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Review article

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Structural neural plasticity evoked by rapid-acting antidepressant interventions

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Abstract

A feature in the pathophysiology of major depressive disorder (MDD), a mood disorder, is the impairment of excitatory synapses in the prefrontal cortex. Intriguingly, different types of treatment with fairly rapid antidepressant effects (within days or a few weeks), such as ketamine, electroconvulsive therapy and non-invasive neurostimulation, seem to converge on enhancement of neural plasticity. However, the forms and mechanisms of plasticity that link antidepressant interventions to the restoration of excitatory synaptic function are still unknown. In this Review, we highlight preclinical research from the past 15 years showing that ketamine and psychedelic drugs can trigger the growth of dendritic spines in cortical pyramidal neurons. We compare the longitudinal effects of various psychoactive drugs on neuronal rewiring, and we highlight rapid onset and sustained time course as notable characteristics for putative rapid-acting antidepressant drugs. Furthermore, we consider gaps in the current understanding of drug-evoked in vivo structural plasticity. We also discuss the prospects of using synaptic remodelling to understand other antidepressant interventions, such as repetitive transcranial magnetic stimulation. Finally, we conclude that structural neural plasticity can provide unique insights into the neurobiological actions of psychoactive drugs and antidepressant interventions.

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Introduction

Dendritic spines are the sites of excitatory inputs in pyramidal neurons[1]. The morphology of dendritic spines is closely related to the presence and strength of excitatory synaptic connections[2], which are important parameters defining the wiring of neural circuits. It has long been appreciated from animal studies that psychoactive drugs can induce synaptic plasticity[3], which can be observed as changes in the density and size of dendritic spines. Typically, for neocortex, these structural modifications occur after the chronic administration of a psychoactive drug[4]. Over the past decade, a series of studies used longitudinal in vivo optical imaging to track dendritic spines in the mouse neocortex across days, showing that a single dose of specific drugs leads to long-lasting structural remodelling. Drugs with this plasticity-promoting quality include ketamine[5–8], serotonergic psychedelics[9,10] and related analogues[11,12], which are either approved or being tested in late-phase clinical trials for their rapid-acting antidepressant effects[13–15]. There is interest from a basic science perspective in understanding how these drugs rapidly promote synaptic rewiring. Incentives also exist for pharmaceutical research, which could leverage structural plasticity to screen new chemical entities that might be candidate drugs for treating mental health conditions.

Depressive disorders present in various forms, ranging from mild, temporary episodes to the most severe and debilitating condition, major depressive disorder (MDD). A particularly challenging subset of MDD is treatment-resistant depression, in which patients fail to respond adequately to first-line antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs), prompting the need for alternative treatments. Growing evidence indicates that MDD is associated with excitatory synaptic deficits[16]. Rapid-acting antidepressant drugs have been postulated to target synapses by enhancing neural plasticity[17], a mechanism that might also be key for other antidepressant interventions, such as electroconvulsive therapy (ECT)[18] and repetitive transcranial magnetic stimulation (TMS)[19]. These interventions can reduce depressive symptoms within days (ketamine[20]) or a few weeks (ECT[21] and accelerated forms of repetitive TMS[22]). Their therapeutic onset is fairly rapid compared with conventional antidepressant drugs (such as SSRIs) and alternative invasive stimulatory techniques (such as deep brain stimulation and vagus nerve stimulation), which can take more than a month to show efficacy. Therefore, given the probable importance of neural plasticity as a potential mechanism of action, preclinical research that delineates antidepressant-evoked effects on the structural rewiring of neurons could shed light on the neurobiology that underlies different treatment options for MDD. Nevertheless, scepticism exists around whether structural plasticity is a sensitive measure or even relevant to the behavioural effects. The adult brain is plastic and synaptic modifications are constantly occurring, particularly after new experiences and learning[23–25]. Moreover, drugs of abuse (for example, cocaine) with negative behavioural consequences are also known to cause structural plasticity of dendritic spines in the neocortex[26,27].

In this Review, we begin by critically reviewing the human data suggesting abnormalities of excitatory synapses in depression, focusing on the prefrontal cortex. We compare the in vivo turnover dynamics of dendritic spines induced by putative rapid-acting antidepressant drugs in preclinical models relative to other types of drugs. We highlight characteristics unique to antidepressant-evoked neuronal remodelling and their potential biological underpinnings. Finally, we discuss the translational opportunities and challenges in using in vivo structural plasticity to advance our understanding of antidepressant interventions.

Excitatory synaptic deficits in depression

To begin, we discuss evidence of synaptic deficits and maladaptive plasticity in studies of humans with depression, as well as the effects of antidepressant interventions on these processes.

Synaptic and morphological abnormalities

The most direct study of the relationship between dendritic spines and MDD in humans uses electron microscopy to analyse post-mortem samples from individuals with MDD and shows fewer spine synapses in layer 2/3 of the dorsolateral prefrontal cortex compared with control individuals[28]. Moreover, there are reports of reduced expression of dendritic markers and synaptic proteins associated with MDD[28,29]. Positron emission tomographic (PET) imaging of synaptic radioligands reveals diminished signals in the prefrontal cortex, anterior cingulate cortex and hippocampus of individuals with MDD compared to healthy individuals, with the amount of synaptic signal reduction correlating with the severity of depressive symptoms[30]. In agreement, in vivo magnetic resonance spectroscopy of individuals with MDD indicates lower glutamate and glutamine concentrations in frontal cortical regions compared with healthy individuals[31,32]. As an integral component in excitatory neurotransmission, such evidence for diminished glutamate signalling is indicative of synaptic deficits in MDD. However, questions remain regarding the origin of the signal from metabolic versus neurotransmitter pools and relative contributions from neuronal and glial cells[33]. Nevertheless, these convergent pieces of evidence suggest excitatory synaptic impairments in MDD.

Genomic and transcriptomic studies have strongly implicated excitatory synaptic dysfunction and prefrontal cortical regions in MDD[34,35]. In a meta-analysis of >1.2 million individuals, three of the eight most significant gene ontology categories associated with depression are synapse assembly, synapse organization and synaptic signalling; of note, some of the included studies used formally diagnosed MDD and others a broader definition of depression[35]. In another large-scale analysis, four out of five gene sets enriched in depression phenotypes encode cellular components for excitatory synapse, neuron spine, postsynapse and dendrite, therefore emphasizing excitatory synaptic function and structure as a major site for perturbance in depression[36]. These and other genome-wide association studies have also identified specific risk genes for depression that could contribute to the synaptic deficits, including those supporting glutamatergic ionotropic and metabotropic receptors, dopamine receptors, calcium-binding proteins[36] and genes involved in synapse development, presynaptic vesicle trafficking and synapse-related major histocompatibility complex function[37]. Bulk and single-cell RNA sequencing of post-mortem brain samples from individuals with MDD and control individuals similarly reveal altered expression of genes related to synaptic transmission and regulation of synaptic plasticity associated with MDD[38,39].

Structural MRI studies of individuals with depression have consistently reported a reduction in cortical thickness and grey matter volume in certain brain regions. These effects were initially described in the hippocampus[40] and subgenual prefrontal cortex[41], but gross volumetric changes are also evident in the anterior cingulate cortex, orbital frontal cortex, amygdala, insula and other locations[42,43]. However, questions about the effect size and whether these changes are a consequence of or a precursor to MDD remain[44]. Moreover, post-mortem samples from individuals with MDD show that the loss of brain volume in the prefrontal cortex is accompanied by abnormalities at the cellular level, which include decreased size and density of neurons and glial cells[45]. Therefore, synapse loss in the neuropil can contribute to a potential

decrease in brain volume; however, it would probably be only one of several relevant factors.

Maladaptive plasticity and functional connectivity

Non-invasive neurostimulation techniques such as TMS can be used to produce motor-evoked potentials (MEPs) in humans. Single-pulse TMS delivers isolated magnetic pulses, primarily for diagnostic or brain mapping, whereas repetitive TMS (including accelerated TMS) involves protocols that apply a series of high-frequency stimulation for therapeutic purposes. Changes in MEPs induced by both single-pulse and repetitive TMS, as measured by electromyography or electroencephalogram recordings, are thought to be indicators of synaptic plasticity in the stimulated circuits. For instance, the paired associative stimulation (PAS) paradigm modulates corticospinal plasticity by applying repeated stimuli to the peripheral median nerve and the contralateral motor cortex[46]. These PAS-induced changes to MEPs can be blocked by administering an *N*-methyl-D-aspartate receptor (NMDAR) antagonist[46], which suggests a mechanism involving excitatory synaptic plasticity. Individuals with MDD show no change in MEP amplitude after paired associative stimulation, unlike healthy control individuals[47]. Of note, the deficient MEP response to PAS observed in MDD can return to a normal level with remission of depression, suggesting that the corticospinal plasticity tracks recovery[48]. Whereas these findings require replication in larger cohorts, the approach reveals a potential deleterious consequence of excitatory synaptic deficits in MDD. Although the PAS paradigm has been primarily demonstrated in the motor cortex, modified protocols have been developed to target the dorsolateral prefrontal cortex, revealing maladaptive neural plasticity in individuals with MDD[49]. These studies are promising but preliminary, with drawbacks including the variation in stimulation effects depending on ongoing brain activity and the large inter-individual and intra-individual variability[50].

Functional MRI is used to investigate circuit-level dynamics in human volunteers. Although resting-state functional connectivity is not a specific readout of synaptic activity, it is an aggregate measure that necessarily incorporates synapse-level signalling. A large consortium study of 1,300 individuals with MDD identifies hypoconnectivity within the default mode network as a hallmark of the disease[51]. Moreover, this result was replicated in another multi-centre study of more than 600 individuals with MDD[52]. Multiple smaller-scale meta-analyses of individuals with MDD have instead reported hyperconnectivity within the default mode network[53,54]. Hypoconnectivity within the frontoparietal network has been reported as a marker of MDD[53], but it was absent in a later investigation[51]. These contradictory results might be attributed to the substantial heterogeneity in the presentation of depression[55–57]; even the most robust depression-associated features have been shown to have extremely small effect sizes when assessed using common neuroimaging modalities[58]. Identifying depression-associated and treatment-associated changes within and between specific networks and circuit pathways remains a crucial yet elusive goal. However, the hope is that advancements in precision functional mapping, using densely sampled longitudinal data from the same individuals, could soon make this goal possible[59,60].

Antidepressant interventions

In individuals with MDD, the effect of antidepressant interventions on excitatory synaptic deficits is not well understood. Non-invasive neurostimulation via repetitive TMS of the left dorsolateral prefrontal cortex has impressive efficacy as an antidepressant treatment[22,61,62]. One study suggests that MEPs are reliably increased in individuals with MDD after repetitive TMS treatment, a strengthening that correlates with the improvement in depressive symptoms[63]. The electrophysiological and symptom changes in individuals with MDD induced by repetitive TMS can be facilitated by co-treatment with D-cycloserine[64], which as a partial NMDAR agonist is expected to boost excitatory synaptic plasticity. Magnetic resonance spectroscopy shows elevated prefrontal glutamate and glutamine metabolite levels in individuals with depression after ketamine treatment[65] and after ECT[66]. The degree of ECT-related change in glutamate levels in the anterior cingulate cortex correlates with the behavioural outcome[67].

The relevance of excitatory neural plasticity to the overall treatment mechanism remains unclear owing to two main challenges for human studies. First, there is the issue of causality, as individuals with depression are often already on a course of SSRIs or might have only recently discontinued psychiatric medications, making it difficult to disentangle and isolate the effect of the treatment being studied. Second, progression is not tracked longitudinally, and currently there is no method available to interrogate synapse-level changes directly and repeatedly in humans. For these reasons, animal models provide a unique opportunity for studying how antidepressant interventions affect neural plasticity. Preclinical studies have shown that dendritic spines in cortical neurons are targeted for remodelling by ketamine[68], classic psychedelics (for example, psilocybin and 5-MeO-DMT)[69,70], ECT[71] and repetitive TMS[72,73]. These early studies have paved the way for studying the effect of antidepressant interventions on structural plasticity, but they have relied on in vitro preparations or histological methods that assess only a single time point. The latest animal studies over the past decade have leveraged chronic imaging to longitudinally track dendritic spines in the brain in vivo, which has been particularly valuable in uncovering the dynamic rewiring process and delineating time courses. These advances are our focus in the next section.

Longitudinal preclinical studies of antidepressant-evoked structural plasticity

Here, we evaluate the nuanced changes of drug-evoked plasticity in mice in response to antidepressant drugs compared with other psychoactive drugs.

Antidepressant drugs versus other drugs

In the mammalian neocortex, most excitatory synapses are located at dendritic spines in pyramidal neurons (Box 1). Laser scanning two-photon-excited fluorescence microscopy can be used to track structural neural plasticity by imaging the turnover of dendritic spines in the mouse (Fig. 1, Box 2). Most in vivo imaging studies of antidepressant treatments so far have focused on antidepressant drugs. Here, to illuminate the crucial plasticity mechanisms, we summarize published studies[5–12,74–80] that have quantified the dynamic turnover of dendritic spines in mice following the administration of putative rapid-acting antidepressant drugs, and we contrast them with responses to other psychoactive drugs (Supplementary Table 1).

We first consider a subset of these experiments in which dendritic spine density in cortical pyramidal neurons has been tracked over many days, surveying three drugs with known and putative therapeutic effects for MDD in humans (ketamine[5], 5-MeO-DMT[10] and psilocybin[9]) and four drugs of other classes (allopregnanolone[74], diazepam[74], zolpidem[74] and cocaine[75]). Spine density data are available longitudinally through 7–15 imaging sessions, covering from pre-drug administration to 15 days after drug administration for ketamine and

Box 1 | Dendritic spines as a proxy for excitatory synapses

Most dendritic spines are sites of excitatory synapses. Upon sensing glutamate released from apposing presynaptic terminals, dendritic spines depolarize and the electrical signals spread for neuronal communication. Biochemically, the unique head-and-neck geometry of a spine suggests that certain molecular signals are compartmentalized to facilitate spine-specific plasticity[162,163]. During postnatal development, synaptic connections are created and pruned, resulting in high spine turnover rates[164]. In the mature brain, structural plasticity slows down to maintain a fairly steady number of synapses[164], yet spines are still malleable in response to novel experience[25] and learning[23,24], or in neuropsychiatric disorders[165]. New dendritic spines emerge after the induction of long-term potentiation[166].

A foundational assumption of this Review is that the existence of a dendritic spine indicates the presence of an excitatory synapse on the cortical pyramidal neuron. Electrophysiology supports this assumption: for example, the frequency of miniaturized postsynaptic currents is considerably increased the day after psilocybin administration in mice, demonstrating enhanced excitatory neurotransmission that corroborates the imaging results showing enhanced spine density[9]. More direct evidence came from electron microscopy that can resolve dendritic spines at high spatial resolution[153,167]. Early studies suggest that 70–95% of the excitatory synapses on cortical pyramidal neurons are formed at dendritic spines[1]. A more recent study shows that only 3.6% of Golgi-stained, optically imaged spines in the mouse neocortex lack synapses[167]. However, whether these spines are functional is less clear, as a recent report based on expansion microscopy and patch clamp recording suggests that up to 25% of the dendritic protrusions in layer 5 pyramidal neurons in the adult mouse brain are filopodia-like, electrically silent synapses[99]. Collectively, these results indicate that almost all dendritic spines contain synaptic elements, with the majority being functional excitatory synapses.

The size of a dendritic spine is thought to be an indicator of synaptic strength. Larger spine volumes are positively correlated with larger postsynaptic densities and a higher number of presynaptic vesicles[153]. Moreover, targeted potentiation of a single spine by glutamate uncaging leads to structural enlargement, which is accompanied by the recruitment of AMPA receptors and increased excitatory postsynaptic currents[168]. Finally, spine head volume is shown to correlate strongly with postsynaptic density area, which directly relates to the amplitude of postsynaptic potential[169]. These results show the tight relationship between spine size and function and stability[2]. Other morphological parameters could have biological implications too, such as the length of the spine neck protrusion, which is expected to affect the compartmentalization of the biochemical signals[162,163].

63 days after drug administration for diazepam. Some experiments test a single dose of the compound whereas others involve chronic administration. The drugs act on various receptors and transporters with distinct synaptic localization (Fig. 2a).

Ketamine, primarily an NMDAR antagonist[81], has been studied at subanaesthetic doses and is an FDA-approved medication for treatment-resistant depression[20,82]. 5-MeO-DMT and psilocybin act on serotonergic receptor subtypes[83,84], which together with other classic psychedelics have long been recognized for their therapeutic potential[85] and are undergoing clinical trials as treatments for MDD and treatment-resistant depression[86–88]. Allopregnanolone, a positive allosteric modulator of the $GABA_A$ receptor via binding on the neurosteroid site[89], is approved for treating postpartum depression. Diazepam and zolpidem, also positive allosteric modulators of the $GABA_A$ receptor[90], are benzodiazepines administered to treat anxiety and insomnia. Cocaine inhibits the uptake of monoamine neurotransmitters, such as via its action on the dopamine transporter, and is a drug of abuse[90]. Here, we list the primary targets, although these drugs probably interact with other receptors. For instance, ketamine and its metabolites can act on NMDAR-independent signalling pathways, including opioid receptors[81].

For visualization, we have extracted data values from published studies (Box 3; Supplementary data). Plotting the results across studies on the same axes reveals that the drugs exert differential effects on dendritic spine density in cortical pyramidal neurons (Fig. 2b). A single dose of ketamine[5], 5-MeO-DMT[10] or psilocybin[9] rapidly increases the spine number density of cortical pyramidal neurons by 12–20% in mice. In the ketamine study, the spine density starts to decline after 2 weeks (ref. 5), but it stays elevated for at least 1 month in the 5-MeO-DMT and psilocybin studies[9,10]. By contrast, allopregnanolone[74] and cocaine[75] also cause the spine density to rise, but the change is more gradual, weaker (increase of ~5%), and occurs after repeated administration. For diazepam[74], 1 week of daily exposure robustly reduces the spine number density, an effect that lasted for 2 months. No detectable change was observed after chronic administration of zolpidem[74].

Timing and dose

One theme that emerges through the comparison is that although drug-evoked changes in spine density can last for days and up to weeks, the initial shift in the dynamic turnover rates of spines is transient. Compounds with putative rapid-acting antidepressant action (ketamine, psilocybin and 5-MeO-DMT) heighten the rate of spine formation for 1–3 days after single-dose administration[5,9,10]. For ketamine, the speed of spine growth has been finely mapped by two studies, which show that higher spine density could be observed at 12 h post-injection, but not earlier[8,91]. Even for other chronically administered psychoactive drugs, spine remodelling kinetics are quick and restricted to the few days after treatment onset. This is true for Δ^9-tetrahydrocannabinol (known as THC), which shows the highest spine gains on day 1 and day 3 despite a 12-day course of administration[80], and is also true for cocaine, in which spine rearrangement is seen within hours after the first injection[75,92]. Likewise, a next-day increase in spine formation in vivo is reported for haloperidol (an antipsychotic that is a D_2 receptor antagonist[77]), 2,5-dimethoxy-4-iodoamphetamine[11] (known as DOI, a substituted amphetamine that is also a phenethylamine psychedelic) and tabernanthalog[11,12] (known as TBG, a novel serotonergic agonist derived from ibogaine).

Dose is probably a major factor in determining the kinetics and effect size of the drug-evoked structural plasticity, but unfortunately

it is often not tested in in vivo imaging studies. For diazepam, a high sedative dose at 5 mg kg^{-1} leads to an immediate loss of spines, whereas a lower anxiolytic dose at 1 mg kg^{-1} has similar but blunted effects[74]. The length of administration matters: 4 weeks of a high dose of diazepam is associated with a more long-term decrease in spine density than the same dose applied for 1 week. Another example is ketamine, in which four studies from four different laboratories have investigated the effects of an antidepressant dose at 10 mg kg^{-1}, either via a single injection[5,7,8] or sub-chronic administration for 2–7 days (refs. 6,7). All studies report a subsequently increased number of cortical dendritic spines. However, when ketamine was tested at a much higher anaesthetic dose (85 mg kg^{-1}) and in conjunction with xylazine, the drug only transiently promoted the formation of filopodia for 1–4 h and had no lasting effect on the dendritic architecture[78]. Furthermore, without testing a range of doses or including another drug as a positive control, it is not easy to interpret a null result. For example, one study reported no effect in the visual cortex following 4 weeks of treatment with the antidepressant drug fluoxetine[76], but perhaps a higher dose would produce a different result.

Wiring or weight changes?

When a new spine is formed, it could arise from de novo spinogenesis or from the conversion of an electrically 'silent synapse' (Fig. 3). During de novo spinogenesis, spine outgrowth occurs at a dendritic location previously lacking synaptic material. This type of glutamate-evoked sprouting can involve the initial growth of a small protrusion[93] that gradually increases in volume with maturation[94,95]. Ketamine has been shown to enhance such glutamate-evoked spinogenesis[91]. The possibility of

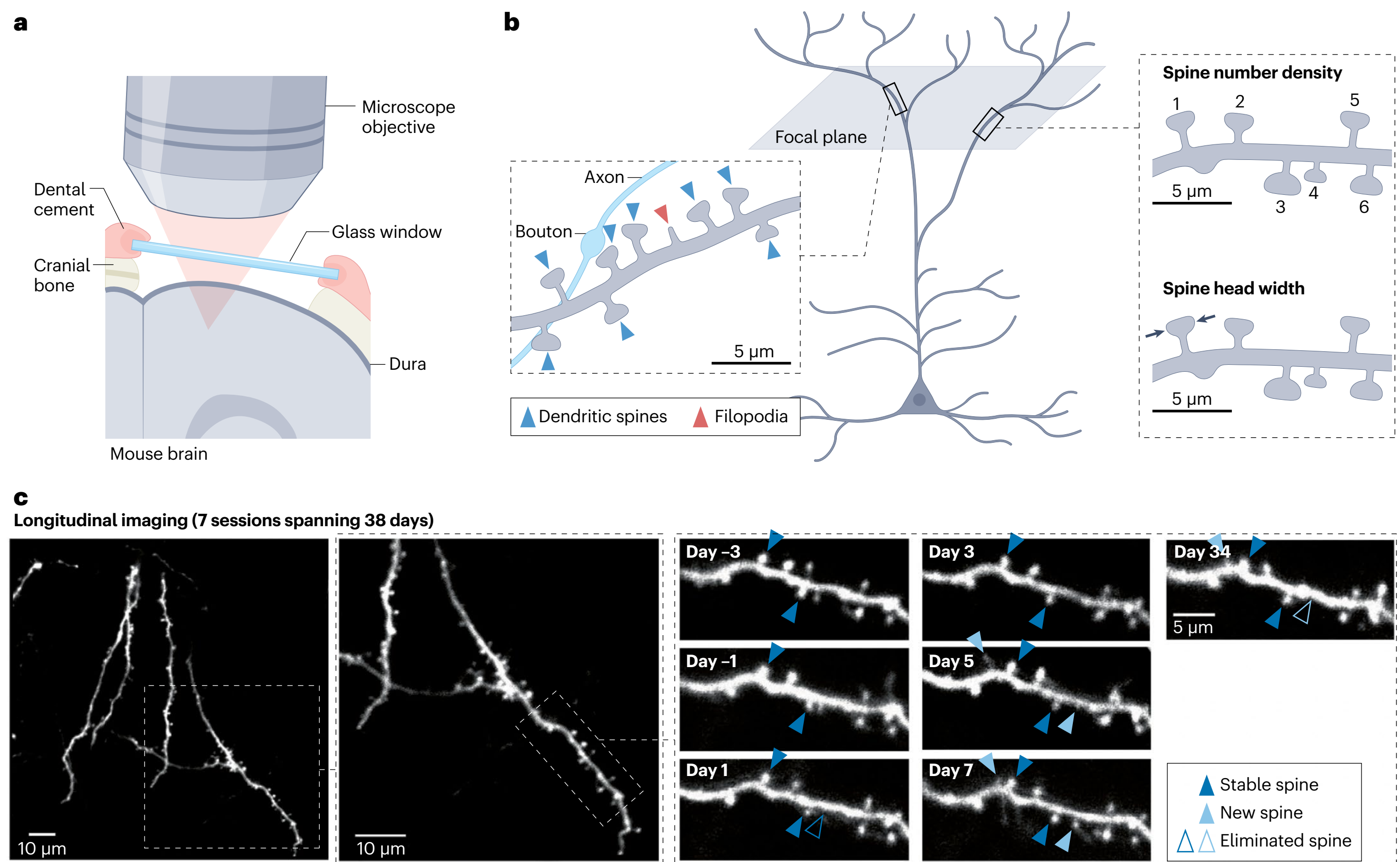


Fig. 1 | Imaging dendritic spines in vivo. a, In animals, such as mice, visualizing and tracking dendritic spines in an intact brain was made possible by multiphoton microscopy[157]. The approach begins with cranial window surgery involving either thinning the skull[158] or removing a bone flap and putting on a 1–10 mm^2 glass cover[116]. The cranial window is essential for optical transmission into the brain. To visualize dendrites at subcellular resolution, mice are typically anaesthetized with the head fixed in a two-photon microscope. Neurons express fluorescent proteins, which are introduced using viruses or by generating transgenic animals. **b**, Cartoon representations of in vivo images obtained from focal planes at the apical tuft of a deep-lying pyramidal neuron. Subcellular resolution imaging enables visualization of dendritic segments including dendritic spines (blue arrowheads), axons and filopodia (red arrowhead). If only a small protrusion is observed, the presence of a spine can be confirmed by checking adjacent images at different depths. Dendritic spines are small structures that protrude off the shaft of a dendrite, with a typical length of <2 µm and a stalk as narrow as 0.1 µm (ref. 159). Spine morphology varies widely, ranging from slender filaments to mushroom shapes[1]. Spine number density, spine head width, or spine brightness (as a proxy of spine volume) can be measured from the images. Thick-tufted layer 5 pyramidal neurons have been estimated to have ~15,000 spines distributed across the apical, oblique and basal compartments of the dendritic tree[160]. The mouse neocortex has an average of 10–15 spines per 10 µm of dendrite[153], although the number differs depending on cell type and dendritic location[161]. **c**, Dendritic spines in the same field of view can be tracked across multiple sessions over weeks. Here, the same field has been tracked in seven sessions over 38 days; persisting stable spines and new spines can be measured. Part **c** is reprinted with permission from ref. 9, Elsevier.

Box 2 | Longitudinal imaging of dendritic spines in the neocortex of living animals

When imaging dendritic spines, expression of fluorescent proteins in a sparse number of neurons is highly desirable to prevent fluorescent dendrites from overlapping and obscuring each other in the images. As such, many studies rely on *Thy1*^{GFP} and *Thy1*^{YFP} mouse strains, which have stable and bright fluorescent protein expression in a sparse subset of neurons[170]. Standard two-photon microscopy offers a spatial resolution of ~0.2 μm laterally and ~1.5 μm axially[171], which is sufficient to visualize dendritic spines, especially those that lie parallel to the focal plane. However, axially oriented spines or thin filopodia can be missed, and small features such as spine neck width cannot be resolved. Nevertheless, spine number density can be quantified from the images on the basis of morphological criteria[116]. Spine size can be estimated on the basis of structural parameters such as spine head width[9,74], although some studies indicate that spine brightness might result in more accurate quantification[94,102]. Some studies classified spines into subtypes (for example, mushroom, stubby and thin)[1], although other ultrastructural analyses suggest that sizes and shapes lie on a continuum rather than being distinct subtypes[172]. Imaging can be repeated for the same field of view; therefore, visualizing longitudinal changes of dendritic architecture in a live animal over an extended period across days and up to several months is feasible.

The number of dendritic spines in the brain is maintained through a balance of the formation of new spines and the elimination of existing spines. Using the longitudinal imaging approach, pioneering studies have investigated the baseline levels of dendritic spine turnover in layer 5 pyramidal neurons in the neocortex of adult mice. One study indicates that dendritic spines are mostly stable, with only ~4% of them turning over each month, suggesting that many spines persist through the lifetime of a rodent[173]. By contrast, another study shows a more dynamic picture, observing that ~20% of dendritic spines have lifetimes of <1 day and ~60% have lifetimes of >8 days, with an estimated mean lifetime of 4 months for the enduring spines[25]. The discrepancy could be due to distinct stable and transient subpopulations of dendritic spines intermixed along cortical dendrites[174]. Moreover, the spine turnover rate can vary for different brain regions; for example, the mean lifetime reported for hippocampal dendritic spines is as short as 1–2 weeks (ref. 175). Overall, these results demonstrate that the structural plasticity of dendritic spines is a process not limited to development but that continues in adulthood.

de novo spine formation in response to ketamine is exciting because it suggests that the drug might increase the number of potential synaptic partners for a given dendritic segment. The prospect for a cell to form synapses with previously unconnected partners confers a high degree of flexibility to the wiring of cortical circuits[96].

Alternatively, new spines could be attributed to the conversion of silent synapses to active synapses. Silent synapses contain NMDAR and are electrically silent[97], but they recruit AMPA receptors upon synaptic activation[98] (Fig. 3). Although silent synapses resemble filopodia and are, thus, easily missed by in vivo two-photon microscopy, most of them have functional presynaptic partners[99]. Therefore, unlike de novo spinogenesis, the wiring for silent synapses has already been laid out, but the connections are dormant. An intriguing idea is that the spine loss observed in depression might not be elimination but rather the conversion of functional synapses into silent synapses, which could then be reactivated by rapid-acting antidepressants. This scenario could explain why several studies in mice have reported that new spines induced by ketamine[7,8] or serotonergic agonists[12] preferentially form at locations where spines were recently eliminated.

Empirically, psychoactive drugs might affect both spine number density and spine size, but the changes could occur in different directions or over different time courses. Following a single dose of psilocybin in mice, both spine number density and spine head width significantly increase[9], whereas diazepam induces significant decreases in these parameters on a similar timescale[74]. By contrast, a single dose of 5-MeO-DMT rapidly elevates spine number but has a minimal effect on the spine size[10]. Of note, after psilocybin treatment, the initial coordinated elevations in spine number and size diverge over time, with spine density remaining enhanced and spine size returning to baseline after a month[9]. However, it should be noted that measurements of spine size might be imprecise owing to methodological limitations (Box 2). Notwithstanding this caveat, an intriguing prospect is that spine number and spine size reflect different forms of synaptic plasticity. This idea is supported by attempts to causally induce structural plasticity. Two-photon glutamate uncaging can be used to study the targeted stimulation of individual dendritic spines. Applying a spike-timing-dependent glutamate uncaging protocol to single spines in mouse brain slices was sufficient to convert silent synapses to functional synapses, but the same protocol was ineffective at enlarging existing spines[99]. This finding suggests that different plasticity processes are responsible for spine formation versus enlargement (Box 4), which could reflect different mechanisms of drug action.

Persistent integration of newly formed dendritic spines

A striking finding in antidepressant-evoked structural plasticity is the sustained increase in dendritic spine density. The extended time course of the circuit rewiring seen in mouse models is reminiscent of the durable therapeutic effects reported in clinical trials. It is tantalizing to think that the clinical outcomes of psilocybin and ketamine treatments could reflect their different effects on spine persistence in preclinical studies. The more long-term effects of psilocybin on dendritic spine density correspond with its ability to provide more sustained relief from depressive symptoms compared with ketamine (Fig. 2). However, the reason behind this persistence of dendritic spines is poorly understood.

Prevailing frameworks of antidepressant drug action have mostly focused on how synaptic connections can be potentiated[17,100], although the theory of a homoeostatic plasticity basis for drug action could speak also to maintenance and stability[101]. In general, some dendritic spines are ephemeral, disappearing within several days after formation, whereas others can remain for months[102]. Moreover, larger dendritic spines are known to be more persistent than smaller ones[102,103]. A drug might selectively favour the growth of larger and more persistent spines. For psilocybin, ~50% and 35% of the newly formed spines induced by drug administration in mice survive for 7 and 34 days, respectively[9], suggesting that a sizable portion of the new connections are stabilized and maintained. These values contrast with the lower rate of persistence (~20%) for ketamine-evoked new spines after 15 days (ref. 5). As another example, diazepam seems to

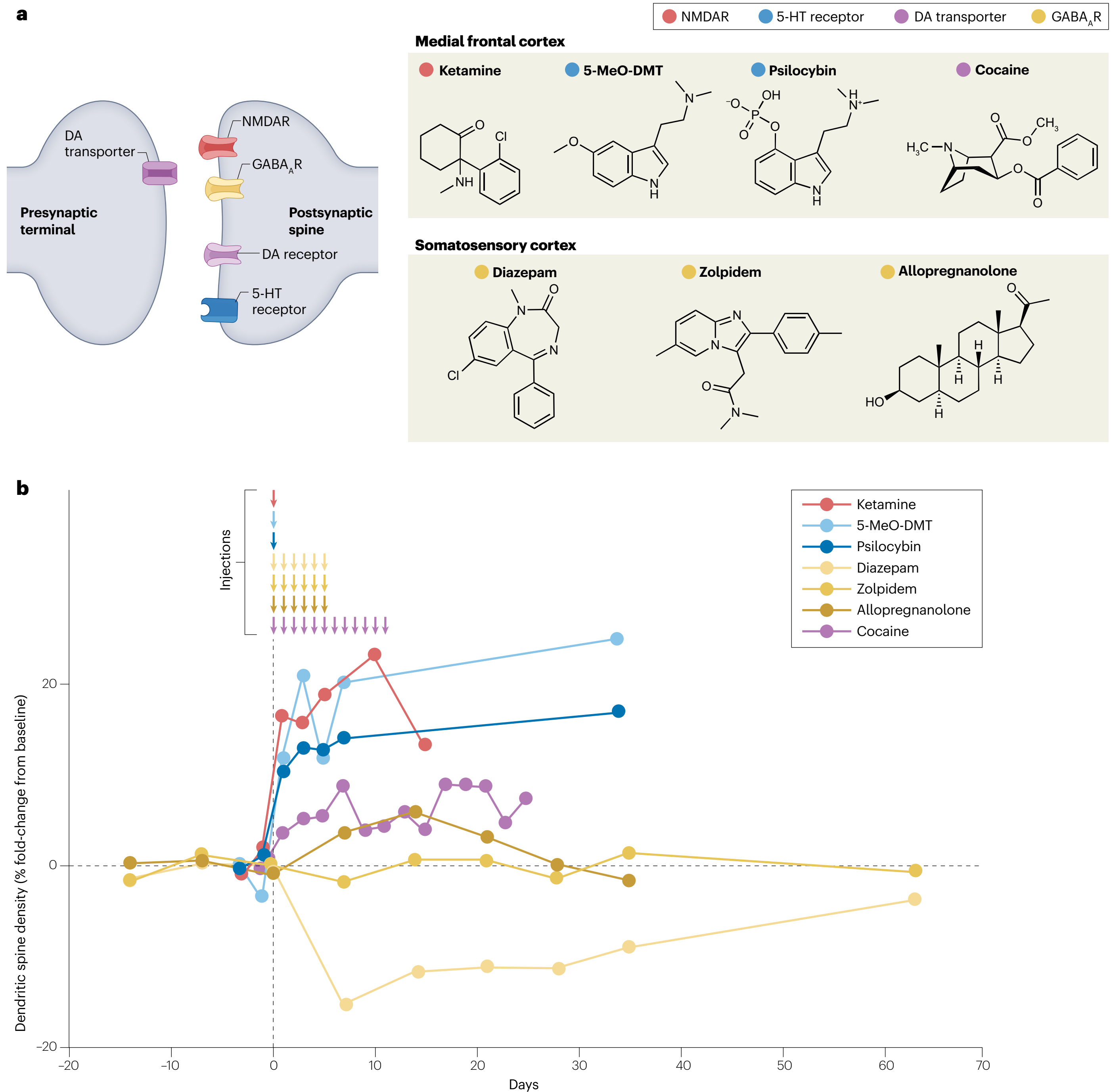


Fig. 2 | Effects of different psychoactive drugs on apical dendritic spines of cortical pyramidal neurons in mice. a, Seven drugs and the putative synaptic localization of their primary target receptors and transporters, for which there are published data on their long-term effects on dendritic spines in mice. **b**, Effects of the drugs on the density of cortical dendritic spines. Data values were extracted from published studies[5,9,10,74,75]. The studies, conducted in different laboratories, all used two-photon imaging to visualize apical dendritic spines of deep-layer pyramidal neurons in the neocortex. The studies differ in the imaged brain region, which was the medial frontal cortex for ketamine, 5-MeO-DMT, psilocybin and cocaine, and the somatosensory cortex for diazepam, zolpidem and allopregnanolone. The studies also differ in the number of drug administrations, indicated by the arrows. To plot the results on the same axes, values were extracted from published plots in pixel measurements using ImageJ and scaled relative to the *y* axis of the respective plot. Scaled drug-treated values were subtracted from scaled vehicle-treated values. All the extracted and subtracted values are provided in the Supplementary data. 5-HT, serotonin; DA, dopamine; GABA$_A$R, GABA$_A$ receptor; NMDAR, *N*-methyl-D-aspartate receptor.

Box 3 | Methodological considerations for published preclinical studies

The published preclinical studies presented in Fig. 2 have methodological differences. The effects of ketamine[5], 5-MeO-DMT[10], psilocybin[9] and cocaine[75] were determined by imaging apical tufts of layer 5 pyramidal neurons in the Cg1 and medial M2 regions of the mouse medial frontal cortex. By contrast, studies of the various $GABA_A$ positive allosteric modulators were done on the mouse somatosensory cortex; however, some results were replicated in a separate experiment that imaged the prelimbic region of the medial frontal cortex[74]. Although imaging dendritic spines in vivo across the entire brain is currently not feasible, future studies could start with a brain-wide characterization of plasticity-related changes, such as an unbiased screening of synaptic proteins or immediate early genes[176,177], before focusing on specific brain regions. Dendritic spines in deep-lying brain regions can be imaged in live animals using a glass microprism[8] or through a cannula[178]. Moreover, the studies have used adult mice with ages ranging from 2–2.5 months for psilocybin[9] to 4–5 months for diazepam[74]. In studies that used younger mice, spine loss was reported for vehicle-treated animals[5,6], which might be due to synaptic pruning from ongoing cortical development, accumulating photodamage from repeated imaging, or the degrading quality of the cranial window over time. The influence of age is an important reason to study drug effects in vivo rather than with dissociated cells in vitro, which contain immature cells reflecting embryonic stages of development.

selectively promote the loss of transient spines while sparing the more persistent, pre-existing spines[74], which might explain why its chronic use impairs learning.

Interactions between stress and dendritic plasticity

An underexplored topic is the interaction between antidepressant interventions and prior experiences that can also evoke neural adaptations. For example, whether antidepressant interventions and stress engage the same or different structural plasticity mechanisms is not currently clear. Chronic stress is a risk factor for depression in vulnerable individuals[104], motivating a variety of preclinical models in which repeated stressors were applied to rodents. These models cannot recapitulate the complexity of depression, but they could still be useful tools for understanding biological mechanisms[105,106].

In the rodent medial frontal cortex, chronic stress causes atrophy of apical dendritic arborizations[107,108] and retraction of dendritic spines[109,110]. Studies from the past 15 years using in vivo longitudinal two-photon imaging have confirmed these structural changes, finding decreased spine density in cortical pyramidal neurons of mice following repeated restraint stress[7,8,111], chronic unpredictable stress[12,112] or chronic corticosterone treatment[8,113]. A heightened rate of spine elimination is a main factor underlying the stress-induced spine loss[111,113], which seems to selectively disturb specific dendritic branches and spatially clustered dendritic spines[8]. Evidence indicates that the effect of ketamine on dendritic architecture depends on prior stress exposures in mice. For example, an increase in the spine formation rate was observed when ketamine was given to mice that were stressed with repeated restraint[7]. However, if ketamine is applied prophylactically before restraint stress, ketamine-treated mice are protected against stress-induced spine elimination, but they no longer have additional new spines. Furthermore, in a tour-de-force demonstration in mice, a single dose of ketamine specifically restored dendritic spines that were eliminated after chronic exogenous corticosterone (the key glucocorticoid hormone of the stress response in rodents) treatment[8]. The spinogenesis occurs after the acute action of ketamine to increase calcium influx at dendritic spines[114] and the induction of synchronous neuronal firing[8] in the medial prefrontal cortex[8], which could be potential pre-requisites to the structural plasticity.

Translational opportunities

Towards an assay for drug screening

Structural plasticity in vivo could be leveraged for preclinical drug discovery, as currently few assays are available with predictive validity for psychiatric disorders[115]. There is value in pursuing a measurement that probes directly inside the brain and yields more nuanced information about drug action than other methods, such as behavioural tests and biochemical assays. Challenges in scaling up in vivo imaging-based drug

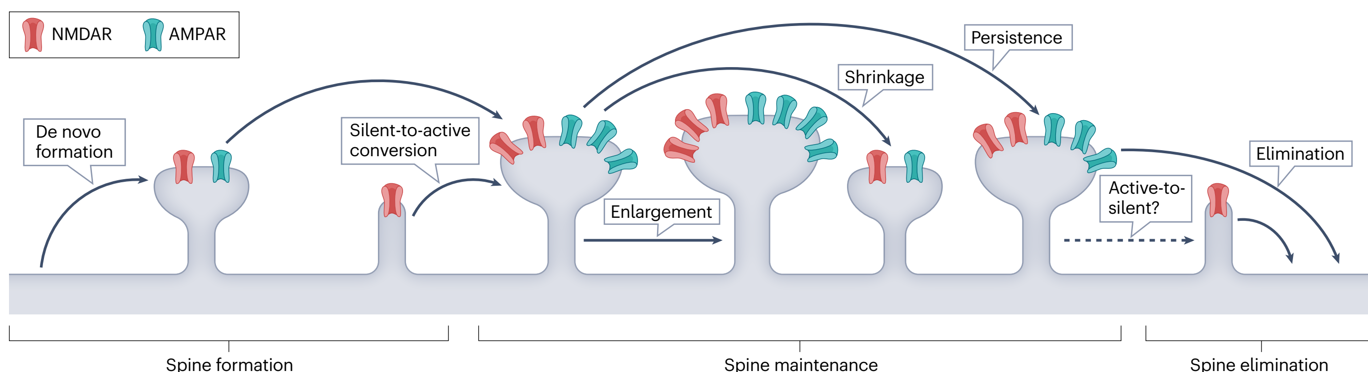
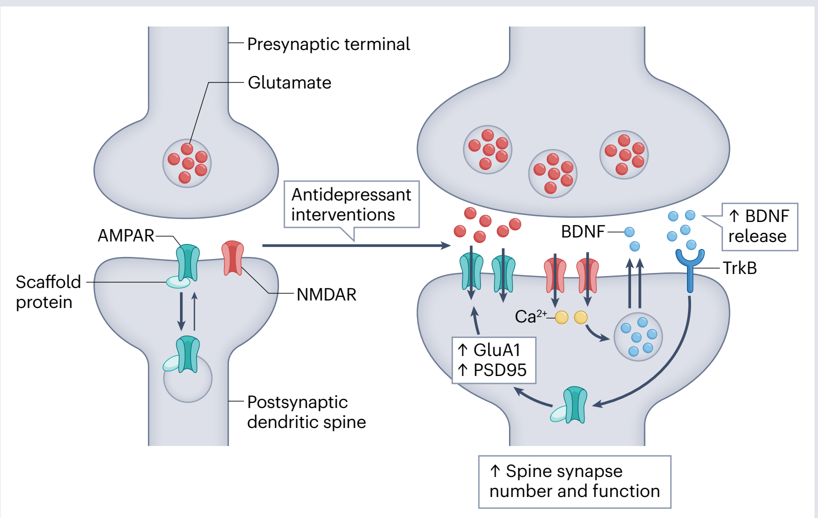


Fig. 3 | The life cycle of a dendritic spine. A functional dendritic spine with a complement of AMPA receptors (AMPAR) and *N*-methyl-D-aspartate receptors (NMDAR) can emerge through de novo formation (spinogenesis) from a location that lacks any synaptic material or conversion from an electrically 'silent synapse', which contains only NMDAR. The size of a dendritic spine can be enlarged or shrunk continually to adjust the synaptic strength. A spine can persist for some time, ranging from days to months, before it is eventually retracted and eliminated. In addition, a potential path could exist for the back-conversion from functional dendritic spine to a silent synapse (dashed arrow).

Box 4 | Molecular factors involved in spine formation and elimination



Spine growth involves the structural expansion of spine volume and can be the result of de novo spinogenesis or enlargement of existing spines. The spine expansion process is well characterized (see the figure), especially through careful studies using two-photon glutamate uncaging for targeted stimulation of individual dendritic spines in brain slices. The general consensus on the enlargement process is a protein synthesis-independent early phase (as demonstrated by applying protein synthesis inhibitors such as anisomycin or cycloheximide and observing no impediment to initial spine enlargement[179]) followed by a protein synthesis-dependent late phase[179]. In the early phase (<60 min), actin and cofilin transiently accumulate in the spine head. From there, actin rapidly undergoes polymerization and actin modifiers are recruited, which enables major reorganization of the actin cytoskeleton to enlarge the spine head. This initial step of actin remodelling involves small GTPases downstream of CaMKII, such as H-Ras, Cdc42 and RhoA[162]. An early, postsynaptic release of brain-derived neurotrophic factor (BDNF) and rapid spine-specific activation of TrkB receptors also occur[180].

The late phase (>60 min) involves protein synthesis including local translation in the dendrites[181]. For example, PSD95 is newly synthesized in response to synaptic stimulation, as shown using novel fluorescent epitope tags[182]. PSD95 and other scaffold proteins are recruited to the spine head, at rates that correlate with the eventual increase in spine volume. The scaffold proteins contribute to the enhanced retention of glutamatergic receptors at synapses, thereby resulting in synaptic potentiation[183]. Of note, these time courses were delineated in vitro but are challenging to confirm in vivo owing to technical limitations. In vivo, newly formed spines might take longer (>1 day and up to 4 days) to become functional synapses[94]. For spine elimination, shrinkage and eventual retraction involve a different sequence of molecular signals[184]. Synapse formation and elimination are additionally regulated by non-neuronal cells such as astrocytes and microglia[185,186]. AMPAR, AMPA receptor; NMDAR, *N*-methyl-D-aspartate receptor. Box 4 figure is adapted from ref. 187, Springer Nature Limited.

screening assays exist owing to low throughput, but advancements in technology and methodology could soon help overcome these limitations. One issue is the image analysis, which involves human observers segmenting regions of interest based on predetermined criteria[116]. Machine learning can automate the process to accelerate the analysis of dendritic spines[117,118]. Moreover, automating the analysis will also improve reliability because currently, different experts can annotate differently and even for the same expert tested weeks apart, intra-rater reliability remains less than ideal[117]. Another viable approach to increase throughput is to first use in vitro high-content imaging of cultured cells to perform a large-scale screen of hundreds of compounds for increased dendritic spine growth or synaptic protein expression[69,119,120], which has the added advantage of potentially including human patient-derived neurons[121], before selecting a few promising candidates for in vivo characterization.

A biomarker for synaptic remodelling

Although visualizing dendritic spines in vivo in non-human primates (such as marmosets and macaques) is possible[122,123], ultimately animal studies are insufficient if the goal is to understand human brain function. The issue of species-specific differences in neuroanatomy is important, given the uniqueness and complexity of the human prefrontal cortex. Moreover, depressive symptoms are not only heterogeneous, but they can also have different trajectories over time. The key to resolving these unknowns will be new methods that can measure synapse-level changes in the human brain while tracking the progression of depressive symptoms.

For studying synapse-level changes in humans, the most promising approach is PET imaging. Novel contrast agents, such as [^{11}C]UCB-J or [^{18}F]UCB-J and [^{18}F]SynVesT-1, have micromolar affinity to synaptic vesicle glycoprotein 2A (SV2A), a synaptic vesicle protein that is expressed in presynaptic terminals throughout the brain. When applied to individuals with MDD, this imaging approach reveals that the severity of depressive symptoms negatively correlates with SV2A density[30]. Moreover, in individuals with MDD with lower SV2A density at baseline, the administration of ketamine leads to a significant increase in the SV2A signal, whereas no change is observed in healthy individuals and in individuals with MDD with no prior evidence of synapse loss[124]. Another study has detected no change in synaptic signal in individuals with late-life depression (over 60 years of age) being treated with first-line antidepressant medications relative to the healthy control group[125]. A caveat of SV2A imaging is that an increase in signal can be due to more synapses or stronger existing synapses, which the method cannot disambiguate. Moreover, longitudinal PET imaging across multiple sessions could be cost-prohibitive, and additional difficulties can arise that are associated with quantification of non-specific SV2A binding[126]. Notwithstanding the challenges, synaptic imaging in vivo in humans is gaining popularity. Similar to preclinical in vivo optical imaging, synaptic imaging in humans might soon make it possible to compare effects across drug types, as data begin to emerge for SSRIs[127] and drugs of abuse[128].

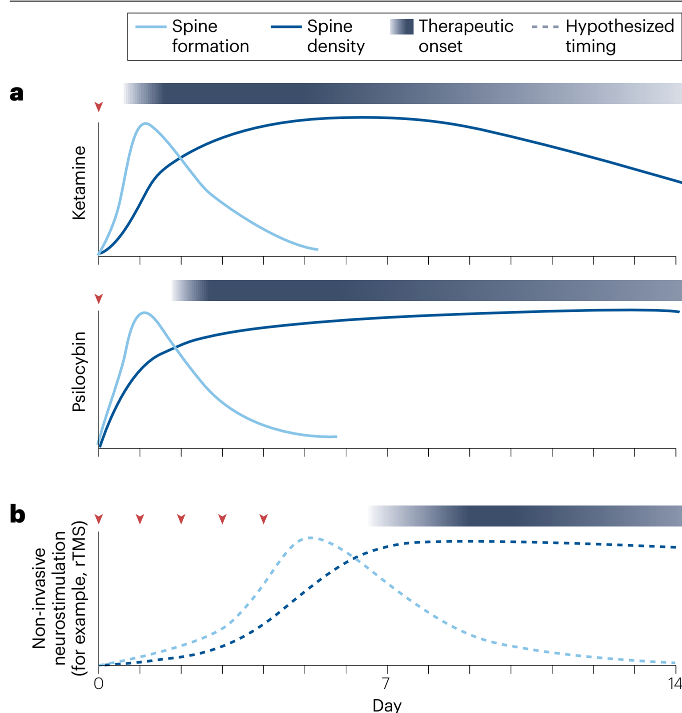


Fig. 4 | Time courses of antidepressant intervention-evoked structural neural plasticity in rodents and therapeutic onset in humans. a, The timing of events after administration of rapid-acting antidepressant drugs, including the elevation in dendritic spine formation rate in the medial frontal cortex in mice (light blue) and the increase in dendritic spine density in the medial frontal cortex in mice (dark blue) as measured by laser scanning two-photon-excited fluorescence microscopy, and the therapeutic onset when a notable decrease in depressive symptoms can be detected for patients measured by Hamilton Depression Rating Scores or Montgomery–Asberg Depression Rating Scale scores with treatment-resistant depression (grey-shaded area) after one or more treatment sessions (red arrowheads). **b**, Similar to part **a** but for accelerated transcranial magnetic stimulation (TMS). The dashed lines denote hypothesized structural plasticity. All profiles are schematic but based on data from published studies (ketamine spine formation and density timing[5,8], ketamine therapeutic onset[20], psilocybin spine formation and density timing[9], psilocybin therapeutic onset[86] and TMS therapeutic onset[22]). rTMS, repetitive TMS.

Gaps and challenges

Causal link from drugs to synapses and from synapses to behaviour

Although rapid-acting antidepressant drugs promote the formation and growth of dendritic spines in animal models, whether this effect is an epiphenomenon or an essential step towards behavioural effects is not understood. An important piece of the puzzle is that the rapid onset and long duration of spine density change in mouse models coincide with the time course of improvements seen in patient depression rating scales (Fig. 4a). Importantly, other evidence suggests that drug-evoked neuronal rewiring could be relevant to behavioural outcomes. The first clue comes from a study of rats that underwent repeated restraint stress, which caused impairments in attentional set-shifting (a measure of attention and cognitive flexibility)[129]. For stressed animals, the loss in dendritic material correlates significantly with the reduction in behavioural performance. A second clue is that in mice administered with cocaine, the persistence of drug-evoked new spines relates to the bias in the conditioned place preference test (a test of preference for an addictive drug)[75]. The third piece of evidence, and the most direct proof so far, comes from experiments involving causal perturbation. Newly formed spines can be selectively manipulated to shrink and collapse using a light-activated tool that disrupts cytoskeleton dynamics[130]. A crucial study has shown that disrupting ketamine-evoked new spines blocks the ability of the drug to alleviate motivated escape behaviour in mice[8]. Intriguingly, the manipulation affects specifically the sustained behavioural responses, but not the acute action, suggesting that ketamine-induced spine formation is more relevant for the long-term clinical effect of the drug. It will be essential for future studies to confirm this causal relationship for ketamine and to further test the link with other antidepressant interventions. Assuming the new excitatory synapses are essential for antidepressant action, questions on what exactly are the downstream mechanisms that enable the new spines to drive behavioural changes remain open.

Towards reverse translation of TMS

Although studies have used in vivo structural plasticity to study antidepressant drugs, a clear opportunity exists here to apply the same approach to understand other interventional psychiatry treatments (Fig. 4b). Consider TMS: in preclinical studies, a single pulse leads to a short-lived and localized increase in spiking activity and then suppression over ~100 ms (refs. 131,132). By contrast, high-frequency repetitive TMS favours excitation and subsequent long-term potentiation of the evoked activity[131]. The consequences on dendrites are interesting to consider. One study shows that a single pulse of TMS reduces sensory-evoked calcium signals in rodent cortical dendrites[133]. The inhibitory effect could arise from the depth dependence of TMS, as dendrite-targeting GABAergic cells in layer 1 are strongly activated, whereas the pyramidal cell bodies in layer 5 are far from the coil and, therefore, not driven[133]. Evidence from preclinical studies in mice now suggests that repetitive TMS could evoke the remodelling of dendritic spines in vivo[134]; however, the effect of clinically relevant high-dose TMS protocols (such as intermittent theta-burst stimulation) on structural neural plasticity remains to be determined. A challenge for reverse translation of repetitive TMS is the vast difference in brain geometry between animals used in preclinical studies and humans. The spatial extent of the magnetic field stimulation should match the brain targets such that the coils used for TMS in humans need to be miniaturized for preclinical experiments.

Which circuits and cell types?

At face value, given the excitatory synaptic deficits in the prefrontal cortex associated with MDD, it seems that the formation of new dendritic spines evoked by rapid-acting antidepressant drugs should be beneficial. However, this view is overly simplistic because not all synapses are equal. The function of synapses in neural circuits crucially depends on the source of the presynaptic input, the subcellular location in the postsynaptic cell and the postsynaptic cell type in different brain regions. Although our discussion focuses on dendritic spines in the frontal cortex, the pathophysiology of depression involves a broad neural circuitry. In humans with depression, maladaptive plasticity and abnormalities are observed in the frontoparietal network, default mode network, salience network and the limbic system[53,135,136]. Increasing evidence also indicates that subcortical structures, such as the amygdala, nucleus accumbens, ventral tegmental area and dorsal raphe, have crucial roles in mediating the diverse symptoms of depression[137]. Preclinical research suggests

there are region-specific differences in the pathophysiology. Although chronic stress causes excitatory synaptic deficits and dendritic spine loss in the rodent medial frontal cortex[109,138], opposing effects have been observed in the amygdala[139,140] and lateral habenula[141,142].

Structural changes have different implications depending on the connectivity of a brain region with the broad neural circuitry; therefore, increased dendritic spine density and spine size should not be interpreted as blanket beneficial attributes. A more probable scenario is that not all new spines are equally important; instead, those that strengthen specific circuit pathways might be particularly crucial for behavioural effects. Future studies should examine how structural plasticity can be modified in a circuit-specific manner by stress[143] and drugs[144,145]. Dual-colour imaging could be used to visualize dendritic spines while, at the same time, identifying the source of presynaptic input[146]. Drug-evoked changes in the distribution of synaptic partners could be systematically assessed across the whole animal brain during monosynaptic anterograde and retrograde viral tracing methods[147,148]. It will be crucial to understand the specific circuit pathways that are targeted by the drug-evoked structural plasticity.

Even within the frontal cortex, the excitatory pyramidal neurons are not a homogeneous population, but they instead consist of subtypes that differ by their projection targets and laminar position[149,150]. Most studies of drug-evoked turnover of dendritic spines rely on transgenic mice that preferentially express fluorescent proteins in the pyramidal tract subtype of pyramidal neurons in layer 5 of the neocortex[150,151], but other neuronal subpopulations could also contribute to antidepressant drug action, such as layer 2/3 pyramidal neurons[28,104]. This limitation could be rectified by using newer methods that enable genetic access to other excitatory cell types in mice, such as viruses with cloned-in cell-specific enhancers[152]. Moreover, pyramidal neurons are embedded within cortical microcircuits, with extensive connections to local GABAergic inhibitory interneurons. Although most synaptic inputs onto dendrites are excitatory, with an estimated excitatory-to-inhibitory input ratio of 20:1 (ref. 153), the few inhibitory connections can powerfully modulate dendritic excitability[154,155]. Therefore, inhibitory connections are postulated to have essential roles in regulating drug-evoked plasticity[100]. Inhibitory synapses are plastic like their excitatory counterparts[156]. The extent to which inhibitory plasticity might contribute to drug action is unknown.

Conclusions

Dendritic spines are the sites for excitatory synaptic connections in cortical pyramidal neurons. Evidence indicates that excitatory synaptic deficits occur in depression. Over the past decade, in vivo longitudinal imaging studies have begun to reveal how drugs with established or putative rapid-acting antidepressant effects in humans, including ketamine, psilocybin and other psychedelic analogues, alter the turnover dynamics and morphology of dendritic spines in the mouse frontal cortex. Different forms of structural remodelling can be measured, such as spine number density, spine size, turnover rates and persistence, with each providing different insights into the underlying biological changes. The future presents exciting opportunities and hurdles for using neuronal rewiring for drug development and reverse translation to understand other antidepressant treatment options with rapid therapeutic onset. For these reasons, in vivo structural plasticity could provide crucial information on the mechanism of action underlying the therapeutic effects of antidepressant interventions.

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