Prefrontal cortex circuits in depression and anxiety: contribution of discrete neuronal populations and target regions

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Abstract

Our understanding of depression and its treatment has advanced with the advent of ketamine as a rapid acting antidepressant and the development and refinement of tools capable of selectively altering the activity of populations of neuronal subtypes. This work has resulted in a paradigm shift away from dysregulation of single neurotransmitter systems in depression towards circuit level abnormalities impacting function across multiple brain regions and neurotransmitter systems. Studies on the features of circuit level abnormalities demonstrate structural changes within the prefrontal cortex (PFC) and functional changes in its communication with distal brain structures. Treatments that impact the activity of brain regions, such as transcranial magnetic stimulation or rapid acting antidepressants like ketamine, appear to reverse depression associated circuit abnormalities though the mechanisms underlying the reversal, as well as development of these abnormalities remains unclear. Recently developed optogenetic and chemogenetic tools that allow high fidelity control of neuronal activity in pre-clinical models have begun to elucidate the contributions of the PFC and its circuitry to depression- and anxiety-like behavior. These tools offer unprecedented access to specific circuits and neuronal subpopulations that promise to offer a refined view of the circuit mechanisms surrounding depression and potential mechanistic targets for development and reversal of depression associated circuit abnormalities.

Depression is ranked by the World Health Organization as one of the world’s most burdensome diseases¹. In the United States alone, over 15% of the population is impacted by depression² resulting in an economic burden over $200 billion³. Current antidepressant medications are ineffective in nearly one third of patients and suffer from weeks long treatment lag⁴. The difficulty in finding candidate genes for depression⁵,⁶ has led to increased efforts to understand brain circuitry underlying depression. Much of this effort has focused on the prefrontal cortex (PFC) and hippocampus as these regions display structural, as well as functional changes potentially triggered by altered glutamatergic and gamma-aminobutyric acid (GABA) transmission⁷,⁸. Reversal of circuit abnormalities in depression by therapies that manipulate neuronal activity provides hope for those with treatment resistant depression.

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For those with treatment resistant depression there are several therapies both in use and under investigation that are designed to modulate neuronal activity. These include non-invasive techniques utilizing electrical stimulation (e.g. electroconvulsive therapy (ECT), transcranial direct-current stimulation (tDCS)) or magnetic fields (e.g. repetitive transcranial magnetic stimulation (rTMS), magnetic seizure therapy (MST)) to transiently manipulate neuronal activity. Alternatively, deep brain stimulation (DBS) is an invasive therapy that produces site specific regulation of neuronal activity through implanted electrodes for continuous manipulation. Many of these therapies appear to impact PFC circuitry as part of the antidepressant response. These therapies typically require numerous treatments for effective relief, though in the case of DBS relief may be almost immediate. Notably the response to ketamine, an effective antidepressant even in treatment resistant cases, also appears to transiently elevate PFC activity indicative of immediate circuit level effects. Importantly, ketamine dramatically reduces depressive symptoms within hours suggesting that an appropriate level of PFC intervention may rapidly induce antidepressant effects. However, ketamine is not without off-target effects that limit its utility as an antidepressant therapy.

The pre-clinical research community studying depression has gained insight on the effects of the aforementioned therapeutic interventions, but these approaches have significant limitations, including regional and cellular specificity. To address these issues, studies are also being conducted with sophisticated, cutting edge cell-type and circuit specific approaches, including optogenetic and chemogenetic tools. These tools offer critical insights into PFC circuitry and the role of neuronal subpopulations that may offer more specific targets for antidepressant therapies. The following examines on the role PFC circuits in depression, with particular focus on information produced by population specific neural manipulations.

PFC pathology in depression

There are numerous lines of evidence demonstrating that PFC circuitry is dysregulated in depression. These include alterations of structure, markers of glutamatergic and GABAergic neurotransmission, and connectivity with downstream structures (for a thorough review of these topics see). Evidence for depression related structural changes in the PFC come largely from secondary measures. For instance, the volume of PFC is reduced in depressed patients and this decrease is correlated with length of illness. Evidence of reduced synapse number is provided by a recent positron emission tomography study reporting decreased levels of ligand binding to synaptic vesicle glycoprotein 2A (SV2A) in depressed patients. This imaging suggests that presynaptic vesicle protein SV2A is reduced across the brains of patients with severe depression, and is associated with altered PFC connectivity. Direct evidence of synaptic loss has been reported in postmortem studies of depressed subjects, including reduced synaptic markers and number of synapses in PFC. Animal models provide further evidence for structural changes, demonstrating that chronic stress exposure, an often used model of depression, decreases spine density and dendrite complexity of medial PFC (mPFC) neurons, and that fast acting antidepressants rapidly reverse the synaptic as well as behavioral deficits caused by stress. Though others have postulated
that such structural changes may eventually lead to neuronal loss to date the literature does not support overt neuronal loss in depression, or models for studying depression\textsuperscript{23, 24}.

Increasing evidence suggests that glutamatergic and GABAergic transmission is altered in depression\textsuperscript{8–25} (for a thorough review of this topic see\textsuperscript{26}). PFC glutamate metabolites are reduced in depression\textsuperscript{27} and postmortem studies demonstrate changes in ionotopic and metabotropic glutamate receptors\textsuperscript{28, 29}. mPFC levels of the GABA synthetic enzyme glutamate decarboxylase-67 are also reduced in postmortem brains of depressed subjects\textsuperscript{30} as are markers of the somatostatin/calbindin (SST) GABAergic subtype\textsuperscript{31, 32}. Cortical TMS studies utilizing motor threshold measurements report reduced cortical GABAergic tone in depressed patients\textsuperscript{33, 34}. Magnetic resonance spectroscopy studies also provide evidence of reduced GABA levels in depressed individuals\textsuperscript{35, 36} and reversal after successful TMS treatment\textsuperscript{37}. Together these findings indicate that neurotransmission within the PFC and communication with downstream targets is dysregulated in depression. Consistent with this, there are reports indicating that default mode network connectivity is elevated in depression, and reduced by successful TMS\textsuperscript{9, 10}, and that functional connectivity between fronto-limbic and fronto-striatal targets may classify depression subtypes and inform response to TMS treatment\textsuperscript{38}. Normalization of frontal activity and functional connectivity is also observed with DBS\textsuperscript{12} and ECT\textsuperscript{11}. It is also notable that the glutamate burst produced by ketamine results from acute, transient pharmacological inhibition of GABAergic transmission, that results in persistent synaptic plasticity that is associated with the antidepressant response well after the acute phase of treatment\textsuperscript{8}.

These findings highlight the importance of balanced PFC function to mental health. Dysregulated glutamatergic and GABAergic transmission within the PFC would be expected to negatively impact cognitive function and emotion through altered local processing of afferent information and generation of efferent activity necessary to communicate with distal structures. Importantly, these findings also point to the importance of the refined understanding of PFC network function and structure that pre-clinical research can provide for future advances in targeted neuromodulation.

Assessing PFC circuits via manipulation of selected neuronal populations

It is challenging to gain insight into the specific brain targets or, circuits, that initiate a therapeutic response following implementation of ECT or other neuromodulatory therapies because the technologies are inherently non-specific. In all electrical or magnetic stimulation techniques the brain area impacted by the manipulation is a function of “dose” (i.e. pulse width, duration, etc.). Broadly, because of the necessity of passing the electrical stimulus directly through the skull ECT may produce electric field strengths throughout the brain volume that are well above neuronal stimulation threshold (Fig. 1A)\textsuperscript{39}. Similarly, though the use of magnetic stimulation bypasses skull shunting to refine the electric field, TMS still offers limited ability to target a specific brain region, especially as “dose” is increased to support neuronal stimulation at greater depth from the skull surface (Fig. 1B)\textsuperscript{40–42}. Invasive DBS allows manipulation of brain activity through surgical placement of electrodes and continuous application of current\textsuperscript{12}. Activity change produced by DBS is non-specific in the area of the electric field, and may also include fibers of passage. Additionally, the
stimulation frequency utilized in DBS is above physiologically sustainable levels making it unclear whether the local modulation by DBS represents activation or depolarization induced-inhibition\textsuperscript{43}. A greater understanding of potential targets for these circuit therapies may therefore be gained through the use of tools that allow for precise targeting of location, and defined activation or inhibition of neuronal activity and even specific cell populations.

Tools available to researchers using preclinical models offer greater precision and offer avenues for understanding the impact of neurostimulation on neural circuits. In recent years a variety of tools have been developed that allow control of neuronal activity through introduction of light sensitive channels (i.e. optogenetics) or engineered receptors sensitive to exogenous ligands (i.e. chemogenetics; designer receptors exclusively activated by designer drugs (DREADDs)). The use of optogenetic or chemogenetic tools in combination with viral-vector strategies enhances the spatial selectivity of modulation when compared to clinically utilized tools for neuromodulation, particularly ECT and TMS. An adenovirus (AAV) carrying an optogenetic or chemogenetic vector may be placed directly into a target region of interest producing very discrete spatial selectivity. This is particularly true with optogenetic techniques where the neuronal manipulation is limited to the area directly below a fiber optic implant (Fig. 1C)\textsuperscript{44}. Further selectivity may be obtained through the use of strategies to control expression in specific cell populations (Fig. 1D, Fig. 2). AAV expression may be gated by packaging specific promoter sequences within the expression vector (Fig. 2A), for example the calmodulin-dependent protein kinase II \textit{(CaMKII)} promoter allows biased targeting of excitatory neurons in a region of interest\textsuperscript{45, 46}. An alternative strategy to gain population specific vector expression employs Cre-recombinase transgenic mice with viral vectors that require Cre-recombinase for expression. This strategy limits viral expression to those cells within an area of interest expressing Cre-recombinase. Injection of viral constructs with retrograde transport properties allows targeting of populations projecting to regions of interest (Fig. 2B), and intersectional strategies are capable of targeting individual projections populations (Fig. 2C). For instance, one might combine a retrograde AAV-Cre viral placement in a terminal region of interest with a Cre-dependent viral placement in the somatic area of interest to target cellular populations of a specific projection pattern (e.g., placement of retrograde AAV-Cre into the basolateral amygdala (BLA) and a Cre-dependent construct into the PFC to target PFC cells that target the BLA\textsuperscript{47}).

Considerations for the use of optogenetic and chemogenetic tools

The use of optogenetic tools allows high fidelity temporally specific activation (i.e., channelrhodopsin (Chr2), light activated cation channel) or inhibition (i.e., halorhodopsin (NpHR), light active Cl- channel) of target cells with light delivery through fiber optic cannula into the region of interest\textsuperscript{48}. Heating of surrounding tissue is a well documented result of light application\textsuperscript{44, 49, 50}, and when sufficient may alter neuronal activity\textsuperscript{44, 50}. This is dependent on the light intensity as well as duration of application. Similarly, blue light of the type used for optogenetic experiments (i.e., ~470nm), may alter transcription, even in the absence of optogenetic constructs\textsuperscript{51}. These findings highlight the need to employ proper controls to account for changes that may be induced by light delivery.
Chemogenetic tools lack the temporal fidelity of optogenetic tools as G protein coupled receptors are engineered to respond to an exogenous ligand and to couple to either stimulatory (e.g., muscarinic receptor M3 to Gq) or inhibitory (e.g., M4 to Gi) signaling pathways in targeted cells; this means that the onset and offset of actions are governed by the pharmacokinetics of the exogenous ligand, and may be better described as altering the firing threshold rather than directly impacting firing. However, because the exogenous ligand can be administered systemically, or directly applied to brain targets through local infusion, the animal does not have to be tethered, as is the case for real time behavior for optogenetic studies. Notably, the absence of light is an advantage to chemogenetic work as the above described concerns for optogenetic work are obviated. This may make chemogenetic approaches a better choice when long-duration stimulation/inhibition is necessary. However, recent evidence demonstrates that the DREADD ligand clozapine-n-oxide (CNO) may be converted to the atypical antipsychotic clozapine. Thus, proper controls must be included in DREADD studies, such as administration of CNO to control animals for analysis of the effects of CNO metabolites on behavior to rule out any off target effects.

Influence of mPFC circuit activity on depression- and anxiety-like behaviors in real-time

Correspondence of rodent mPFC and definition of areas to be discussed

In an effort to delineate the scope of the review it is necessary to define the regions being considered. We will focus on aspects of mood in depression, and the role of PFC in regulation of mood related behaviors. As detailed above, numerous studies have demonstrated functional and structural alterations in the PFC of depressed patients that likely play a significant role in dysfunction of mood related behaviors. There is much debate as to whether the rodent PFC is similar to PFC in non-human primates and humans. For the purposes of this review we focus on studies of the rodent mPFC an area encompassing prefrontal (PL), infralimbic (IL), and anterior cingulate cortex (ACC). The rodent IL (also referred to herein as ventral medial mPFC (vmPFC)) appears to correspond to the subgenual cortex area (i.e. Brodmann 25) and is an important component of the striatal emotion processing network that is conserved across species. More dorsal regions of the rodent mPFC (i.e. PL and ACC) appear to correspond to ACC though there is more debate here. The mPFC plays a critical role in behavior, as a central hub that receives input from cortical, thalamic, and limbic regions and sends outputs to structures that regulate emotion, fear, and stress responses such as the amygdala, habenula, and dorsal raphe nucleus (DRN). Segregation of function between ventral and dorsal regions of mPFC is evident from work involving fear expression and extinction learning and also appears in the response to rapid-acting antidepressants described below. Research in mPFC related to fear is often conceptualized in terms of Post Traumatic Stress Disorder and will not be covered (for review see). Similarly, while orbital frontal cortex is likely important to depression given its role in choice based on expected outcome the current review will not discuss research focused on this brain region. Instead, we will focus on recent optogenetic and chemogenetic studies that have begun to separate the roles that specific cellular populations play within the mPFC in the regulation of depression-related behaviors in rodent models. The vast majority of these studies utilize opto- or chemogenetic manipulations at the time of testing (i.e. in
real-time) and the interpretation of results are based on the acute nature of the manipulation (Fig. 3A). Previous reviews have detailed the impact of manipulating brain regions other than the mPFC on depression-like behavior\textsuperscript{62}; the current review will focus on manipulations of mPFC cell populations as well as mPFC efferent and afferent fibers. Because of the high comorbidity, we include studies assessing anxiety, as well as depression-related behaviors.

**Involvement of mPFC in active versus inactive coping strategies**

Numerous tests of depression-like behavior incorporate a stressful challenge that provides a means to assess active and inactive behavioral periods. In the forced swim test (FST) this is achieved by placing a rodent (rat or mouse) in a container filled with water to a sufficient depth that the animals cannot support themselves and must choose between active swimming and climbing, or inactive floating. In mice, the tail suspension test (TST) also contrasts active struggling against periods of inactivity. Experiments employing learned helplessness models (rat or mouse) utilize prior uncontrollable stress to generate a behavioral state in which animals do not engage in active behavior that would allow them to escape a mild foot shock. Depression-like behavior following uncontrollable stress is also evident in reduced exploration of a novel juvenile (rat or mouse). In each of these tests the amount of inactivity has in the past been described as reflecting helplessness or despair, however more recent interpretations feature a transition between active and passive coping that is impacted by prior experience as well as learning during the test session\textsuperscript{63}. The mPFC’s role in action selection, in addition to the well documented effects of stress on the mPFC\textsuperscript{21}, has led to numerous investigations into the role of mPFC cellular populations in these models.

Photostimulation of glutamatergic cells within the mPFC has produced mixed results. An initial study utilizing the CamkIIa promoter to target glutamatergic neurons within the mPFC observed no effect of photostimulation during the FST\textsuperscript{64}. However, photostimulation of mPFC terminals in the DRN decreased immobility, an antidepressant response, while photostimulation of mPFC terminals in the lateral habenula (LHB) increased immobility\textsuperscript{64}. This finding clearly demonstrates the additional insights obtainable through sub-population specific targeting, in this case based on different mPFC projections to the DRN vs. LHB. Later studies have observed reduced immobility in the forced swim test when Thy-1 expressing neurons were targeted for photostimulation\textsuperscript{65}, and when mPFC neurons that receive ventral hippocampal (vHipp) input were activated with Gq DREADDs\textsuperscript{66}. Immobility was also reduced in the TST when vesicular glutamate transporter-2 (\textit{vGlut2}) neurons were photostimulated\textsuperscript{67}. The reason for the differences are not clear, but presumably are related to targeting different populations of excitatory neurons with the different promoters used for these studies. In addition, the stimulation parameters could account for differences in the effects observed with somatic stimulation. This is exemplified in work targeting IL mPFC projections to the medial dorsal thalamus (MDT). Photostimulation of this subpopulation with gamma bursts timed to IL oscillatory activity reduced immobility in the TST, but similar stimulation not timed to oscillatory activity did not, and more rapid stimulation produced an increase in immobility\textsuperscript{68}. The different and even opposing effects of different
stimulation frequencies (Table 1) provides further insight that is especially relevant to DBS paradigms that use only very high frequency (110 Hz) stimulation.

There have been only a handful of studies utilizing these approaches to target mPFC afferents. In a study attempting to determine the locus of ketamine’s antidepressant effects, photostimulation of vHIPP terminals in the mPFC reduced immobility, but only when DRN was inactivated. This study also reported that in ketamine treated rats, inhibition of the vHIPP-mPFC population increased immobility, effectively reversing the ketamine response. In contrast, inhibition of the MDT-mPFC population did not alter the response to ketamine, demonstrating circuit specificity. However, in the absence of prior ketamine administration MDT-mPFC stimulation using a Gq DREADD has been reported to reduce immobility.

There is strong evidence that dopaminergic neuronal activity regulates the transition between active and passive strategies, however to our knowledge this effect has not been examined at mPFC terminals from midbrain dopamine neurons.

**Involvement of mPFC in social avoidance and exploration**

Normal social interactions are disrupted in depressed patients, and social avoidance or exploration are commonly used measures for studying depression in rodent models. In social defeat stress, animals are subjected to repeated defeat followed by prolonged periods of sensory contact with a dominant agressor. During later testing animals susceptible to this paradigm demonstrate social avoidance operationalized as less time investigating a novel conspecific. Beyond social avoidance, this model also produces anhedonic behavior in susceptible animals demonstrated as reduced preference for sucrose, increased anxiety, metabolic changes, and continued corticosterone reactivity, making it an attractive model for studying depression. Alternative models utilize uncontrollable stress exposure to produce a reduction in juvenile exploration time at testing. Numerous studies point to a role for mPFC in the development of these depression-like behaviors. Following demonstration of reduced immediate early gene levels in the mPFC of depressed patients, an indirect marker suggesting reduced neuronal activity, Covington et al implemented a stimulation protocol that induced an increase in immediate early gene levels that were reduced by exposure to social defeat stress. Stimulation of all neurons, glutamatergic and GABAergic, in the mPFC of mice exposed to social defeat stress reduced social avoidance, as well as anhedonia determined in the sucrose preference test, consistent with an antidepressant response.

Similarly, others have shown that unilateral left, but not right, stimulation of glutamatergic neurons in the PL mPFC reduces social avoidance. However, utilizing step function opsins (SSFOs) that asynchronously elevate mPFC excitability, others have shown that increased mPFC activity produces deficits in juvenile exploration and reduced sucrose preference, though this was not observed when neurons were activated using DREADDs. The latter studies were conducted in unstressed animals, and as such the differences may be due to prior stress exposure altering mPFC network dynamics and therefore the response to stimulation. Alternatively, differences in stimulation parameters or variations in the subpopulation of neurons targeted may be the cause of the contradictory outcomes (Table 1).

Studies of mPFC afferents support a role for mPFC circuitry in altered social interaction. Use of optogenetic constructs to bidirectionally control BLA neurons projecting to the...
mPFC in unstressed animals demonstrated that photostimulation reduced juvenile interaction, while photoinhibition had an opposite effect\(^8\). Results from other studies raise the possibility that BLA to mPFC responses are amplified by stress exposure. Exposure to chronic stress decreases mPFC apical dendritic complexity and spine number and function, while basal dendrites, which are targeted by BLA are unchanged by stress\(^8\). This may lead to an increase in BLA control after stress. Dopaminergic afferents to mPFC also appear to play a role in social avoidance. Inhibition of ventral tegmental area (VTA) projections to mPFC increased social avoidance in animals following a sub-threshold social defeat paradigm\(^8\). This is consistent with reports demonstrating reduced dopamine\(^8\), \(^8\), and dopamine D1 receptor signaling\(^8\) in the mPFC following social defeat. Together these studies of mPFC afferents demonstrate a clear role for mPFC in social avoidance and highlight a stress reactive circuitry that may be targeted for treatment of stress related illnesses such as depression.

Studies involving mPFC efferents highlight important projection regions impacting the response to social defeat. Hultman\(^8\) and colleagues identified an mPFC-amygdala connection as critical to maintaining synchrony in an mPFC-amygdala-VTA circuit. Increasing activity in amygdala neurons that receive input from mPFC reduced social avoidance in susceptible mice. mPFC projections to the nucleus accumbens (NAc) and DRN may also impact social behavior. Photostimulation of mPFC terminals in the NAc during avoidance testing produced an antidepressant effect, increasing social interaction in animals that had undergone social defeat stress exposure\(^8\). mPFC projections to the DRN have been shown to bidirectionally modify social defeat outcomes. Challis et al stimulated or inhibited the mPFC-DRN pathway during the sensory contact period after daily defeat and observed that increasing activity in this pathway increased subsequent social avoidance, while inhibition produced the opposite antidepressant-like effect\(^8\). A similar finding is observed in the DRN, where inhibiting GABAergic interneurons, the target of mPFC projections, during the sensory contact period blocked the effect of social defeat\(^8\). Notably, manipulation of GABAergic interneurons in the DRN during testing had no effect on social avoidance after social defeat, highlighting the importance of this pathway in adaptation to continuing social defeat.

Stress associated adaptations in the mPFC-DRN pathway have also been linked to prior experience with stressor controllability where activation of this pathway during uncontrollable stress produces outcomes similar to those where stress is controllable, leading to levels of interaction with a juvenile conspecific that are similar to that observed in unstressed animals\(^8\), \(^9\), \(^9\). It is interesting that this differs and in fact is opposite to the effects of activating the mPFC-DRN pathway during the sensory contact period following social defeat. This may be due to the chronicity of manipulation, multiple experiences in social defeat versus single exposure to uncontrollable stress, and/or the nature of the stressor during neural manipulation (i.e. shock or sensory contact). Together, these findings highlight important contributions of mPFC and its downstream targets in social avoidance, and demonstrate the utility of optogenetic tools for determining likely sites of adaptation to stress experience, while also demonstrating the importance of the timing of circuit manipulations to the outcomes observed during behavioral testing. Additionally, the mPFC-DRN pathway appears important in regulating the response to defeat experience in Syrian Hare and Duman
hamsters, though a thorough examination of this literature is beyond the scope of the review.92–94.

Involvement of mPFC in anxiety-like behavior

Approximately half of the individuals diagnosed with depression also have a comorbid anxiety disorder95, and this combination results in reduced rates of treatment efficacy. Optogenetic and chemogenetic work in the mPFC has largely focused on approach-avoidance conflicts when investigating the role of mPFC in anxiety-like behavior. In the elevated plus maze (EPM) and open field test (OFT) typical exploration behavior is opposed by safety cues to avoid open spaces, such as the open arms of the plus maze, or against the center of the open field. Animals that spend more time exploring the open arms of the EPM or the center of the open field are considered less anxious. The use of these tests is supported by demonstrated anxiolytic effects with compounds that produce effective anxiolysis in humans, and similar results between tests96, 97.

In the novelty suppressed feeding test (NSF) a food restricted animal is placed into an open field and the time to enter the center and consume a food pellet is recorded. Longer latencies to approach and eat the pellet are an indication of greater anxiety. There is very little evidence demonstrating that acute stimulation of mPFC principle neurons alters anxiety-like behavior in the EPM or OFT, using optogenetic75, 98 or chemogenetic techniques79. However, mPFC single unit recordings are known to signal anxiogenic locations on the EPM, and show coupling to vHIPP oscillatory activity99. Consistent with this, photoinhibition of vHIPP inputs to the mPFC reduces anxiety-like behavior in EPM, OFT, and NSF100. A similar manipulation of MDT-mPFC circuitry had no effect100.

Bidirectional effects of manipulating BLA projections to mPFC have also been observed. Photostimulation of BLA neurons that project to mPFC produces an anxiogenic response, while inhibition of these BLA-mPFC projection neurons is anxiolytic80. As noted above, the influence of BLA-mPFC input appears to be augmented by stress and normalized by ketamine treatment81, and the vHIPP input to mPFC has also been implicated in the antidepressant effects of ketamine66. From vmPFC, projections to the basomedial amygdala (BMA) are involved in anxiety: vmPFC-BMA stimulation produces an anxiolytic effect in the OFT and EPM, while inhibition produces an anxiogenic effect98. In contrast, stimulation of mPFC neurons projecting to the NAc had no effect on anxiety-like behavior in the OFT87, but was effective in reducing social avoidance, demonstrating differential effects of these projection neurons on social avoidance versus anxiety. Together this work demonstrates the need to target discrete neuronal populations by input/output region, as clear effects of mPFC on anxiety-like behavior are observed only when populations targeted by, or projecting to, distinct regions are targeted.

Involvement of mPFC GABAergic neurons in models of depression

Altered excitation/inhibition balance within the mPFC is emerging as a potential causative factor in depression, and restoration has emerged as a hypothesized therapeutic outcome with rapid acting antidepressants such as ketamine8, 32. However, there have been few studies directly assessing the role of GABAergic populations in depression. As described above, SST neurons are a subpopulation of GABAergic neurons that gate input activity at

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the level of dendrites, and are reported to be decreased in postmortem PFC of depressed subjects. Recent efforts to understand SST regulation of anxiety- and depression-like behavior have utilized DREADD mediated inhibition of dorsal mPFC SST neurons after both acute and chronic inhibition. Acute administration of CNO to produce DREADD mediated inhibition of SST interneurons at the time of testing increased anxiety like behavior on the EPM, and increased time to consumption in the cookie test, a measure of increased anhedonic behavior. These positive results and consistent trends in other measures such as EPM and NSF led to a significant increase in what the authors termed behavioral emotionality. In contrast, administration of CNO twice per day for 3 weeks produced reduced behavioral emotionality. Consistent with this result, SST interneuron ablation reduced behavioral emotionality before, and after chronic unpredictable stress.

Parvalbumin (PV) neurons gate pyramidal cell activity at the cell body, placing this GABAergic interneuron subtype in position to limit communication with targets downstream of the mPFC. There have been limited investigations targeting this population in studies of depression. However, Perova et al observed reduced excitatory drive onto PV cells following footshock training prior to learned helplessness testing. These authors then utilized a DREADD approach to inhibit PV interneurons during training and testing and observed an increase in escape failures. These findings indicate a role for PV interneurons in gating mPFC activity during uncontrollable stress that opposes the development of helpless behavior. Given the importance of interneuron populations to regulating mPFC function deeper investigations into the role of these interneuron subtypes in models of depression and treatment response are warranted.

**Sustained effects of mPFC circuit manipulations**

The vast majority of optogenetic work has focused on real-time modulation of neuronal activity as it relates to circuit function and behavior. This makes sense as the multitude of opsins allow high fidelity control of neuronal activity in discrete regions along user defined time-scales. However, there are an increasing number of examples where optogenetic protocols are used to induce forms of plasticity at synapses of interest that persist well after the light application (Fig. 3B). For instance 1 hz photostimulation has been used to induce synaptic long term depression (LTD) in the lateral amygdala and reduce responding to fear conditioned cues 24 hours later. These authors also demonstrated that 100 hz photostimulation effectively produced synaptic long term potentiation and reversed the effects produced by 1 hz optogenetic LTD. Studies of depression-like behavior have also used this approach. Optogenetically induced LTD at vHIPP-NAc synapses reduces social avoidance tested 45 minutes later, an effect not observed at mPFC-NAc synapses. Persistent effects are not limited to optogenetic techniques. Three weeks of CNO administration in mice expressing inhibitory DREADDs in mPFC SST neurons produced antidepressant effects outside of the drug active period, as did 5 weeks of CNO administration in mice expressing excitatory DREADDs in excitatory neurons of entorhinal cortex.

These studies demonstrate the utility of optogenetic and chemogenetic tools to produce sustained changes in behavior when used in a way that is somewhat analogous to clinical
treatments for depression, including TMS, DBS, and ketamine. While the utility of optogenetic and chemogenetic tools in patients remains to be seen, these techniques may identify specific synaptic targets that reverse depressive phenotypes in a way that is not achievable with the indiscriminate circuit modulatory approaches available for human use. Completing this type of work may then inform clinical studies using different treatment paradigms. For instance, recent pre-clinical work in the field of addiction has demonstrated that sensitization of D1 medium spiny neuron responses to cocaine may be abolished by optogenetic stimulation using a frequency (12 hz) sufficient to produce LTD. This effect was not evident with DBS at the same frequency. However, co-administration of a D1 antagonist in addition to DBS produced mGluR dependent LTD, and a behavioral response similar to that generated with optogenetically applied LTD. The authors produced a framework for causally linking plasticity produced by circuit manipulations to persistent changes in behavior. Key to this framework is identifying the synaptic plasticity associated with the disorder of interest, in this case cocaine associated synaptic potentiation, and designing therapeutically effective protocols to reverse these changes.

There have been multiple studies in the mPFC that may be informative within a framework linking neuroplasticity produced by circuit manipulations to persistent behavioral change that may be useful in understanding depression and designing more focused and efficacious treatments. Kumar et al. pointed to hypotheses suggesting that DBS and TMS associated activation of descending mPFC circuits drives the antidepressant response. The authors demonstrate that acute photostimulation of deep layer anterior PL projection neurons modulates limbic system oscillatory power and synchrony, and that ~4 hz stimulation, 5 minutes daily for 14 days, reduced anxiety tested 10 days after termination of the stimulation protocol, but did not impact social avoidance after social defeat stress. Interestingly, a pair of studies, using 20 hz photostimulation, 20 minutes per day over 5 days, of neurons in deep layers of the posterior ACC/PL observed pro-depressant and anxiogenic effects for up to 5 days after stimulation. The rationale and design of the latter study was based on evidence that ACC/PL neuronal activity is increased in models of chronic pain that also produce depression-like behavior. Together these studies demonstrate that there may be regional selectivity in the mPFC response to activation and highlight the need to gain a better understanding of how different photostimulation protocols of mPFC subregions influence depression-like behavioral outcomes (Table 2).

The discovery of ketamine’s antidepressant actions was a major therapeutic advance, and has had a major conceptual impact on the field by demonstrating that pharmacological agents can rapidly (within hours) alleviate the symptoms of depression. This has shifted the focus of drug discovery toward the development of glutamatergic, as well as GABAergic agents that can produce rapid effects on these neurotransmitter systems that lead to sustained synaptic and circuit level plasticity. Ketamine produces a burst of glutamate in the mPFC in rodent models, and an antidepressant response that occurs within hours and lasts for approximately one week indicating that brief, mPFC circuit activation produces rapid and sustained therapeutic effects. A pair of studies have employed optogenetic and chemogenetic techniques to pursue the mechanism behind this effect. Fuchikami et al. used muscimol inactivation to demonstrate the necessity of IL mPFC for ketamine’s antidepressant effects. To mimic the rapid and transient increase of extracellular

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glutamate caused by ketamine, they photostimulated glutamatergic neurons in IL mPFC at 10 hz for one hour (1 minute on/1 minute off). They found that photostimulation of IL, but not PL, mPFC produced antidepressant effects in the FST, SPT, and NSF tests observable 24 hours after photostimulation and still present 17 days after the manipulation. Also consistent with the synaptic effects of ketamine, this photostimulation paradigm significantly increased the number and function of spine-synapses in the mPFC, demonstrating structural as well as behavioral consequences.

A second series of studies demonstrated that co-administration of a D1 dopamine receptor antagonist blocked the antidepressant behavioral actions of ketamine, and used a DREADD inactivation approach to demonstrate that activity of the Drd1 expressing neuronal population in the mPFC was necessary for the antidepressant response to ketamine. In addition, the results demonstrated that stimulation of the mPFC Drd1 population was also sufficient to generate an antidepressant response, as was photostimulation of mPFC Drd1 terminals in the BLA. In contrast, inhibition of the Drd2 expressing mPFC neuronal population did not block the response to ketamine, and photostimulation of the Drd2 population did not produce antidepressant effects. The Drd1 and Drd2 neuronal populations segregate separate classes of excitatory cells that display different electrophysiological, morphological, and projection characteristics. Repeated stress paradigms attenuate working memory via a reduction in the activity of Drd1 expressing pyramidal cells in mPFC. In addition, repeated stress exposure alters excitability and synaptic inputs onto Drd1 and Drd2 cells, and causes atrophy of Drd1 expressing neurons. These studies provide key insights into a discrete population of neurons in the mPFC and the projections of these neurons to the BLA that are necessary and sufficient for the rapid antidepressant actions of ketamine. This type of cell and circuit specific information, as well as evidence for D1 receptor involvement could aid in the development of novel therapeutic interventions. Further studies on the impact of Drd1 photostimulation on stress associated synaptic and behavioral changes could provide additional key information for drug development.

Considerations for future studies

The studies detailed herein provide clear evidence that mPFC neurons and projection target regions regulate depression and anxiety related behaviors in pre-clinical models. Additionally, this work provides examples of the informative nature of pre-clinical studies to treatment modalities such as DBS or ECT that lack the selectivity of pre-clinical tools. For instance, the stimulation paradigm is critical to the behavioral outcome. Manipulation of frequency and duration of stimulation may have strikingly different effects on behavior. Similarly, while studies report that indiscriminate acute stimulation of the mPFC during testing does not appear to impact anxiety like behavior, significant effects are observed when subpopulations of mPFC neurons are targeted. These results highlight the advantage offered by viral targeting of specific cellular populations, and again point to the difficulty of interpreting negative results, in this case when large populations of neurons are targeted. With this in mind, and to generate further rationale for clinical studies using tools for brain stimulation, null results in optogenetic work should be confirmed with additional experiments using alternative stimulation parameters. Due to the challenges inherent in human studies it is important that pre-clinical studies clearly delineate the
neuronal population, subregion, and stimulation paradigm to provide information that can be used to design and refine clinical interventions to produce more selective circuit effects in patients. For instance, these types of studies may be informative for TMS therapies where both high and low frequency stimulations are being used with mixed results\textsuperscript{113-116}, or may inform the development of targeted pharmacotherapies that are designed to impact specific aspects of the circuit response as is ongoing with the development of compounds seeking to mimic ketamine’s rapid antidepressant action while limiting off-target effects\textsuperscript{117}.

Another important takeaway is that optogenetic and chemogenetic manipulations can produce persistent neuroplasticity changes that may impact behavior (Table 2)\textsuperscript{60, 118, 119}, highlighting the need to control for prior exposure to these paradigms that could impact subsequent behavioral testing. For instance, studies utilizing repeated neuronal modulation through multiple separate test sessions should take care to determine if the effects observed are due to the acute manipulation rather than a sustained effect of prior manipulations. However, the ability to produce sustained synaptic and behavioral responses indicates that the clinical interventions could be designed to produce similar effects. For example, optogenetic or chemogenetic tools could be used to study population specific effects in brain regions targeted by DBS, which would provide specific frequency and duration settings for further refinement of this therapeutic intervention. Information in specific neuronal populations within these regions could also be used to identify novel therapeutic targets. Additionally, sustained behavioral effects of photostimulation are clearly different than those obtained with acute manipulations. For example, sustained anxiolytic actions are observed well after photostimulation of glutamatergic neurons in the mPFC\textsuperscript{60, 65}, and require subpopulation specific control in acute situations\textsuperscript{98, 100}. Understanding the mechanistic drivers that produce these sustained effects could also inform the refinement of clinical targets and pharmacological treatments in the future.

A final area to consider in future efforts is in the study of circuit and cell population specific effects underlying the actions of prophylactic agents. Notably, ketamine has been demonstrated to have prophylactic effects when administered prior to uncontrollable stress exposure in male and female rats\textsuperscript{74, 120}, an effect governed by mPFC to DRN circuitry. Similarly, ketamine administered 1 week before social defeat, chronic corticosterone exposure, or learned helplessness training blocked the post-stress expression of depression-like behavior\textsuperscript{121}. Prophylactic ketamine also limits learned fear expression when administered one week, but not one month, or one hour before training\textsuperscript{122}. Interestingly, further study in this paradigm demonstrated changes in neurometabolite levels in PFC and hippocampus only in animals given ketamine prophylactically and that were fear conditioned\textsuperscript{123}. These studies further demonstrate the importance of mPFC circuitry in regulation of depression- and anxiety-like behavior and highlight the utility of ketamine to studies of brain-adaptive changes associated with the antidepressant response. To our knowledge studies examining whether optogenetic/chemogenetic stimulation may produce similar prophylactic effects have not been undertaken.
Conclusions and future directions

The combination of optogenetic and chemogenetic tools with viral tools that allow cell population specific control allows powerful insight into the diverse role of mPFC neuronal populations in pre-clinical studies of depression-like behavior (Fig. 3). The results described here highlight the impact of specific neuronal populations in discrete mPFC subregions driving active responding, social avoidance, and anxiety-like behavior (Table 3). The results also demonstrate that in addition to identifying circuitry that is acutely involved in behaviors, it is possible to produce prolonged changes in synaptic function, morphology, and depression and anxiety related behaviors. Together these studies provide key information to help direct clinical interventions such as DBS and TMS, as well as in development of targeted pharmacotherapies. Key to these type of advances will be incorporation of tools to identify the physiological changes in neuronal activity after manipulation, such as in vivo imaging using two-photon microscopy and fiber photometry, as well as multielectrode electrophysiology. Combined these efforts provide an exciting opportunity to advance our knowledge of the neuronal and circuit level determinants underlying depression- and anxiety-like behaviors as well as novel therapeutic interventions.

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Conflict of Interest

We declare that Dr. Duman has consulted and/or received research support from Naurex, Lilly, Forest, Johnson & Johnson, Taisho, and Sunovion. The remaining authors have no competing financial interests.

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Fig. 1.
Regional specificity of modalities for transiently manipulating neuronal activity a. Model estimates of electric field strength (E) above stimulation threshold (E_{th}) for conventionally applied (bilateral) ECT at 800mA. Stimulation strength on the cortical surface, and representative coronal and axial slices from realistic head models are shown (adapted from\textsuperscript{39}) b. Model estimates of electric field strength for 1ms TMS pulse applied at 120% of the leg motor threshold. Red and purple areas indicate power above neuronal activation threshold (adapted from\textsuperscript{42}) c. Iso-contour lines depicting monte-carlo estimated light spread and intensity at 10mW output power with typically utilized cannula diameter (62\textmu{}m top, 200\textmu{}m bottom) and light wavelengths for optogenetic stimulation demonstrates the discrete area below the implanted fiber optic cannula expected to be directly impacted by light.
delivery (adapted from\textsuperscript{44}) \textbf{d}. The effects of optogenetic stimulation may be further refined using viral vectors with population specific promoters or Cre-recombinase dependence.
Fig. 2.
Viral strategies for targeting neuronal populations. **a.** Targeting a population using promoter specific or Cre-dependent AAV and somatic or terminal manipulation **b.** Targeting a population of cells that project to a region of interest using retrograde AAV and somatic manipulation **c.** Intersectional AAV strategy to limit AAV vector expression to a discrete projection population.
Fig. 3.
Pre-clinical neuronal manipulation methods and mPFC circuitry implicated in depression like behavior. **a.** Optogenetic or chemogenetic manipulations may take place at the time of testing or **b.** may precede testing. **c.** mPFC afferent (grey) and efferent (black) circuitry with behaviors reported to be impacted by optogenetic and/or chemogenetic manipulations indicated. **d.** mPFC cellular populations with behaviors reported to be impacted by behaviors reported to be impacted by optogenetic and/or chemogenetic manipulations indicated.
Table 1:
Overview of studies using optogenetic/chemogenetic techniques in real-time to manipulate mPFC circuitry in studies of anxiety and depression

| Soma Targeting | Neuromodulatory approach | Target Population | Effect | Test |
|----------------|---------------------------|------------------|--------|------|
| Covington, 2010 | ChR2; 40ms of 100hz per 3 second period, 1–2mW | Glutamatergic and GABAergic | Antidepressant | Social avoidance, SPT |
| Lee, 2015 | ChR2; 5ms 10hz, 1mW | Glutamatergic | Antidepressant | Social avoidance |
| Warden, 2012 | ChR2; 5ms 20hz, 3mW | Glutamatergic | No effect | FST |
| Yizhar, 2011 | SSFO; light delivery produces asynchronous excitability enhancement | Glutamatergic | Pro-depressive | Social exploration |
| Perova, 2015 | Gi DREADD; 10mg/kg CNO prior to training and testing | PL Parvalbumin neurons | Pro-depressive | Learned helplessness |
| Adhikari, 2015 | Chα2; 5ms 1mW, 10Hz | vmPFC Glutamatergic | No effect | OFT, EPM |
| Ferenczi, 2016 | SSFO; light delivery produces asynchronous excitability enhancement | Glutamatergic | Pro-depressive | SPT, Social exploration |
| Warthen, 2016 | Gq DREADD; 0.5–2.5mg/kg CNO | Glutamatergic | No effect | Social exploration, OFT |
| Son, 2018 | ChR2; 1 second 100hz per 4 second period | vGluT2 | Antidepressant | TST |
| Soumier, 2014 | Gi DREADD; 5mg/kg CNO | PL Somatostatin neurons | Pro-depressive | Test battery net result |
| Kumar, 2013 | ChR2; Neuron matched (~4hz), 2mW | PL mPFC Thy-1 | Antidepressant | FST |
| Carreno, 2016 | Gq DREADD; 0.5mg/kg CNO | mPFC neurons that receive vHIPP input | Antidepressant | FST |

| Afferent Targeting | Neuromodulatory approach | Target Population | Effect | Test |
|--------------------|---------------------------|------------------|--------|------|
| Felix-Ortiz, 2016 | ChR2; 5ms 5mW, 20hz | BLA - mPFC | Anxiogenic, Pro-depressive | EPM, OFT, Social exploration |
| NphR; 5mW, constant | | BLA - mPFC | Anxiolytic, Antidepressant | OFT, Social exploration |
| Chaudhury, 2013 | NphR; 8 seconds on - 2 seconds off | VTA - mPFC | Pro-depressive, No effect | Social avoidance, SPT |
| Chα2, 0.5Hz 15ms and 20Hz 40ms | VTA - mPFC | No effect | Social avoidance, SPT |
| Padilla-Coreano, 2016 | Arch; Continuous, 10 mW, 2 minutes off – 2 minutes on | vHIPP - mPFC | Anxiolytic | EPM, OFT, NSF |
| Carreno, 2016 | ChR2; 20hz for 10 minutes, 30mW | vHIPP - mPFC | Antidepressant with DRN inactivation | FST |
| Padilla-Coreano, 2016 | eNpHR3.0; Continuous, 10 minutes | vHIPP - mPFC | Pro-depressive | FST |
| Miller, 2017 | Gq DREADD; 1mg/kg CNO | MDT - mPFC | Antidepressant | FST, TST |

| Efferent Targeting | Neuromodulatory approach | Target Population | Effect | Test |
|--------------------|---------------------------|------------------|--------|------|
| Warden, 2012 | ChR2; 5ms 20hz, 10–20mW | mPFC - DRN | Antidepressant | FST |
| | mPFC - LHB | No effect | Pro-depressive | FST |

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| Neurmodulatory approach | Target Population | Effect | Test |
|-------------------------|------------------|--------|------|
| Adhikari, 2015<sup>58</sup> | eNpHR3.0; 10mW | vmPFC - BMA | Anxiolytic | OFT, EPM |
| Bagot, 2015<sup>87</sup> | ChR2; 4hz, 15–20mW | mPFC - NAc | Antidepressant | Social avoidance |
| Hultman, 2016<sup>66</sup> | Gq DREADD; 1mg/kg CNO | mPFC – amygdala | Antidepressant | Social avoidance |
| Challis, 2014<sup>48</sup> | ChR2; 25hz, 10 ms, for 10 minutes, 10mW | PL mPFC - DRN | Pro-depressive | Social avoidance |
| Challis, 2014<sup>48</sup> – this manipulation was performed during the social defeat sensory contact period not testing | Arch; 20 minutes continuous, 10mW | PL mPFC - DRN | Antidepressant | Social avoidance |
| Carlson, 2017<sup>68</sup> | ChETA; Closed loop, gamma bursts timed to IL oscillations | IL mPFC - MDT and MDT soma | Antidepressant | TST |
| Carlson, 2017<sup>68</sup> | ChETA; Gamma bursts not timed to IL oscillations | IL mPFC - and MDT soma | No effect | TST |
| Carlson, 2017<sup>68</sup> | ChETA, 14hz | IL mPFC - and MDT soma | Pro-depressive | TST |
| Dolzani, 2018<sup>74</sup> – effects observed from ketamine antidepressant baseline | Real-time, Gi DREADD; 3mg/kg CNO during exposure to uncontrollable stress | PL - DRN | Pro-depressive | Social exploration |

Anterior cingulate cortex (ACC), basolateral amygdala (BLA), basomedial amygdala (BMA), dorsal raphe nucleus (DRN), elevated plus maze (EPM), forced swim test (FST), infralimbic (IL), lateral habenula (LHB), medial dorsal thalamus (MDT), medial prefrontal cortex (mPFC), nucleus accumbens (NAc), novelty suppressed feeding (NSF), prelimbic (PL), somatostatin (SST), sucrose preference test (SPT), ventral hippocampus (vHIPP), ventral medial prefrontal cortex (vmPFC), ventral tegmental area (VTA), vesicular glutamate transporter (vGlut)
Table 2:
mPFC optogenetic/chemogenetic manipulations with sustained effects on behaviors used to study anxiety and depression

| Neuromodulatory Approach | Target Population | Effect | Test          |
|--------------------------|-------------------|--------|---------------|
| Kumar, 2013\textsuperscript{65} | ChR2; Neuron matched (~4Hz), 2mW, 5 minutes daily for 14 days after social defeat | PL mPFC Thy-1 | No effect anxiolytic | Social avoidance EPM |
| Soumier, 2014\textsuperscript{101} | Gi DREADD; 0.5mg/kg CNO 2x per day for 3 weeks including test days | PL mPFC SST | Antidepressant | Test battery |
| Friedman, 2014 | Chr2; 5 20Hz pulses per 10 second period, 20 minutes per day for 5 days | VTA - mPFC | Antidepressant; No effect | Social avoidance; SPT |
| Barthes, 2015\textsuperscript{106}, 2017\textsuperscript{107} | Chr2; 20Hz 40ms 4–5mW for 8 seconds followed by 2 seconds off. 4 days for 30 minutes per day. | ACC mPFC Thy-1 | Pro-depressive | NSF, Marble burying, Splash test |
| Fuchikami, 2015\textsuperscript{60} | Chr2; 10Hz 15ms, 5mW, 1 minute on – 1 minute off for 60 minutes | IL mPFC glutamatergic | Antidepressant | FST, SPT, NSF |
| | | PL mPFC glutamatergic | No effect | FST, SPT, NSF |
| Hare, 2019\textsuperscript{47} | Chr2; 10Hz 15ms, 5mW, 1 minute on – 1 minute off for 60 minutes | vmPFC Drd1 neurons | Antidepressant; No effect | FST, EPM, NSF SPT |
| | | vmPFC Drd2 neurons | No effect | FST, EPM, NSF SPT |
| | | vmPFC Drd1 - BLA | Antidepressant | FST, NSF |

Anterior cingulate cortex (ACC), basolateral amygdala (BLA), elevated plus maze (EPM), forced swim test (FST), infralimbic (IL), medial prefrontal cortex (mPFC), novelty suppressed feeding (NSF), prelimbic (PL), somatostatin (SST), sucrose preference test (SPT), ventral medial prefrontal cortex (vmPFC), ventral tegmental area (VTA)
mPFC circuitry impacting behaviors used to study anxiety and depression

### Table 3:

| Behavior | Target Population | Reference |
|----------|-------------------|-----------|
| Forced swim - Animal is placed in an inescapable beaker of water of sufficient depth to prevent contact with the bottom of the beaker. Time immobile during a short test is measured. Increased time immobile is interpreted as a passive coping strategy. | PL, mPFC, Thy1, IL,vmPFC Glutamatergic, vmPFC Drd1, - BLA, mPFC - DRN, mPFC - LHB, vHIPPP - mPFC, MDT - mPFC | Kumar, 2013<sup>36</sup> Fuchikami, 2015<sup>50</sup> Hare, 2019<sup>17</sup> |  |
| Tail suspension - Animal is suspended by the tail. Time immobile during a short test is measured. Increased time immobile is interpreted as behavioral despair or a passive coping strategy. | mPFC vGlut2, IL, mPFC - MDT, MDT - mPFC | Son, 2018<sup>70</sup> Carlson, 2017<sup>88</sup> Miller, 2017<sup>69</sup> |
| Social avoidance - Experimental mice are placed into a cage with larger, more aggressive, conspecific and allowed to interact for a short period of time. This interaction is followed by an extended period of sensory contact without physical contact. The interaction and sensory contact are repeated over multiple days. On test, time spent investigating a novel animal separated by a mesh enclosure is measured. Reduced time investigating the novel animal is interpreted as evidence of social avoidance. Time investigating the novel animal may also be contrasted against time spent investigating the mesh enclosure without a target present. | mPFC, PL mPFC Glutamatergic, Pl mPFC Glutamatergic, PL mPFC - DRN, mPFC - Nac, mPFC - amygdala, VTA - mPFC, VTA - mPFC | Covington, 2010<sup>73</sup> Lee, 2015<sup>58</sup> Challis, 2014<sup>58</sup> Bagot, 2015<sup>37</sup> Hultman, 2016<sup>68</sup> |  |
| Social exploration/Juvenile exploration - Experimental mice are placed into a box with multiple chambers. In one chamber is a conspecific in a mesh enclosure. Reduced time spent investigating the conspecific is interpreted as evidence of abnormal social behavior. Investigation time may be contrasted against time spent investigating an empty enclosure. | mPFC Glutamatergic, PL mPFC - DRN, BLA - mPFC | Yizhar, 2011<sup>78</sup>, Ferenczi, 2016<sup>77</sup> Dolzani, 2018<sup>74</sup> Felix-Ortiz, 2016<sup>60</sup> |
| Sucrose preference/Sucrose consumption - Animals are habituated to a sucrose solution, typically 1–2%, prior to testing. On test animals are given free access to bottles containing sucrose and water. The amount of each liquid consumed are measured. Reduced sucrose consumption as a percentage of total liquid consumed is interpreted as evidence of anhedonia. | PL, mPFC, Thy1, IL, mPFC Glutamatergic | Kumar, 2013<sup>65</sup> Hare, 2019<sup>17</sup> Adhikari, 2015<sup>98</sup> Felix-Ortiz, 2016<sup>63</sup> Padilla-Coreano, 2016<sup>100</sup> |
| Elevated plus maze - Animals are given access to a plus shaped arena elevated above the floor. Two opposing arms have walls (‘closed’), and two are ‘open’. Increased time spent in the open arms during the test is interpreted as reduced anxiety | PL, mPFC, Thy1, IL, mPFC Glutamatergic | Kumar, 2013<sup>65</sup> Soumier, 2014<sup>101</sup> Hare, 2019<sup>17</sup> Adhikari, 2015<sup>98</sup> Felix-Ortiz, 2016<sup>65</sup> Padilla-Coreano, 2016<sup>100</sup> |
| Open field test - Animals are given access to a square enclosure and allowed to freely explore. Time spent in the center of the field is measured. Animals that spend more time exploring the center of the field as opposed to the periphery are interpreted as being less anxious. | vMPFC - BMA, BLA – mPFC, vHIPPP – mPFC | Adhikari, 2015<sup>98</sup> Felix-Ortiz, 2016<sup>65</sup> Padilla-Coreano, 2016<sup>100</sup> |
| Novelty suppressed feeding - Food deprived animals are given access to a square enclosure with a piece of food in the center. Time spent to approach the food and take a bite is measured. Animals with shorter feeding latencies are interpreted as being less anxious. | ACC mPFC Thy1, IL, mPFC Glutamatergic, vmPFC Drd1, vHIPPP - mPFC | Barths, 2015<sup>108</sup>, 2017<sup>107</sup> Fuchikami, 2015<sup>50</sup> Hare, 2019<sup>17</sup> Hare, 2019<sup>17</sup> Padilla-Coreano, 2016<sup>100</sup> |

Anterior cingulate cortex (ACC), basolateral amygdala (BLA), basomedial amygdala (BMA), dorsal raphe nucleus (DRN), elevated plus maze (EPM), forced swim test (FST), infralimbic (IL), lateral habenula (LHB), medial dorsal thalamus (MDT), medial prefrontal cortex (mPFC), nucleus accumbens (NAc), novelty suppressed feeding (NSF), prelimbic (PL), somatostatin (SST), sucrose preference test (SPT), ventral hippocampus (vHIPPP), ventral medial prefrontal cortex (vmPFC), ventral tegmental area (VTA), vesicular glutamate transporter (vGlut) Manipulations in bold demonstrate sustained effects.

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