak et al. 2016).

Cellular Mechanisms in Animals

There have been far fewer studies in experimental animals aimed at determining the prefrontal locus of action of monoaminergic antidepressants, and those that have are evenly spread across healthy controls and chronic stress models. Perhaps even more surprisingly, there have been very few studies that have compared different types of monoamine-targeting antidepressants, with the vast majority focusing on the relatively selective SSRI, fluoxetine. In most

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cases, fluoxetine is given systemically to match treatment regimes in the clinic, and medial regions of the PFC (mPFC) have been the primary focus. The rodent PFC is much less complex than in humans and other primate species, but anterior cingulate, prelimbic, and infralimbic cortex are thought to share some cytoarchitectural, functional, and anatomical features with the primate anterior cingulate, dorsomedial, and ventromedial PFC, respectively. However, very often the precise region within mPFC is not detailed, and rarely are different regions compared. Moreover, the OFC has been largely ignored, despite changes occurring within this region, both in patients with depression and in stress-induced models of depression in rodents. What is clear from these studies, though, is that fluoxetine has a marked impact on a range of measures of physiological function within mPFC. In intact animals, prolonged daily treatment with fluoxetine for anything between 2–4 weeks has been reported to alter the excitatory-inhibitory balance in the prelimbic cortex, with an increase in pyramidal cell firing and reduction in interneuron firing (Yin et al. 2021). In particular, chronic fluoxetine has been shown to reduce selectively parvalbumin but not other GABAergic interneurons within mPFC (Ohira et al. 2013; for opposite effects on mPFC parvalbumin neurons *in vitro*, see Zhong and Yan 2011). The accompanying reduction of perineuronal nets, a marker of neuronal maturation suggests one aspect of antidepressant action may be to reinstate a juvenile state of plasticity. A de-maturation of astrocytes has also been reported alongside dynamic changes in 5-HT1A receptors and upregulation of brain-derived neurotrophic factor (BDNF), which is also argued to be consistent with long-term neurotrophic effects (Song et al. 2021). Comparison of citalopram, a relatively selective SSRI, with the mixed antidepressant, trazadone (which is not only an SSRI but also a serotonin 2A/B receptor antagonist with effects on histamine and alpha-1 adrenergic receptors), found comparable effects on clock genes in mPFC but differentiable effects on BDNF and TrkB receptors. Only trazodone increased these in the mPFC while citalopram's effects were unique in the nucleus accumbens and amygdala, respectively (Carboni et al. 2022). When task-based firing patterns of mPFC have been investigated, chronic treatment with fluoxetine has been associated with overall reductions in firing related to the reward-predicting stimulus, likely related to a less redundant encoding capacity and a less robust encoding of information (Pereyra et al. 2021). The hypothesis that this may reflect increased flexibility, however, remains to be determined.

Of more relevance to our understanding of the actions of chronic treatment for ameliorating anxiety and depression are their effects on stress-induced models of anxiety and depression-like symptomatology in animals. In the majority of examples, regardless of the nature of the stressor (physical, social, or physiological), anxiety- or depression-like behavioral changes induced by the stressor are ameliorated or prevented by chronic treatment with the SSRI, fluoxetine. Such treatment can also ameliorate the accompanying changes in mPFC function brought about by the stressor, such as the downregulation of

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cytosolic proteins and upregulation of nonsynaptic mitochondria (Filipović et al. 2022) and reductions in BDNF protein levels (Misztak et al. 2021), the latter consistent with the effects of chronic fluoxetine in normal controls (Song et al. 2021). Moreover, such treatment also reverses the reduced gap junction function specifically within prelimbic cortex, reported to occur after chronic unpredictable mild stress (Xia et al. 2023) as well as the gliogenesis that occurs after chronic social defeat stress (Czéh et al. 2007). Where a mixed 5-HT drug has been used, vortioxetine, (targeting 5-HT receptors and the 5-HT transporter), this has been shown to reverse the inhibitory effects of chronic mild stress and chronic social defeat on mTORC1 signaling, important for protein synthesis and plasticity (Li et al. 2023). Chronic fluoxetine also reverses the desensitization of α2-adrenoceptors within mPFC following chronic slow release corticosterone (Horrillo et al. 2019) and inhibits microglial activation, regulates the Notch signaling pathway, and inhibits the inflammatory response within mPFC in a liposaccharide model of depression in Parkinson disease in rats (Zhang et al. 2022). In contrast, in a PTSD model involving severe acute footshock, the efficacy of chronic fluoxetine to reverse the subsequent increase in immobility in the forced-swimming test was only associated with its ability to also reverse the accompanying increases in the expression of the immediate early gene, cfos, in the amygdala, but not the prelimbic cortex or anterior cingulate (Cg1) (Yu et al. 2020).

A limitation of the above studies, which will be seen to be a reoccurring limitation throughout this review, is the lack of repeatability and comparability. The vast range of cellular mechanisms that have been studied across intact and stress-induced animal models makes it difficult to provide a comprehensive synthesis. However, effects on the variety of plasticity mechanisms available to the central nervous system is a common theme which likely underlies the changes in functional connectivity following successful treatment in patients.

The Rapidly Acting Antidepressant, Ketamine

The majority of individuals with depression will not show a full response to their first monoamine-targeting antidepressant trial (Rush et al. 2006). These limitations led investigators to pursue other antidepressant mechanisms that might yield more rapid responses, even in treatment-resistant individuals. Motivated by evidence that glutamatergic signaling in the PFC and other stress-sensitive brain regions may be altered in depression (Auer et al. 2000; Duman et al. 2019; Sanacora et al. 2004), these efforts led to clinical trials of ketamine, an NMDA receptor antagonist and dissociative anesthetic. In one early trial, seven patients with severe depression received an intravenous infusion of a subanesthetic dose of ketamine or saline on two separate days, and investigators observed potent antidepressant effects just six hours after treatment that persisted for at least three days (Berman et al. 2000). Larger-scale clinical

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trials with more robust placebo controls followed, confirming rapid and potent antidepressant effects (Cohen's $d > 1.4$) in both unipolar and bipolar depression that persisted in some individuals for up to a week (Diazgranados et al. 2010; Murrough et al. 2013; Zarate et al. 2006, 2012). This led in 2019 to FDA approval of esketamine, an intranasal formulation of ketamine's (S) enantiomer, for treatment-resistant depression.

Here, we consider insights gained into the molecular and circuit-level mechanisms of ketamine's actions within the PFC from studies in animal models before reviewing insights gained from neuroimaging studies.

Molecular Mechanisms

In preclinical rodent models, early studies in this field showed that ketamine's antidepressant properties are most likely mediated in part by effects on neuronal function and synapse formation in the mPFC. As has been the case with investigations into the monoamine-targeting antidepressants, mPFC has been the primary focus for many ketamine studies in mice and rats with no studies to date having examined these mechanisms in OFC. Studies have shown that ketamine causes a rapid increase in the expression of glutamatergic AMPA receptors, PSD95, and other synaptic markers in the prelimbic area of PFC in rats that correlated with changes in depression-related behavior (Li et al. 2010a). The same study in rats showed that these effects are mediated by NMDA receptor antagonism and are blocked by a prefrontal cortical infusion of rapamycin, implicating downstream effects on the mTOR signaling pathway. However, a subsequent study showed that unexpectedly, when rapamycin was systemically infused alongside ketamine in patients with depression, the antidepressant effects were not attenuated (Abdallah et al. 2020b). This may be related to confounding effects of a systemic infusion on inflammation, which may not occur with direct infusion into the PFC.

Ketamine's antidepressant effects are also driven by neurotrophic signaling. A parallel series of studies showed that ketamine's antagonism of NMDA receptor signaling enhances activity-dependent release of BDNF by de-suppressing its translation within neurons (Autry et al. 2011). Ketamine's effects on depression-related behavior, in turn, require BDNF and its receptor, TrkB (Autry et al. 2011; Lin et al. 2021). Enhanced activity-dependent release of BDNF may be especially important for sustaining ketamine's effects over time, through downstream effects on methyl-CpG-binding protein 2 (MeCP2) phosphorylation, which is required for maintaining ketamine's effects on behavior and long-term synaptic potentiation (Kim et al. 2021). In addition, at least one report indicates that ketamine's effects on BDNF signaling may be driven not only by NMDA receptor antagonism but also by direct binding to its receptor TrkB (Casarotto et al. 2021), an effect potentiated by astrocyte-derived cholesterol. Ketamine's interactions with the TrkB receptor facilitated BDNF signaling in active synapses and increased the expression of TrkB on

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dendritic spines. Conversely, mutating a specific ketamine-binding motif in the TrkB receptor blocked the effects of ketamine on depression-related behavior, synapse function, and plasticity. Interestingly, fluoxetine and a variety of monoamine-targeting antidepressants were also found to bind directly to TrkB, but different compounds accumulated at different rates in mPFC tissue (specific subregions were not studied here), suggesting one potential mechanism by which ketamine may elicit rapid antidepressant effects while fluoxetine and other SSRIs operate on slower time scales.

Importantly, ketamine is not a selective NMDA receptor antagonist; its effects on depressive symptoms and PFC function may also be driven by its other pharmacological properties. Recent studies have shown that hydroxynorketamine, an active metabolite of ketamine, may act to promote synapse formation and antidepressant effects through direct effects on AMPA receptors (Zanos et al. 2016), although other studies point to a role for NMDAR inhibition by hydroxynorketamine (Suzuki et al. 2017). Furthermore, ketamine is also a mu opioid receptor (MOR) agonist and its effects may be mediated in part by opioid receptor signaling systems (discussed further below). Together, these studies indicate that ketamine acts to relieve depressive symptoms rapidly via multiple molecular mechanisms, including NMDA receptor antagonism, activity-dependent BDNF release, and other neurotrophic signaling. Downstream effects on MeCP2 phorphorylation, in turn, play a critical role in synaptic potentiation and sustaining the antidepressant behavioral effects over time.

Circuit-Level Mechanisms

The studies reviewed above underscore a molecular mechanism involving NMDA receptor antagonism and activity-dependent BDNF signaling that culminates in prefrontal cortical synapse formation, implying a causal role for synaptogenesis in mediating its antidepressant effects. Until recently, it was challenging to test this hypothesis directly, but new approaches for *in vivo* imaging and optogenetics have made such studies possible (Figure 13.2). For example, two-photon laser-scanning microscopy combined with chronically implanted cranial windows or microprisms, which provide optical access to the PFC (Andermann et al. 2013; Low et al. 2014), have enabled researchers to characterize the time course of synaptogenesis after ketamine treatment precisely. One such study showed that ketamine has rapid effects on the formation of dendritic spines, microscopic protrusions from neuronal dendrites that usually contain functional synapses, and that these effects on prefrontal spinogenesis were rapid and persisted for at least two weeks (Phoumthipphavong et al. 2016), when the vast majority of dendritic spines will contain functional synapses (Holtmaat and Svoboda 2009; Knott et al. 2006). This study focused on the dorsal medial frontal cortex (also known as M2), which approximates the primate premotor cortex, indicating that ketamine's effects on synaptogenesis may be more generalized across cortical areas than previously appreciated.

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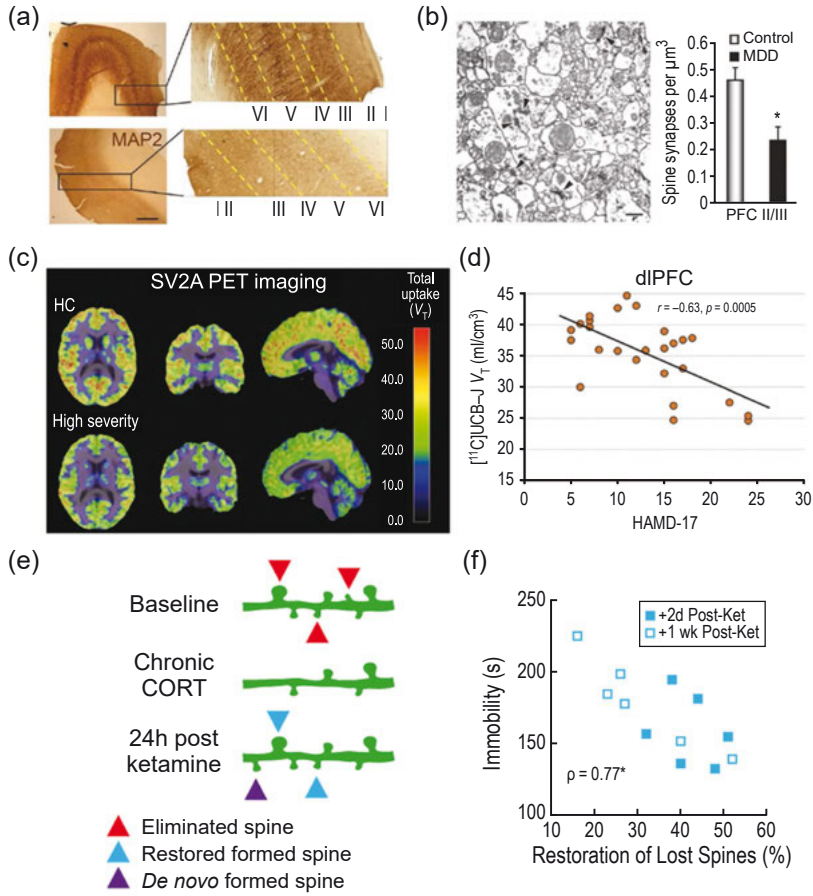

Figure 13.2 Ketamine rescues dendritic spine loss in PFC. (a) Immunohisto-chemistry images from postmortem human brain tissue showing reduced expression of the synaptic protein MAP2 in the dorsolateral PFC from a patient with depression (lower image) compared to an individual without depression (upper image). (b) Electron micrograph of synapses (marked by arrowheads) in layer II/III of the dlPFC in a depressed patient (left). Synapse density was reduced in MDD compared to control individuals (right). (c) SV2A PET imaging reveals reduced synapse density in patients with severe depression compared to healthy controls. (d) Synapse density (as indexed by [^{11}C] UCB-J V_T) correlated with depression severity (as indexed by HAMD-17). (e) Schematic showing how chronic corticosterone exposure eliminates postsynaptic dendritic spines in mice (red arrowheads), and ketamine restores spines to their original position (blue arrowheads). (f) Restoration of lost spines by ketamine was correlated with maintenance of ketamine's antidepressant-like effects on immobility in the tail suspension test. Panels (a) and (b) were adapted with permission from (Kang et al. 2012), panels (c) and (d) with permission from Holmes et al. (2019), and panels (e) and (f) with permission from Moda-Sava et al. (2019).

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Of note, in almost all experiments from the studies reviewed above, ketamine was administered to "unstressed" mice or rats (i.e., in the absence of a chronic stress treatment), so it is unclear to what extent ketamine might engage prefrontal targets differently in a chronic stress state. Furthermore, it is unclear how ketamine effects on synapses and circuit function relate to those induced by stress. One recent study addressed these questions, showing that ketamine acts in a targeted way to reverse some effects of chronic stress and that prefrontal spinogenesis is required for supporting ketamine's antidepressant effects (Moda-Sava et al. 2019). Two-photon imaging showed that chronic stress causes spatially clustered, dendritic branch-specific synapse loss in the mPFC, and that ketamine acts in a targeted way to restore lost spines. These effects were observed in all three mPFC subregions, including ACC, prelimbic cortex, and infralimbic cortex. They were associated with parallel effects on functional connectivity and neuronal activity in multicellular ensembles that were disrupted in a neuroendocrine model of chronic stress, restored by ketamine, and required for driving motivated escape behavior. Unexpectedly, ketamine's effects on circuit function and behavior were evident just three hours after treatment and preceded its effects on spine formation, which did not emerge until 12 hours after treatment; this indicates that new spines were not required for initiating ketamine's antidepressant effects. However, using a newly developed optogenetic tool to selectively delete newly formed synapses, Hayashi-Takagi et al. (2015) showed that prefrontal synaptogenesis was required for sustaining ketamine's effects on prefrontal circuit function and behavior over time. Of note, these effects were specific: deleting newly formed synapses did not interfere with ketamine's effects on sucrose preference behavior, indicating a specific role for prefrontal synaptogenesis in sustaining effects on some depression-related behaviors (e.g., motivated escape behavior) but not others.

If prefrontal synaptogenesis is required only for sustaining ketamine's antidepressant effects, what then are the circuit-level mechanisms that initiate those effects? This is an outstanding question for the field, but converging data from several studies indicate that GABAergic interneurons may be involved. In one study, cell-specific deletion of GluN2B, an NMDA receptor subunit in somatostatin (SST)- or parvalbumin (PV)-expressing interneurons, but not glutamatergic pyramidal cells, in the prelimbic and infralimbic regions of mPFC was sufficient to block ketamine's effects on depression-related behavior (Gerhard et al. 2020). Another study went on to show that antidepressant-dose ketamine suppresses the activity of SST interneurons in the anterior cingulate and dorsal PFC, reducing dendritic inhibition and enhancing calcium signals in prefrontal pyramidal neurons (Ali et al. 2020). Together, these studies suggest that ketamine may initiate its antidepressant effects by silencing SST interneurons, disinhibiting prefrontal pyramidal neurons (Ali et al. 2020; Gerhard et al. 2020), and restoring multicellular ensemble events, which may in turn drive the formation of new synapses that sustain these effects over time (Moda-Sava et al. 2019). Future studies will be required to test this model.

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An alternative approach, recently adopted in studies in nonhuman primates, has studied the efficacy of antidepressants on their ability to ameliorate specific symptoms induced by select targeted interventions highly associated with depressive illness. Specifically, systemic ketamine given 24 hours earlier ameliorated the anticipatory anhedonia (blunted appetitive arousal) but not the heightened anxiety induced by overactivation of caudal scACC (Alexander et al. 2019, 2020) in marmosets. By applying chemogenetics to obtain pathway specificity, it was shown that the anticipatory anhedonic effects could be localized to overactivation of the subcallosal-accumbens pathway and not the subcallosal-amygdala pathway, and that ketamine could ameliorate the anhedonia through its actions at the level of the nucleus accumbens (Wood et al. 2023). In the next section, we discuss how these findings are consistent with a recent imaging study in humans in which ketamine differentially blocked scACC hyperactivity to positive, but not negative, processing in depressed patients (Morris et al. 2020). Studies such as these open up the possibility of differentiating the actions of distinct classes of antidepressants on symptoms induced by specific network dysfunction.

Human Neuroimaging Correlates of Ketamine's Antidepressant Effects

Despite the relatively underdeveloped PFC of rats and mice upon which most experimental studies have been performed, converging data from human neuroimaging studies indicate that similar mechanisms may be operative in patients with depression. Magnetic resonance spectroscopy (MRS) is a tool that provides for the direct, noninvasive measurement of specific neurotransmitters in the living human brain. MRS studies have shown that depression is associated with a reduction in glutamate and glutamine availability in the dmPFC, ACC, and vPFC (Auer et al. 2000; Hasler et al. 2007; Moriguchi et al. 2019; Rosenberg et al. 2005). Ketamine acts to reverse these deficits, causing a rapid increase in glutamate availability in the PFC (Abdallah et al. 2018; Milak et al. 2016, 2020) and ACC (Rowland et al. 2005). While most MRS studies to date have not been able to resolve region-specific effects of ketamine on glutamate signaling in specific subregions of the PFC, future studies employing larger magnetic field gradients may be able to resolve such effects. This could be useful for characterizing associations between glutamate signaling and changes in specific PFC-dependent behavioral domains.

More recently, the development of new ligands for PET have enabled new approaches to studying synapse function directly and noninvasively in the human brain. PET studies of radioisotope binding to synaptic vesicle glycoprotein 2A (SV2A) have shown that depression is associated with reduced synapse density in the anterior cingulate and dlPFC (Holmes et al. 2019). A similar approach showed that ketamine reduces metabotropic glutamate receptor availability (mGluR5), which may be a compensatory response to a surge in glutamate release (Esterlis et al. 2018). These effects were most pronounced

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in the anterior cingulate, medial PFC, OFC, and striatum, among other areas. Unexpectedly, an SV2A-PET study of patients before and 24 hours after ketamine did not observe any significant effects on synapse density at the group level (Holmes et al. 2022). However, a post hoc exploratory analysis found that patients with lower prefrontal synapse density prior to treatment did show a significant increase in synapse density 24 hours after ketamine, consistent with effects reviewed above in rodent models, indicating that ketamine may act in a targeted way to restore synapses lost during chronic stress (Duman et al. 2019; Moda-Sava et al. 2019; Phoumthipphavong et al. 2016).

A host of effects on PFC function after ketamine treatment have been identified through fMRI. In one study, for example, anterior cingulate activity in response to fearful faces was reduced in depressed patients compared to healthy controls, and the magnitude of this effect correlated with increased likelihood of later responding to ketamine (Salvadore et al. 2009). In accord with its effects on synapse formation in rodents, ketamine appears to modulate functional connectivity in the human brain as well, as indexed by changes in the degree to which low-frequency fluctuations in the fMRI BOLD signal are correlated between brain regions. Previous work showed that an area of the dmPFC ("the dorsal nexus"), which is functionally coupled with three depression-related brain networks (the default mode network, the frontoparietal cognitive control, and the rostral affective network) exhibits increased functional connectivity in depression (Sheline et al. 2010), and subsequent work showed that ketamine rescues those effects, reducing dorsomedial prefrontal functional connectivity (Scheidegger et al. 2012). Indeed, ketamine has been shown to impact within and between connectivity of the default mode, affective, reward, central executive, and salience networks as well as for activity within these networks to act as biomarkers of treatment response (reviewed in Demchenko et al. 2022). "Global brain connectivity"—a distinct measure indexed by correlating the BOLD signal in a given region with every other area of gray matter and averaging across areas—has recently been employed to study treatment predictors in depression. Originally it was used to identify reductions in the PFC and increases in posterior midline structures, including the posterior cingulate cortex and precuneus in depression (Abdallah et al. 2017). It has subsequently identified a unique brain connectome fingerprint that predates and predicts the response to the slow-acting antidepressant, sertraline, and preliminary evidence suggests it also predicts response to ketamine (Nemati et al. 2020). Elaboration of this approach has since identified a ketamine-induced connectivity fingerprint from control subjects that at one week posttreatment predicts the success of sertraline at eight weeks (Abdallah et al. 2020a), highlighting the overlap of action of slow- and fast-acting antidepressants at the level of prefrontal connectivity.

In summary, the studies reviewed above indicate that ketamine's effects on molecular signaling, synapse formation, and circuit formation in rodent models are probably associated with pronounced effects on prefrontal network

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function and functional connectivity in the human brain. Most notable is the considerable overlap in the prefrontal networks affected by both ketamine- and monoamine-targeting antidepressants.

Mu Opioid Receptor Signaling As a Therapeutic Target

The studies reviewed above indicate that ketamine acts to restore lost synapses in the PFC by antagonizing NMDA receptor signaling and potentiating BDNF and TrkB signaling. Still, as noted above, ketamine has numerous other pharmacological properties that could also be involved. Among these is MOR agonism. In a recent study, Bonaventura et al. (2021) screened over 100 receptors and enzymes and found that ketamine had potent interactions of comparable magnitude with both MORs and NMDARs. To test whether MOR signaling might be required for mediating ketamine's antidepressant effects, Williams et al. (2018) co-treated depressed patients with intravenous infusions of ketamine and naltrexone, which antagonizes both mu and kappa opioid receptors, or with ketamine alone. They found that naltrexone blocked the antidepressant effects of ketamine without interfering with its dissociative properties (Williams et al. 2018), and it also disrupted ketamine's therapeutic effects on suicidal ideation (Williams et al. 2019). In a similar study of five patients with comorbid depression and alcohol use disorder, naltrexone did not interfere with the antidepressant effects of ketamine (Yoon et al. 2019), but it was unclear to what extent these benefits were attributable to ketamine versus naltrexone, which is an established treatment for substance use disorders. Thus, additional studies are required to resolve these discrepancies. Taken together, these results are consistent with the hypothesis that ketamine's antidepressant effects may involve MOR signaling, at least in patients without comorbid substance use disorders.

Preclinical studies lend further support to this hypothesis. In one study, Bonaventura et al. (2021) used esketamine (an S-ketamine enantiomer) to activate MOR signaling, and converging behavioral data showed that it was reinforcing in rats as measured by self-administration and conditioned place preference. PET studies in the same report showed that esketamine stimulated dopamine release in the mPFC, lending further support to an MOR-associated reinforcing mechanism. Likewise, Klein et al. (2020) showed that opioid antagonists blocked the effects of ketamine on depression-related behavior and hyperactivity in the lateral habenula in rats. Finally, Samuels et al. (2017) showed that tianeptine, an atypical antidepressant with an unknown mechanism of action, also requires MOR signaling for mediating its antidepressant behavioral effects. Interestingly, tianeptine-induced MOR signaling had opiate-like effects on reward processing and analgesia but did not lead to tolerance or withdrawal, indicating that distinct mechanisms—possibly involving distinct circuits or cell types—may be involved in mediating MOR-dependent antidepressant effects versus MOR-driven reinforcement and addiction potential.

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Although these latter studies did not examine prefrontal function, they lend further support to the hypothesis that MOR signaling may be a viable target for developing new antidepressants and warrant further study.

Psychedelic Compounds

A growing body of work has begun to investigate the therapeutic potential of psilocybin and other psychedelic compounds, building on early work in the 1950s and 1960s (Vollenweider and Kometer 2010). Two randomized controlled trials published in 2016 triggered renewed interest in this topic, showing that psilocybin—the primary psychoactive compound in hallucinogenic Psilocybe mushrooms—had potent antidepressant and anxiolytic effects in patients with life-threatening cancer that emerged rapidly after a single dose and persisted for six months in many individuals (Griffiths et al. 2016; Ross et al. 2016). Subsequent small-scale open-label studies extended these antidepressant effects to individuals with severe treatment-resistant depression unrelated to a medical diagnosis (Carhart-Harris et al. 2016; Davis et al. 2021). In 2021, a larger study confirmed these observations in a randomized controlled trial, showing that psilocybin was statistically superior to escitalopram for achieving sustained remission (Carhart-Harris et al. 2021). Although the conclusions that can be drawn from these studies are associated with some important caveats—including small sample sizes (ranging from 12 to 59 patients) and technical difficulties in providing a convincing placebo control for a hallucinogenic drug—they are also an important step forward in efforts to develop other rapid-acting antidepressants in addition to ketamine.

Our understanding of the underlying mechanisms is still developing. Like ketamine, a single dose of psilocybin is sufficient to drive rapid and sustained increases in postsynaptic dendritic spine density, accelerated spine formation, and enhanced glutamatergic neurotransmission in a region of the dorsal frontal cortex in mice that is analogous to primate premotor cortex (Hesselgrave et al. 2021; Shao et al. 2021). These effects emerged within one day of treatment, correlated with antidepressant-like behavioral effects, and persisted in an attenuated form for at least one month. Interestingly, while the hallucinogenic and psychotomimetic effects of psilocybin in humans are widely understood to be driven by direct effects on serotonergic (5-HT2A) signaling (Kwan et al. 2022), the antidepressant effects may be driven by other mechanisms. For example, in mice, pretreatment with ketanserin, a potent 5-HT2A receptor antagonist, blocked the effects of psilocybin on head-twitch behavior (a commonly used screening assay for hallucinogenic potential) but did not interfere with effects on depression-related behavior or spine formation (Hesselgrave et al. 2021; Shao et al. 2021). Also, in accord with the hypothesis that the therapeutic and hallucinogenic properties of psychedelic compounds might be dissociable, other studies have identified structural analogs of psychedelic compounds that

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have no effect on head-twitch behavior in mice but retain their therapeutic effects on depression- and addiction-related behavior (Cameron et al. 2021; Dong et al. 2021).

Very few studies have systematically examined the network-level substrates of these effects in humans, but those that have suggest that psilocybin may alter functional connectivity in prefrontal cortical areas. In one such study, psilocybin or placebo was administered to 15 healthy volunteers and a significant decrease in functional connectivity between the dorsomedial PFC and posterior cingulate cortex was observed (Carhart-Harris et al. 2012). Subsequently, Carhart-Harris et al. (2017) showed that psilocybin treatment in 19 patients with treatment-resistant depression caused an increase in functional connectivity between ventromedial prefrontal and lateral parietal areas of the default mode network, as well as decreased cerebral blood flow in the amygdala and increased amygdala BOLD responses to emotional faces (Roseman et al. 2018). Finally, a third study in depressed patients showed that psilocybin treatment caused a rapid decrease in network modularity measures derived from functional connectivity data and involving multiple areas of the PFC—effects that may have been especially pronounced in 5-HT2A receptor-rich areas (Daws et al. 2022).

Current Limitations and Future Strategies

While considerable insights have been gained into the actions of antidepressants on PRC function at the molecular, cellular, network, and behavioral levels of analysis, we have not yet identified the critical factors that determine the differential responsivity of individual patients to antidepressants. Overwhelming evidence suggests that a wide variety of prefrontal regions and their associated circuits act as both mediators and predictors of antidepressant efficacy (Figure 13.1b) and that changes in plasticity and thus connectivity within and between functional circuits underlie symptom improvement. Although selected regions or circuits have been implicated at the level of individual studies, these differ across studies. One of the challenges in synthesizing findings across studies is that different approaches are used to acquire and analyze data. It is very rare to see one study attempt to prospectively replicate another—a major need for the field going forward. Furthermore, most studies tend to involve relatively small samples, on the order of tens of subjects, especially when an antidepressant treatment is involved; this may lead to false positives, inflated effect sizes, and varying results across studies (Elbau et al. 2023; Marek et al. 2022; Schmaal et al. 2020). Moreover, the primary outcome measure is nearly always a change in the global depression score with little focus on specific symptom recovery; the latter on occasion proving effective at identifying subtypes and parsing heterogeneity of depression (Drysdale et al. 2017; Goldstein-Piekarski et al. 2022; Spielberg et al. 2013, 2014; Williams 2016; Xia et al. 2018). In addition, the

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lack of placebo controls is very often a major caveat, alongside the relatively few studies that have directly compared antidepressant therapies. The ethical constraints on such studies is, of course, enormous because of the vulnerability of the patients under study, especially if they are treatment resistant. This makes direct comparison of rapidly acting antidepressants with the more traditional monoamine-targeting antidepressants, controlling for past treatment and overall depression severity, fraught with difficulties. This is where additional insights can be obtained from experimental studies in animals but surprisingly, direct comparisons of different antidepressants has so far been relatively rare. So, too, have comparisons across prefrontal brain regions, including the OFC, even though all these regions have been associated with stress-related changes; although not all in the same direction. For example, stress has been reported in some cases to potentiate synaptic plasticity and connectivity in the OFC compared to the reductions most often associated with medial PFC (reviewed in Pizzagalli and Roberts 2022). The extent to which antidepressant mechanisms are conserved across species is also unknown. Thus, future studies would benefit from a greater comparative approach, not only at the level of the different pharmacotherapies but also the distinct prefrontal/orbitofrontal regions and the distinct symptom-related behavioral functions.

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