VIEWPOINT

How can drug discovery for psychiatric disorders be improved?

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Abstract | Psychiatric disorders such as depression, anxiety and schizophrenia are leading causes of disability worldwide, and have a huge societal impact. However, despite the clear need for better therapies, and major advances in the understanding of the molecular basis of these disorders in recent years, efforts to discover and develop new drugs for neuropsychiatric disorders, particularly those that might revolutionize disease treatment, have been relatively unsuccessful. A multidisciplinary approach will be crucial in addressing this problem, and in the first *Advances in Neuroscience for Medical Innovation* symposium, experts in multiple areas of neuroscience considered key questions in the field, in particular those related to the importance of neuronal plasticity. The discussions were used as a basis to propose steps that can be taken to improve the effectiveness of drug discovery for psychiatric disorders.

At the end of 2005, experts from across the field of neuroscience — including stress, memory, molecular pharmacology, animal behaviour, pharmacogenetics, imaging, and drug discovery and development — met for the first *Advances in Neuroscience for Medical Innovation* (ANMI) symposium (see BOX 1 for a list of the participants). The aim of the symposium was to discuss why drug discovery for psychiatric disorders has been relatively unsuccessful compared with other areas in recent years, to highlight recent advances in our understanding of psychiatric disorders (BOX 2; FIGS 1–7), such as those related to the role of neuronal plasticity, and, from this, to propose how the effectiveness of drug discovery for such disorders could be enhanced. Research relevant to five key questions was discussed:

- Why have efforts in the discovery of new drugs for psychiatric disorders been relatively unsuccessful compared with other disease areas?
- How could advances in our understanding of neuronal plasticity be translated into novel therapeutic approaches?
- How could imaging improve our understanding of psychiatric disorders and the development of novel drugs?
- How could current preclinical models of psychiatric disorders be revised to improve their predictivity?
- How could patients be best defined to improve the likelihood of clinical success?

In this report, opinions of participants on these questions following the meeting are presented, and recommendations for improving drug discovery and development for psychiatric disorders are put forward (BOX 3).

Question 1

Why have efforts in the discovery of new drugs for psychiatric disorders been relatively unsuccessful compared with other disease areas?

Husseini Manji: There are many reasons that could underlie the lack of success in the development of new drugs for psychiatric disorders. First, psychiatry has a diagnostic and classification system that is not based on aetiology, neurobiology, epidemiology, genetics, or response to medications, but rather on a constellation of signs and symptoms[1,2]. The Diagnostic and Statistical Manual[3] (DSM-IV) is based on clusters of symptoms and characteristics of clinical course that do not necessarily describe homogeneous disorders, but rather reflect final common pathways of different pathophysiological processes involving genetic and environmental contributors[1,4]. The heterogeneity implicit in the current classification schemes is a probable reason for the limited success of clinical studies, at the levels of treatment, neurobiology and genetics[1,2]. A primary goal of future research in this area must be to develop a diagnostic system based on aetiology[5].

Second, target validation represents one of the main barriers for central nervous system (CNS) drug discovery and development in general, and psychiatric disorders in particular. In many ways, the entire drug discovery and development process in psychiatric disorders is one of target validation, with the ultimate validation of the target occurring when the drug is widely prescribed on the market. For depression, the absence of adequate animal models has forced the field to focus on available paradigms, most of which involve exposure of normal animals (without human depression vulnerability genes) to various forms of acute or chronic stress[6]. And ironically, the search for novel targets for antidepressants has typically involved searching for proteins and genes that are altered in these models by stress and that show reciprocal regulation by current reuptake-inhibitor antidepressants.

Other reasons for a lag in psychiatric medicine are manifold. In addition to the sheer complexity of the CNS, they include lack of a defined pathology, no direct tissue accessibility, and the daunting fact that the complexity of behaviour is not simply the sum of its parts[7]. For example, the highly variable symptom compilation that is used

to define depression, and the highly variable course of the illness and its response to various treatments, indicate that depression is comprised of numerous disease states of distinct aetiology and perhaps distinct pathophysiology. Indeed, it has been argued that if one could biopsy someone with depression, it is far from clear where one would obtain the biopsy[8]. Moreover, given the heterogeneity of the illness, different regions might well be involved in different individuals.

David Diamond: Attempts to treat psychiatric disease suffer a great disadvantage in comparison with those that treat diseases involving non-neural systems. Specific types of cancers, for example, can be identified on the basis of the expression of physiological and histological abnormalities. Diseases of the mind, however, are typically diagnosed with subjective behavioural tests that categorize, but can not rigorously identify, specific psychiatric disorders.

For example, depression and post-traumatic stress disorder (PTSD) are not identified on the basis of abnormalities in blood chemistry, but are determined subjectively by diagnostic tests. Co-morbidity further complicates the diagnosis and treatment of psychiatric diseases. That is, depressive symptoms are often co-morbid with disorders such as PTSD, bipolar disorder, thyroid disease and Cushing's disease as well as neural degenerative disorders such as Parkinson's disease. In addition, depression can be caused entirely by an organic abnormality, such as a disturbance of the endocrine system (for example, chronically elevated cortisol levels), or can develop as a response to intense adverse life events in a person with a predisposing physiological, developmental or genetic abnormality. Finally, the genetic and developmental history of a depressive person can influence the effectiveness of pharmacotherapy. So, the complex aetiology, subjective diagnosis of mental disorders and idiosyncratic responses to pharmacotherapy makes the discovery of new drugs for the treatment of psychiatric disorders highly challenging.

György Buzsáki: The standard approach for psychiatric drug discovery is based on 'receptor models' of diseases, but most successful drugs are nonspecific for many known receptor types. As homologous behavioural models do not exist, what is the next-best substitute?

My view is that brain-generated 'spontaneous' ensemble patterns in cortical structures (FIG. 3) are the source of cognition[9], and impairment of proper temporal organization might underlie the various deficits associated with psychiatric diseases[9,10]. If so, drug screening methods that possess the temporal resolution of cell-assembly cooperation are needed. Network-level temporal correlations are often expressed in the form of oscillations[11]. Network oscillations are robust phenotypes that are well-preserved throughout mammalian evolution, and are specifically and differentially affected by a large spectrum of psychotropic drugs[9], and so these patterns can be used in early screening. For the assessment of specific mechanisms that affect the spatio-temporal patterns of neuronal assemblies, large-scale recordings of multiple single neurons now offer unprecedented opportunities[12]. These high-resolution methods can provide insights into network-level mechanisms of action, even when field-recording assessments fail to reveal changes, and when synapse or channel-level mechanisms are not understood. Impairment of temporal coordination of neurons is a probable cause

Box 1 | **Symposium participants**

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of mental deterioration, and drugs affecting the temporal domain should be actively sought. The experimental techniques, such as tetrode electrodes, are available, but skilled systems researchers are scarce even in academic institutions, and are virtually non-existent in industry.

Anthony Grace: One of the primary difficulties is the absence of appropriate animal models. This is due to at least two factors: deficiencies in understanding the nature of the pathology in humans that causes the disorder; and limitations in characterizing a complex, uniquely human disorder with complex affective and cognitive components in non-human species. Even in our attempts to evaluate such disorders in humans, caution must be applied to imaging or post-mortem studies, as a difference between the afflicted individual and the norm could be attributed to at least four mechanisms: first, the difference reflects the actual aetiology of the disorder; second, the difference is due to drug treatment; third, the difference is due to compensations in the brain that occur secondary to the disease state; or fourth, the differences are due to long-term pathological alterations caused by the disorder. Therefore, defining the appropriate target based solely on studying humans with the disorder will be problematic.

Working from our current understanding of drug action might also be difficult in refining drug treatments. Few drugs other than L-DOPA (3,4-dihydroxy-L-phenylalanine) have been developed for neurological disorders based on a rational therapeutic hypothesis (in this case, alleviating the dopamine deficiency characteristic of Parkinson's disease by providing a prodrug of dopamine). For example, antipsychotic drugs such as the dopamine antagonists were identified serendipitously, and are not likely to represent the most effective therapeutic regimen. Indeed, it has been suggested that antipsychotic drugs achieve their efficacy not by reversing an underlying deficit in schizophrenia, but instead by producing an off-setting deficit in a system (that is, the dopamine system) that is being abnormally regulated by the regions demonstrating the key pathology (that is, the cortical glutamatergic system; for a review, see REF. 13). For this reason, antipsychotic drugs seem to produce the same physiological actions in normal animals as they do in humans with schizophrenia[14]. By contrast, the comparatively more subtle effects produced in the physiology of a normal system by the latest generation of antidepressant drugs might actually reflect a reversal of a primary

Box 2 | **Advances in understanding neuronal complexity**

Advances at the molecular, cellular and systems levels relevant to drug discovery for psychiatric disorders were described at the symposium.

Molecular level

- Prochiantz described how a new family of signalling molecules — homeoproteins, such as Engrailed, which are transported across biological membranes and regulate translation and transcription — operate at different periods of brain development, and also in the adult. As these homeoproteins have the ability to transduce cells and are associated with several diseases, they could be used as therapeutic proteins[27].
- Manji highlighted how several intracellular signalling pathways could converge on crucial 'nodes' with major consequences — the nodal enzymes are targets for drugs, particularly agents used in bipolar disorders (for example, inhibition of glycogen synthase kinase 3β (GSK3β) by lithium or valproate) (FIGS 1,2). By modulating β-catenin, GSK3β can have major effects on neuronal plasticity, and lithium can rapidly reverse frontal grey-matter atrophy in patients with bipolar disorder[67]. These compounds and antidepressants can modify α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor trafficking and therefore fast transmission and long-term potentiation and long-term depression[67] (LTP and LTD).

Cellular level

- LTP and LTD are two forms of neuronal plasticity that might underlie memory formation and stress-induced amnesia. Stress blocks LTP and enhances LTD in the hippocampus and prefrontal cortex. The stress-induced enhancement of LTD might act as a 'switch' to reverse LTP-related memory storage processes. Collingridge reported that a GSK3β inhibitor (SB415286) blocks LTD. Diamond reported that predator stress impaired hippocampus-dependent memory and suppressed the learning-induced increase in dendritic spine density in the hippocampus[17,68]. The brain regions most sensitive to the influence of stress on neuronal plasticity —hippocampus/prefrontal cortex/amygdala — are all crucially involved in psychiatric disorders.
- Some crucial parts of the brain are beginning to be well defined at a cellular level. For example, all the neurons, interneurons, transmitters and some of the receptors are known in the CA1 area of the hippocampus (Supplementary information S2 (figure)) with their main inputs and outputs[77]. However, even at the receptor level there is immense complexity. For example, 19 different $GABA_A$ (γ-aminobutyric acid A) receptor subunits have been discovered that are heterogeneously expressed in the CNS, which permits the differential expression of ~20–30 receptor isoforms that are physiologically and pharmacologically distinct. The behaviours mediated by different $GABA_A$ receptors are being revealed by transgenic approaches identifying targets for therapeutic intervention (FIG. 6).

Systems level

- Grace described how the hippocampus, amygdala and prefrontal cortex interact at a systems level and that psychiatric disorders such as schizophrenia arise from a disturbance of neuronal balance in them, with stress potentially being an important factor (FIG. 5).
- The complexity of neuronal firing in these brain areas can be analysed in several ways. Buzsáki described how the brain can generate spiking activity, independent of incoming information, most often in the form of network oscillations[9] (FIG. 3). Impairment of temporal coordination of neurons is a likely cause of mental deterioration, and drugs affecting the temporal domain should be actively sought after[11].
- Imaging studies reinforce this systems approach because these circuits are directly modified in depression. Mayberg reported that in depressed patients the key brain area Cg25 and its connections to the hypothalamus, brainstem, prefrontal cortex and hippocampus are dysregulated[61] (remarkably similar to the hippocampal-infralimbic circuits in which LTP is inhibited by stress). Electrical stimulation of these brain areas immediately reversed mood and amotivational features of depression in severely depressed, drug-resistant individuals[60] (FIG. 7).
- Dolan reported that the effect of mood on cognition could be detected by imaging the amygdala and that this was an excellent measure for early clinical studies in drug development.
- The changes in development trajectory of neuronal development in individuals with high and low IQ, child-onset schizophrenia and various psychiatric disorders can be measured with precision and are crucial to understanding the disorders and their treatment[45].
- There was a consensus about the importance of particular brain areas . Output from the hippocampus to the medial prefrontal cortex (subgenual cingulate: BA25 in humans and non-human primates, infralimbic in rodents) were seen as key areas for blood-flow changes in mood disorders and schizophrenia (FIG. 7). Weinberger reported that the same brain areas were associated with expression of polymorphisms of key genes associated with psychiatric disorders (for example, polymorphisms of the serotonin transporter, catechol-*O*-methyltransferase (COMT) and brain-derived neurotrophic factor (BDNF)). The coherence of the brain areas implicated and the proof of concept in humans, following electrical stimulation, therefore encourages the creation of entirely new animal models to screen a new generation of therapeutic agents.

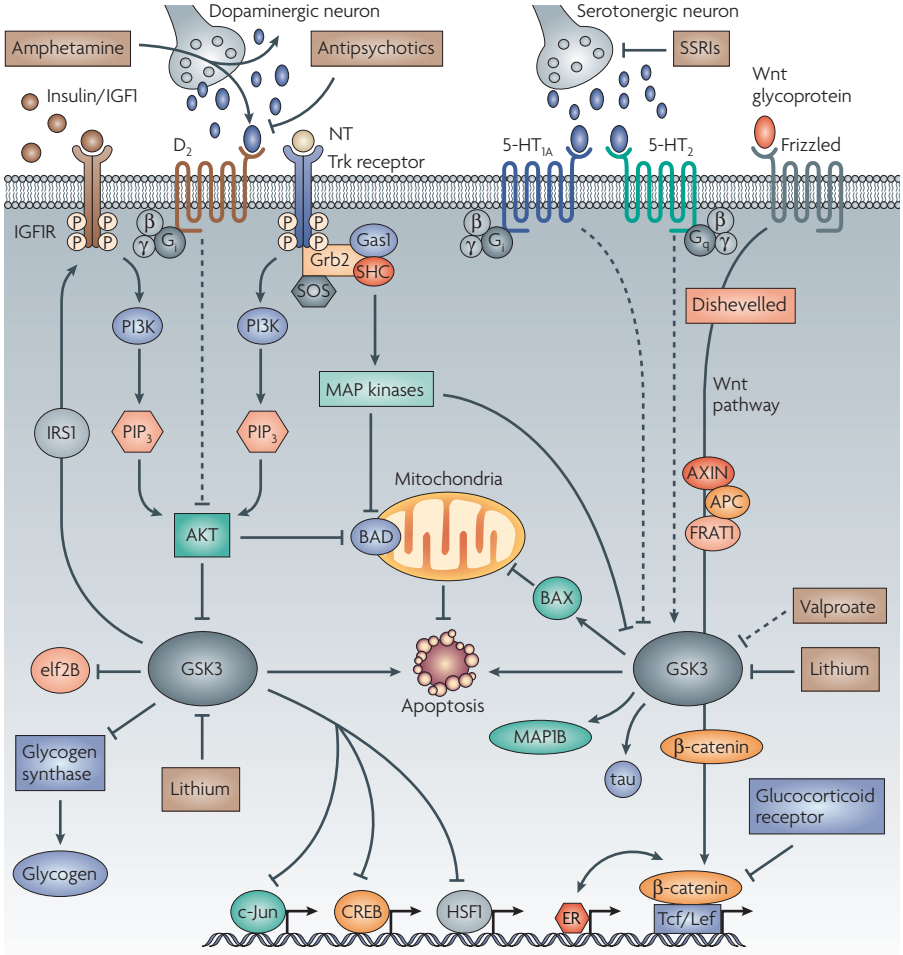

Figure 1 | **Convergence of signalling pathways on crucial 'nodes': implications for drug targets.** The figure depicts some of the major intracellular signalling pathways involved in regulating neural and behavioral plasticity. Most neurotransmitters and neuropeptides activate G-protein-coupled receptors (GPCRs). Endogenous growth factors such as brain-derived neurotrophic factor (BDNF) use different types of signalling pathways. BDNF binds to and activates its tyrosine kinase receptor (TrkB); this facilitates the recruitment of other proteins (SHC, SOS), which results in the activation of the ERK-MAP kinase cascade (via sequential activation of Ras, Raf, MEK, Erk, and Rsk). In addition to regulating several transcription factors, the ERK-MAP kinase casade, via Rsk, down-regulates BAD, a pro-apoptotic protein. Enhancement of the ERK-MAP kinase cascade might have effects similar to those of endogenous neurotrophic factors; one potential strategy is to use inhibitors of MAP kinase phosphatases (which would inhibit the turn-off reaction) as potential drugs with neurotrophic properties. Another pathway gaining increasing recent attention in adult mammalian neurobiology is the Wnt signalling pathway. Wnts are a group of glycoproteins active in development, but now known to play important roles in the mature brain. Binding of Wnts to the Wnt receptor (WntR) activates an intermediary protein, Dishevelled, which regulates a glycogen synthase kinase (GSK3β). GSK3β exerts many cellular effects; it regulates cytoskeletal proteins, including tau, and is also important in determining cell survival/cell death. GSK-3β has recently been identified as a target for the actions of lithium. GSK3β also regulates phosphorylation of β-catenin, a protein that when dephosphorylated acts as a transcription factor at LEF (lymphoid enhancer factor) sites. GSK3 regulates a variety of signalling pathways, including IGF1, neurotrophic factor and Wnt signalling. As shown in the figure, medications useful for the treatment of mood disorders have both direct and indirect effects on GSK3, and GSK3-regulated pathways. β-catenin, activated through the Wnt pathway, interacts with the intracellular oestrogen receptor (ER), which also effects transcription of an independent set of genes. These distinct pathways have convergent effects on cellular processes such as bioenergetics (energy metabolism), neuroplasticity, neurogenesis, resilience and survival. So, lithium (and other medications) might act by enhancing these processes through inhibition of GSK3. Gi refers to Gi/Go; Gq refers to Gq/G11. APC, adenomatous polyposis coli; BAD: Bcl-2/Bcl-2 associated death promoter; Bcl-2, B cell lymphoma-2; CREB, cyclic AMP response element binding protein; ER, oestrogen receptor; GSK, glycogen synthase kinase; HSF1, heat shock factor-1; IGF1, insulin/insulin like growth factor; Lef, lymphoid enhancer factor; NT, neurotrophins; PI, phosphoinositide; SSRIs, selective serotonin reuptake inhibitors; TCF, T cell-specific transcription factor; Trk, tyrosine receptor kinase. Adapted from REF. 67.

deficit that is not extant in the normal animal[13]. Therefore, it might be that the development of the most effective therapeutic agents in psychiatry will depend on testing in an appropriate animal model, in which the drugs would be capable of acting on the primary site of the pathology.

Maurizio Popoli: A major reason is that there has been a lack of sufficient translational efforts applying new basic research findings and technology to molecular pharmacology and biological psychiatry, target discovery and validation, and clinical research. We have learned much in recent years about post-receptor signalling mechanisms, regulation of gene expression, epigenetic mechanisms, integrated mechanisms of synaptic plasticity, and the identification of new biomarkers for vulnerability and drug response and resistance by global genomics and proteomics. However, a considerable amount of current pharmaceutical research is still focused on the stereotypical 'receptor–ligand' interaction.

As a result, most of the 'novel' drugs in psychiatry are still compounds that principally act on neurotransmitter receptors or transporters. It would be interesting to intensify the research on intracellular targets by seriously analysing the challenges for target selectivity and compartment specificity, which such compounds might require. However, it seems evident that, in contrast to other fields such as cancer, much of the psychiatry research in drug companies in the last two decades has been directed towards replication and implementation of already known mechanisms, with perhaps the only notable exception to this being the atypical antipsychotics.

Question 2

How could advances in our understanding of neuronal plasticity be translated into novel therapeutic approaches?

David Diamond: The great capacity for the brain to reorganize its connections at all stages of life enables us to store a vast amount of information, but it also makes the brain vulnerable to a disturbance in neuronal connectivity in response to stressful experiences. For example, PTSD is a psychiatric disease that is characterized by pathologically strong memories of a horrific experience, such as wartime combat or rape. In this disorder, unbridled neuronal plasticity activated by trauma generates intrusive memories that can haunt the patient for the remainder of his or her life (for a review, see REF. 15). Moreover,

PTSD is typically co-morbid with depression, thereby complicating treatment approaches, which must address both the depressive symptoms and the hypervigilance common to PTSD. So, excessive neuronal plasticity and brain atrophy are the result of a dysregulation of neuronal plasticity in stress-related psychiatric disorders.

Recent research has implicated the glutamatergic system in excessive, as well as essential, neuronal plasticity, normal learning processes and stress-related pathophysiology[16]. The challenge in this area of research has been to develop treatments that attenuate the neurotoxic features of glutamatergic-mediated plasticity, while maintaining the capacity for the glutamate system to allow learning-related plasticity. For example, tianeptine (Stablon; Servier), is a well-described antidepressant that has been shown to attenuate glutamate-induced hyperexcitability. Tianeptine blocks stress-induced atrophy and also reverses stress-induced memory impairments[17]. We have found that tianeptine blocked the inhibitory effect of stress on memory and neuronal plasticity in the hippocampus (FIG. 4), without interfering with learning under non-stress conditions[17]. So, our findings with tianeptine are consistent with other studies identifying the control and stabilization of glutamatergic neuronal plasticity as a promising avenue of research into pharmacotherapy for stress-related psychiatric disorders.

Maurizio Popoli: Recent findings have suggested that hyperactivation of glutamate neurotransmission in some cortical and limbic areas might occur in depression and stress-related disorders. Conversely, stabilization of glutamate neurotransmission by a reduction of depolarization-evoked release of glutamate might be a common effect of different antidepressants, perhaps representing a component of their therapeutic action[18]. These drugs might work by limiting excessive release of glutamate when this is induced by stressful neuronal activation. The effect could be reinforced by alteration of the balance between excitatory and inhibitory neurotransmission, due to selective action of antidepressants on glutamate versus GABA release.

Down-regulation of glutamate release by chronic antidepressants was found to be accounted for by a redistribution of the SNARE protein syntaxin-1 between αCaM kinase II and Munc-18, with a stronger interaction of syntaxin-1 with Munc-18 suggesting a decreased efficiency of the presynaptic machinery. A key role in these modifications was attributed to a redistribution (induced by chronic antidepressants) of the Thr286 phosphorylation of αCaM kinase II in synaptic terminals (Supplementary information S1 (figure).

So, presynaptic proteins regulating glutamate release, such as αCaM kinase II, might represent targets for novel antidepressant drugs. Whether stress-induced upregulation of glutamate release follows similar mechanisms is being investigated. A primary goal would be to identify the anchoring protein(s) for αCaM kinase II in synaptic vesicles, because this might allow the design drugs targeting this kinase and interfering with glutamate release.

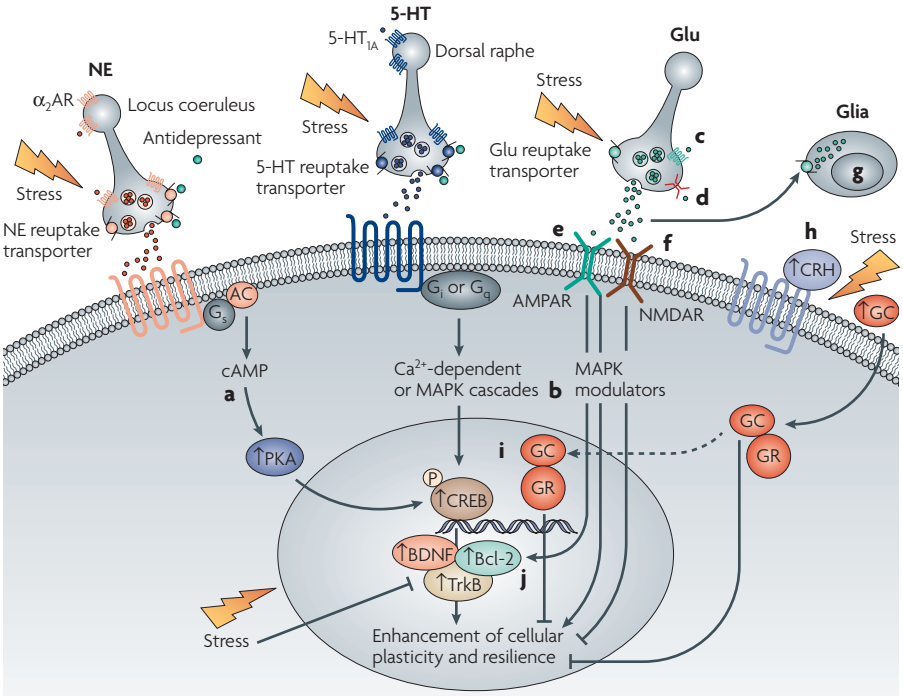

Figure 2 | **Enhancing cellular plasticity and resilience to treat severe mood disorders.** The figure shows the multiple targets by which cellular plasticity and resilience might potentially be regulated in the treatment of severe mood disorders. Genetic/neurodevelopmental factors, repeated affective episodes (and likely elevations of glucocorticoids (GCs)) and illness progression might all contribute to the impairments of cellular resilience, volumetric reductions and cell death/atrophy observed in mood disorders. Bcl-2 attenuates apoptosis by sequestering proforms of death-driving cysteine proteases (called caspases), by preventing the release of mitochondrial apoptogenic factors such as calcium, cytochrome *c* and AIF (apoptosis-inducing factor) into the cytoplasm, and by enhancing mitochondrial calcium uptake. Antidepressants regulate the expression of brain-derived neurotrophic factor (BDNF), and its receptor TrkB. Both TrkA and TrkB use the PI3-kinase/Akt and ERK-MAP kinase pathways to exert their neurotrophic effects. The ERK-MAP kinase cascade also increases the expression of Bcl-2 via its effects on CREB (cyclic AMP response element binding protein). **a** | Phosphodiesterase inhibitors increase the levels of pCREB. **b** | MAP kinase modulators increase the expression of the major neurotrophic protein, Bcl-2. **c** | Metabotropic glutamate receptor 2/3 agonists modulate the release of excessive levels of glutamate (Glu). **d** | Drugs such as lamotrigine and riluzole act on Na^+ channels to attenuate glutamate release. **e** |AMPA potentiators upregulate the expression of BDNF. **f** | NMDA antagonists such as ketamine enhance plasticity and cell survival. **g** | Novel drugs to enhance glial release of trophic factors and clear excessive glutamate might be useful for the treatment of depressive disorders. **h**,**i** | Corticotropin-releasing hormone (CRH) and glucocorticoid receptor antagonists attenuate the deleterious effects of hypercortisolaemia and CRH antagonists might have other beneficial effects in the treatment of depression via non-HPA mechanisms. **j** | Agents that upregulate Bcl-2 (for example, lithium, valproate or pramipexole) would be postulated to have considerable utility in the treatment of severe mood disorders, and other disorders associated with atrophic changes. AC, adenylate cyclase; AMPAR, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; Bcl-2, B-cell lymphoma 2; 5-HT, 5-hydroxytryptamine; MAP, mitogen-activated protein; NE, noradrenaline; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A. Adapted from REF. 5.

Anthony Grace: Many, if not most, psychiatric disorders emerge as a developmental consequence of an underlying genetic predisposition interacting with environmental factors (FIG. 5). The fact that the pathology is probably present from birth, but does not become expressed until late adolescence or early adulthood, suggests that the disorder is not a

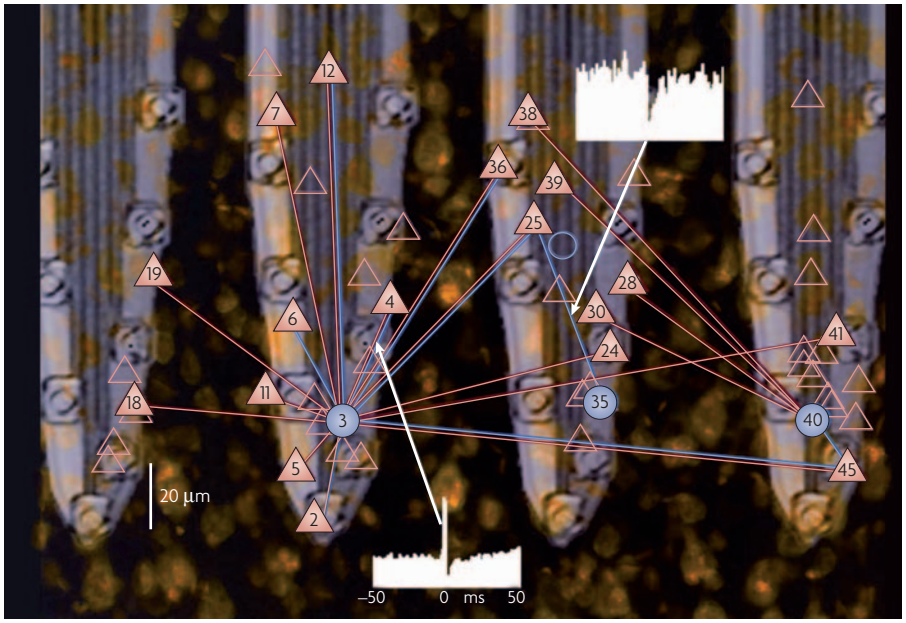

Figure 3 | **Brain-generated ensemble patterns in cortical structures.** Functional topography within the recorded population in the somatosensory cortex of the rat. Filled symbols: participating pyramidal cells (red triangles) and interneurons (blue circles). Empty symbols: neurons not connected functionally. Red line: monosynaptic excitation; blue line: monosynaptic inhibition. Note that interneurons (for example, 3 and 40) are activated by large numbers of pyramidal cells and an interneuron inhibits several local and distant pyramidal cells. The relative position of the neurons was determined by calculating the 'centre of mass' of spike amplitude recorded from multiple sites. Inserts: Cross-correlograms between an interneuron–pyramidal cell pair (interneuron 35 inhibits spiking activity of pyramidal cell 25) and reciprocally connected pair (interneuron 3 inhibits pyramidal cell 4; pyramidal cell 4 excites interneuron 3). Large-scale recordings and network analysis offer a high-resolution tool for the assessment of drugs on assembly patterns. For a review, see REF. 12.

direct result of the lesion, but instead is due to a series of alterations that are triggered by the lesion[19,20]. Studies of neonatal brain lesions that only produce pathological changes postpubertally is consistent with this model (for example see REFS 21,22). If such disorders are indeed due to altered plasticity during maturation, then it might be that the most effective means to address this pathology is through drugs that target such plasticity. In addition, it is important not to overlook the pathological changes that can arise owing to stress, because stress has been identified as an important variable both in the symptomatology and aetiology of several major psychiatric disorders. Perhaps circumventing stress-induced pathological changes might be an important means for preventing the transition from a genetically induced predisposition to the expression of the disorder later in life[23,24].

Alain Prochiantz: The implication of developmental processes in neurological and psychiatric diseases is attracting the interest of many. One concept is that fragile mental states have a developmental origin. For example, sensitivity to some forms of schizophrenia could be associated with the inability to open a critical period for plasticity, allowing individuals to respond to changes in hormonal status that accompany puberty. The demonstration that homeogene levels of expression control the opening and closure of the critical period for binocular vision[25] might be a first step in understanding how developmental genes, expressed late in development and in the adult, might participate in the aetiology of psychiatric diseases.

A second consideration is that many parts of the nervous system are submitted to cell renewal or morphological plasticity. It seems probable that factors that contribute to cell division, differentiation and survival during development also contribute to this adult 'silent development', and therefore to the ability of individuals to emotionally adapt to an ever-changing world. When it comes to neurological diseases, a good example is the role of developmental genes, such as *NURR1*, *PITX3*, *LMX1B* and Engrailed (*EN*) in the development and survival of midbrain dopaminergic neurons[26]. An interesting twist in the story is that homeoproteins are also signalling molecules with the ability to pass between cells. They can therefore be used, in principle, as therapeutic proteins with intracellular activities at the levels of translation and transcription. Through the latter activities they can also help in the identification of new therapeutic targets. Finally, the sequences necessary for homeoprotein secretion and internalization have been used to address hydrophilic compounds into live cells *in vivo*[27], thereby enriching the repertoire of accessible targets.

Peter Somogyi: Defining the spatio-temporal rules that govern cortical activity is the key to explaining normal and pathological events involving the cortex. Cortical pyramidal cells receive multiple glutamatergic inputs from several functionally distinct sources, most of them of intracortical origin. The thalamocortical glutamatergic innervation forms only a minor part of glutamatergic inputs to any cortical cell (for a review, see REF. 28). All glutamatergic inputs in a region, including the local axon collaterals of the pyramidal cells, also innervate GABA-containing (γ-aminobutyric acid) neurons, which form about 20% of all cortical neurons. These serve many functions, including gain setting, input scaling, spatial cellular activity pattern formation, neuronal population synchronization and the selective post- and/or presynaptic modulation of voltage-sensitive ion channels, as well as the regulation of synaptic plasticity. Many, but not all, of these functions involve inhibition. The GABA-containing neurons show great diversity in terms of the distribution of their axons and dendrites, as well as the expression of molecules related to intercellular signalling such as neuropeptides, calcium-binding proteins and neurotransmitter receptors. There is no agreement on the number of GABA-containing neuronal types and their particular roles, and only more multidisciplinary work can clarify their position in the cortical network.

The layout of the basic cortical circuit is best seen in the hippocampal formation, in which a relatively homogeneous population of pyramidal cells are innervated by a few extrinsic and intrinsic glutamatergic afferents, which mostly segregate into distinct layers innervating different domains of the pyramidal cells (Supplementary information S2 (figure)). This laminar specificity allows the delineation of GABA-containing neuronal classes and their targeted analysis.

The multiplicity of glutamatergic and GABAergic cell types shows that these two neurotransmitters are released independently from each cell class with distinct temporal dynamics for normal operations. Selective coordination abnormalities are expected from the malfunctioning of specific neuronal

classes. To learn what each cell class contributes to the temporal dynamics of the cortex, we should try to modulate the output of the entire population of a given cell type selectively and reversibly on various time scales. Indeed, such a method would be useful to test hypotheses for many psychiatric disorders involving changes in the activity of specific cell classes also outside the cortex. The fast reversible regulation of targeted neuron classes is being developed using a genetically altered mouse line insensitive to the benzodiazepine agonist, zolpidem, in which zolpidem sensitivity will be restored in specific neuronal classes[29]. Short- or long-term zolpidem treatment affecting only the selected neurons might then reveal the contribution of the cell class to behaviour or, with local application, to the function of specific brain areas.

Jeremy Lambert: Although the $GABA_A$ receptor is an important clinical target, many of the currently used therapeutics are relatively non-selective and interact with the majority of the 20–30 $GABA_A$ receptor isoforms estimated to be expressed in the mammalian CNS. Consequently, such drugs are associated with a range of side effects that limit their clinical utility. The results of experiments with transgenic mice have encouraged the development of new classes of $GABA_A$ receptor modulators that are subtype-selective. Such 'knock in' mice have been engineered to express $GABA_A$ receptor isoforms, which by virtue of mutating critical amino acids are rendered insensitive to benzodiazepines and certain general anesthetics[30,31]. For example, this approach has demonstrated the sedative and anxiolytic actions of diazepam to be mediated by different $GABA_A$ receptors (FIG. 6). Such results are consistent with those obtained with $GABA_A$ receptor subtype-selective drugs[31]. The future generation of mice in which the receptor mutation is restricted to specific neurons should help in understanding the role of particular neuronal circuits in behaviour.

Per Svenningsson: Corticostriatal neural plasticity is dysfunctional in several psychiatric and neurological conditions, including schizophrenia, attention-deficit hyperactivity disorder (ADHD) and Parkinson's disease. At the postsynaptic site, dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) has a crucial role in regulating corticostriatal synaptic plasticity[32]. Four distinct phosphorylation sites for protein kinase A (PKA), cyclin-dependent kinase 5 (CDK5), casein kinase 1 (CK1) and CK2, respectively, determine the function of DARPP-32 (REF. 33). Recent studies have emphasized an integrative role of DARPP-32 in dopaminergic, serotonergic and glutamatergic neurotransmission[34]. It has, for example, been found that the pharmacologically distinct psychomimetics D-amphetamine, lysergic acid diethylamide (LSD) and phencyclidine (PCP) regulate the phosphorylation state of DARPP-32 at the PKA and CK1 sites in a similar manner[35]. In mice with a genetic deletion of DARPP-32 or with point mutations in phosphorylation sites of the PKA and CK1 sites of DARPP-32, the effects of D-amphetamine, LSD and PCP on two behavioural parameters involving corticostriatal neurotransmission — sensorimotor gating and repetitive movements — were strongly attenuated. These data indicate that multiple kinase/phosphatase pathways converge on DARPP-32 and that this protein represents a nodal point regulating corticostriatal neural plasticity (FIG. 1). Future studies on genetic

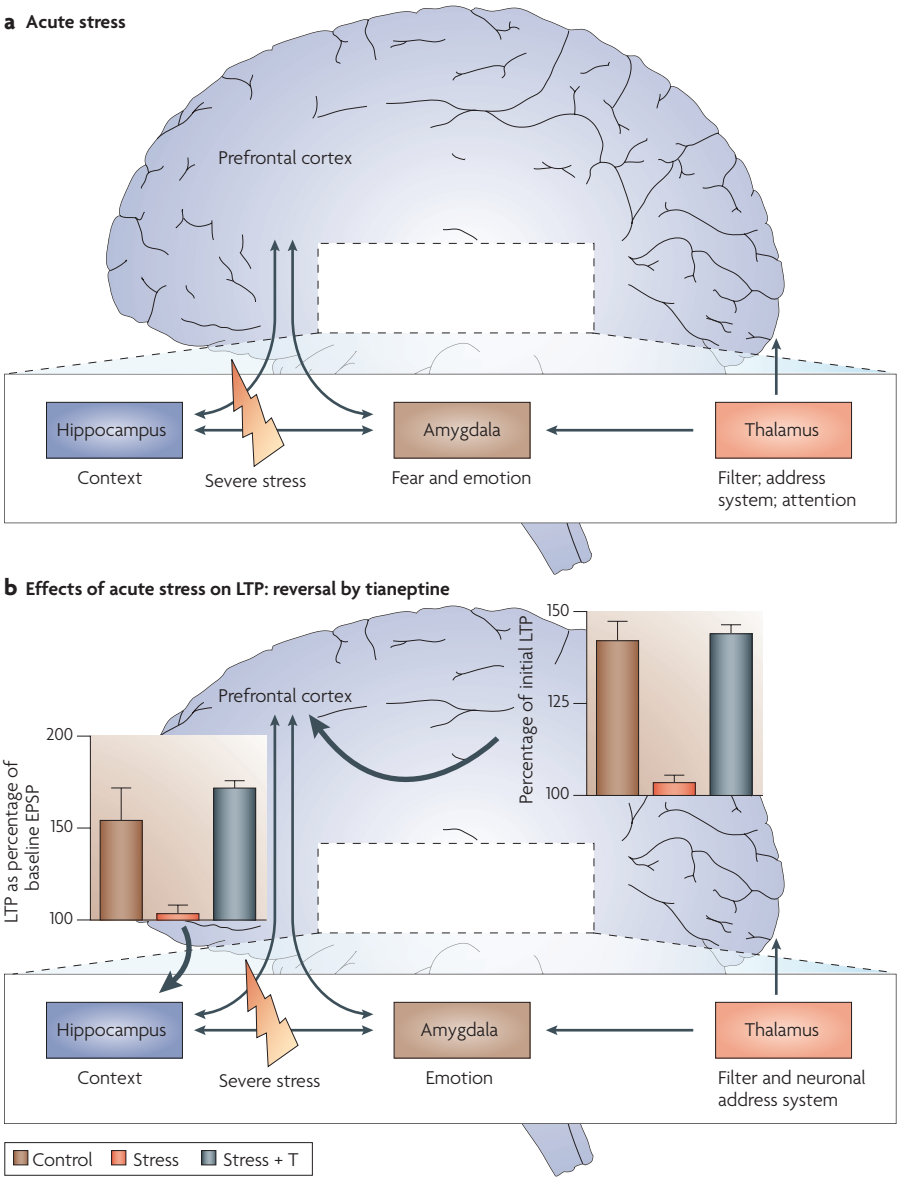

Figure 4 | **Reversal of the suppressive effects of stress on neuronal plasticity in the hippocampus and prefrontal cortex by antidepressant treatment.** Panel **a** illustrates the finding that severe stress interferes with the functioning of the hippocampus and prefrontal cortex. Panel **b** illustrates findings from different laboratories that have all shown that tianeptine (an antidepressant that has been shown to stabilize glutamate activity under stress conditions) blocks the inhibitory effect of stress on memory and neuronal plasticity (long-term potentiation, LTP) in the hippocampus and prefrontal cortex. The inset graphs show that stress suppressed LTP in each of these two structures (red bar), which was reversed to control levels (brown bars) by acute tianeptine (T) treatment (grey bars)[17]. EPSP, excitatory postsynaptic potential. Adapted from REFS 17, 69.

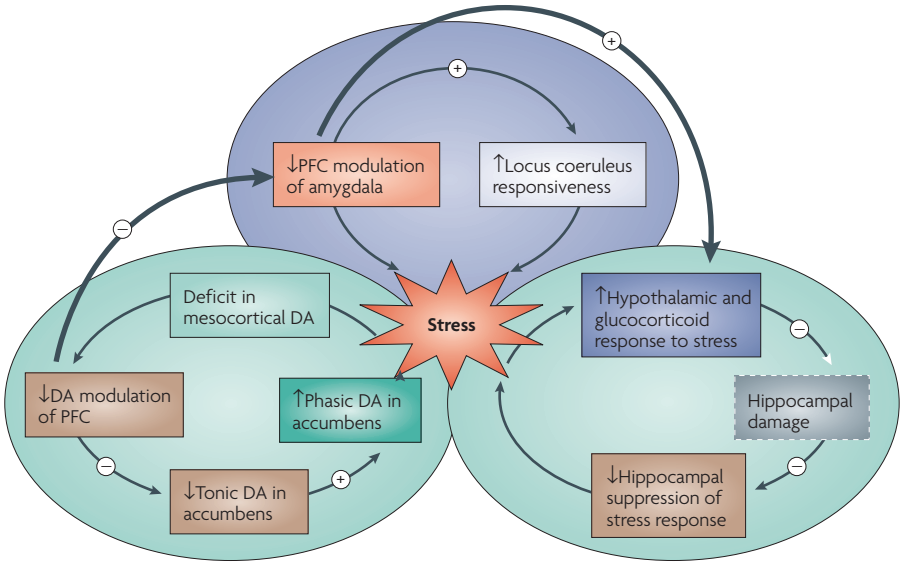

Figure 5 | **A proposed model regarding the interaction between stress, the limbic system, and the delayed onset of schizophrenia.** In this model, a deficit in prefrontal cortical function, potentially related to abnormalities in mesocortical dopamine (DA), will prevent stress-induced, DA-mediated activation of the prefrontal cortex (PFC). As a result, there is a failure of the PFC to modulate subcortical responses to stress. The resultant failure of the PFC to down-modulate the amygdala and regulate accumbens DA dynamics will lead to a positive feedback increase in the stress response. The non-attenuated activation of the amygdala will also lead to abnormal activation of the locus coeruleus, causing increased brain noradrenaline release and additional stress exacerbation. Together, this will trigger increases in glucocorticoid release, resulting in hippocampal damage. The prefrontal deficit therefore enables moderate stress levels to initiate a positive-feedback cascade of events during adolescence that leads to hippocampal damage and the onset of schizophrenia symptomatology in the young adult. Adapted from REF. 23.

linkage and/or polymorphism(s) in the *DARPP-32* gene in patients suffering from schizophrenia or other neuropsychiatric disorders will provide important information on the role of DARPP-32, and the kinase/phosphatase cascades that regulate the function of this protein in human disease conditions.

Jean-Antoine Girault: In recent years, considerable progress has been made in understanding the intracellular signalling pathways that mediate the actions of neurotransmitters. For example, the pathways that control synaptic plasticity have been characterized to a reasonable extent. This knowledge opens a number of novel potential therapeutic avenues, as many key components in these pathways are targets for drugs. Work in other areas, such as cancer therapy, has demonstrated that it is possible to design molecules with a high degree of selectivity for signalling molecules, such as protein kinases. Although many signalling pathways are ubiquitous and involved in numerous functions, it is theoretically possible to achieve specificity by hitting cell-type-specific isoforms of some enzymes. In addition, signalling pathways are organized in networks in which the most sensitive nodes, which are crucial for specific functions, differ from one cell type to the other (FIGS 1,2). It is therefore conceivable that blockade of a signalling enzyme will achieve relatively selective functional effects.

Several laboratories, including our own, have studied the signal transduction mechanisms involved in brain reward systems as targets for drugs of abuse[36,37]. These systems, which are controlled by simple chemical stimuli, provide excellent models for studying neuronal plasticity *in vivo*. As a proof of concept, recent work in our laboratory shows that a single systemic dose of a protein kinase inhibitor (a mitogen-activated protein kinase/ERK kinase (MEK) inhibitor, which inhibits the extracellular signal-regulated kinase (ERK) pathway) is able to reverse previously drug-induced learned behaviour, provided it is administered in the appropriate environmental conditions[38] (Supplementary information S3 (figure)). This demonstrates the feasibility of powerful and yet selective pharmacological intervention targeting intracellular signalling pathways *in vivo* with interesting behavioural consequences. Further investigations should allow the identification of targets relevant for neuropsychiatric conditions.

Question 3
How could imaging improve our understanding of psychiatric disorders and the development of novel drugs?

Richard Frackowiak: The role of imaging in developing new drugs for psychiatry has to be determined practically. However, there are a number of promising avenues that merit immediate consideration. The first is the idea that imaging can provide reliable disease markers to aid preclinical diagnosis of a state or trait. If a trait results in a specific effect on local brain function or anatomy, the definition of homogeneous patient populations for therapeutic trials of agents designed to postpone disease onset would be feasible. Another strategy is to follow a functional or structural imaging correlate of disease activity in time as a disease develops, or perhaps even beforehand, and to test candidate disease-modifying drugs by monitoring whether they modify the evolution of imaging features. Both strategies depend on having imaging methods that are reproducible and sensitive, but above all are as free of bias as possible.

Magnetic resonance imaging (MRI) provides a technology for non-invasive repetitive imaging that carries no risk of harm to patients or volunteers[39]. The analysis of repeated structural or functional scans is not difficult. Validated automated (and therefore observer-bias free) methods of image preprocessing and analysis are freely available to the community (for example, statistical parametric mapping). Studies of biomarkers in preclinical neurodegenerative disorders have been validated with normal subjects harbouring the polyglutamine mutation of the Huntington gene that is predictive of future disease onset[40]. Specific focal structural changes have been found; for example, related to visuo-spatial memory usage in London taxi-drivers and in training motor skills[41].

Such results are especially interesting because most radiology departments have MRI scanners that can make simple T1-weighted structural brain scans. No special equipment modifications are required, so translation of experimental structural imaging methods (for example, voxel-based morphometry) into clinical and pharmacological departments should be simple. A problem is the use of sophisticated experimental imaging techniques for individual patient studies. Practically, only studies based on representative sample populations that generate group inferences are possible at present. However, this is a relatively unimportant issue because screening for drugs and monitoring their effects, even in preclinical studies, implies

population, or group, studies and inference before any application to individuals. A great advantage of human brain imaging is its sensitivity to the relative change of structure or function over time in response to stimulation or modification by drugs. Meaningful data can be generated and inferences made from small test populations (of the order of tens of subjects rather than thousands) and in relatively short times. That is not to say that brain imaging will replace clinical trials, but it is probably going to become a very efficient way of screening candidate drugs in small populations rapidly and efficiently. However, candidate agents will still need toxicological studies, which might become less demanding when administration to small numbers of people over relatively short times rather than mass administration to patient populations over years is being considered. The result could be a considerable decrease in the cost and an increase in the scope of human drug discovery.

Functional brain imaging with MRI also suggests a way of investigating pathophysiological mechanisms and how these are modulated by drugs. For example, when components of the normal motor system are 'conditioned' with repetitive transcranial magnetic stimulation (TMS), there is a profound functional reorganization that is associated with trivial or no change of motor performance[42]. This reorganization resembles that found after local brain injury or ischaemia. In conditioned subjects the pattern of activation reverts to normal within an hour or two. This process is being characterized with sequential imaging and correlated with other measures of motor performance and physiology. If a standard pattern is discovered, as seems probable, then a model of brain reorganization pertinent to brain-injured patients can be proposed in normal volunteers. The screening of drugs that promote reorganization will then become a much simpler matter. Although futuristic as an idea, it is certainly not beyond the realms of possibility.

It is almost certain, given the efficacy of drugs that target the diffuse ascending neuromodulatory system — for example, serotonergic and cholinergic agents — that the ability to measure the 'strengths' of functional connections between distant cortical areas with functional MRI will also be exploited to measure how these drugs cause their effects. Monitoring changes of inter-regional brain activity over time, both in the presence and absence of potential disease-modifying drugs, presents a technical challenge, but one that has already been shown to work in principle[43]. The idea that drugs change function in brain systems is *a priori* more rational than the idea that activity in a single disease-specific region is altered. Many psychiatric diseases could turn out to be disorders of frontal lobe interactions with other brain regions. The ability to quantify these interactions and to detect significant changes in them that are disease-, drug- or time-dependent is very promising.

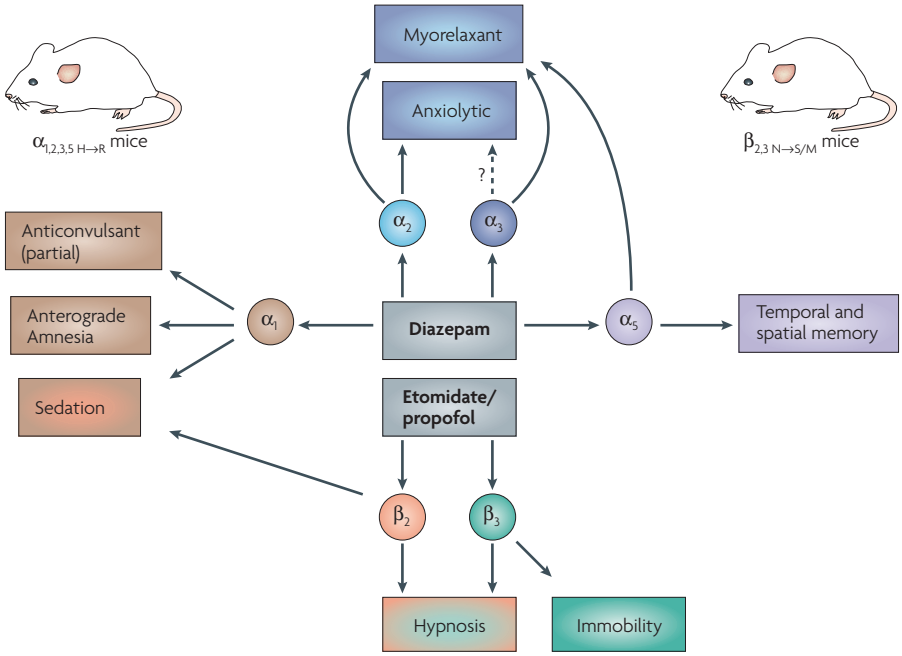

Figure 6 | **$GABA_A$ receptors and behaviour.** Distinct $GABA_A$ receptors mediate the behavioural effects of diazepam and etomidate. Studies utilizing mice in which specific α- or β-subunits have been genetically engineered to be insensitive to diazepam or etomidate, respectively, reveal the behavioural repertoire of these agents to be mediated by different receptor isoforms[30,31].

There is therefore much that imaging studies can do and show in relation to drug discovery — from demonstrating pathophysiological correlates to detecting preclinical markers, measuring inter-regional connection strengths, screening for disease-modifying drugs and monitoring their therapeutic effects. The future of drug discovery could therefore be profoundly affected by the intelligent use of structural and functional MRI. There are other techniques becoming established, notably automatic detection of white-matter lesion load and fibre tracking using MRI-based diffusion weighted imaging. It is a paradox of the field that the excitement generated by new things to image and ways of imaging them sometimes results in a paucity of systematic applications of perfected techniques to problems in applied medicine or therapeutics.

Jay Giedd: Neuroimaging consistently shows group average neuroanatomical or neurophysiological differences between subjects categorized according to various DSM or International Classification of Diseases (ICD) diagnostic criteria[44]. However, the large overlap between individuals in different groups greatly limits its current diagnostic utility.

The search for biomarkers in psychiatric illness has been the hallmark of psychiatric research in recent years. Biomarkers could be used to confirm diagnosis, establish subtypes or monitor the effectiveness of interventions. The lack of such biomarkers has contributed to the lag in progress compared with disease states such as cancer and has been used by some to question even the reality of psychiatric disorders. There is considerable optimism that neuroimaging, as a bridge between the molecular and behavioural levels of inquiry, could help to serve this need.

An important component of interpreting neuroimaging findings is to discern whether the differences are related to the disease itself, compensatory processes related to the disease, to treatment interventions, or to other factors. Such knowledge is best obtained from longitudinal studies by examining how specific neurobiological features evolve together with clinical features. Also, as many psychiatric disorders are increasingly conceptualized from a neurodevelopmental perspective, it will be important to consider not only the final imaging differences between groups but also the developmental trajectory. For instance,

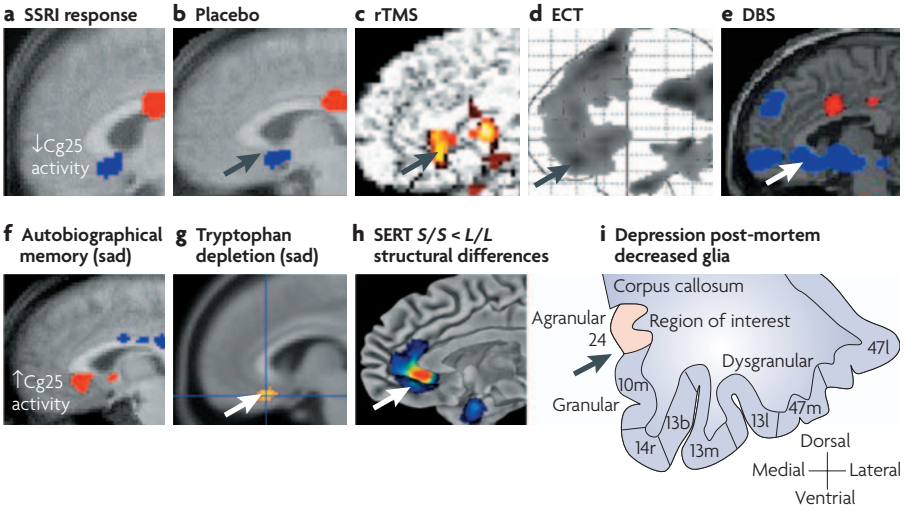

Figure 7 | **Converging evidence implicating the subgenual anterior cingulate region (Cg25) in major depression.** Top row: common imaging pattern of glucose metabolic or blood flow decreases in Cg25 with antidepressant response to various interventions. Images demonstrate group change patterns relative to the baseline depressed state for each treatment response to a serotonin reuptake inhibitor (SSRI)[70] (**a**), placebo[71] (**b**), repetitive transcranial magnetic stimulation[72] (rTMS) (**c**), electroconvulsive therapy[73] (ECT) (**d**) , and high-frequency deep-brain stimulation[60] (DBS) (**e**). Bottom row: common pattern of Cg25 blood flow increases with induction of transient sadness induced by both recollection of a personal sad memory[70] (**f**) and tryptophan depletion[74] (**g**) in healthy subjects. Anatomical differences in Cg25 distinguish healthy subjects homozygous for the *S* allele of the serotonin transporter promoter gene (a putative risk factor for depression) relative to *L*/*L* carriers[75] (**h**). Area of decreased glial number in post-mortem studies of depressed patients relative to non-depressed subjects[76] (**i**). Individual images courtesy of Helen Mayberg (panels **a**, **b**, **e**, **f**), Mark George (panel **c**), Mitch Nobler (panel **d**), Peter Talbot (panel **g**), Dan Weinberger (panel **h**), and Dost Ongur (panel **i**).

IQ is best predicted not by the final cortical thickness in the adult state but by the peak age of cortical thickness and the characteristics of its developmental trajectory during childhood[45].

Another aspect of development that might be relevant for drug discovery is that different regions and circuitry of the brain undergo periods of maximum plasticity at different ages. For instance, a selective serotonin reuptake inhibitor might have quite different effects on the development and long-term characteristics of the serotonin system depending on the age of the person at the time it is administered. Although brain neuroanatomy is moulded by both progressive and regressive events throughout development, it is during adolescence that the net regressive processes, such as synaptic pruning, overcome the net progressive processes, such as dendritic and axonal arborization, resulting in a thinning of the cortex. Early events in these processes might have greater downstream effects and the dynamic activity of brain reorganization during late adolescence could be related to the observation that the peak age of onset for psychotic, mood and anxiety disorders all occur during this time.

Question 4

How could current preclinical models of psychiatric disorders be revised to improve their predictivity?

Maurizio Popoli: This could be achieved by engineering new animal models, which, rather than being straightforward knockouts or transgenics, are made up of animals carrying a genetic vulnerability (such as a gene dosage) with good face, construct and predictive validity, interfaced with validated stress paradigms. It is important and desirable, if possible, to reproduce to some extent the gene–environment interaction that is believed to be central to human depression.

David Diamond: Preclinical models of psychiatric disorders, such as major depressive disorder and PTSD, commonly involve stress manipulations carried out on normal adult animals. One problem with these approaches is that they do not incorporate the well-documented findings that genetic or developmental influences predispose a subset of the population to develop psychiatric disorders in response to a trigger (traumatic) stimulus later in life[46]. Preclinical models of psychiatric disorders should therefore take into account the genetic and/or developmental predisposition to express the disorder, in conjunction with an effective and ethologically relevant trigger stimulus, to induce changes in brain and behaviour in animals that have features in common with psychiatric disorders.

Anthony Grace: The development of an animal model for psychiatric disorders is particularly challenging because of the nature of the disorder. First, we do not know the specific pathophysiology of major psychiatric disorders in humans, making them difficult to model in animals. Second, because these disorders are distinctly human and are expressed in terms of complex human cognitive and emotional disturbances, their assessment in animals is challenging. And third, the genetic bases of these disorders would make them difficult to replicate in other species.

Nonetheless, there are several approaches that are useful. One method that has been used, and which has provided much of the initial progress in drug design to date, is to examine the physiological and pharmacological consequences of drugs known to either mimic schizophrenia in humans (for example, PCP[47] or amphetamine[48]), or to examine the mechanism of action of drugs known to have therapeutic efficacy in schizophrenia[14,49,50].

A different and perhaps more effective approach has been to mimic the aetiological factors that are thought to lead to schizophrenia in humans. In disorders such as schizophrenia, foetal stress during the second trimester has been associated with increased predisposition for schizophrenia. In rats, treatment with a mitotoxin at an equivalent time point has been shown to lead to states in the adult that resemble several features of schizophrenia[21,51–53]. Although such treatment has substantial face and construct validity, the widespread nature of the deficit makes defining the exact pathology difficult. However, this could be further evaluated by examining the impact of lesions of specific brain areas that are known to be affected by the toxin. Results can then be compared with human imaging and postmortem studies. Such interchange between basic animal models and human pathophysiology can help to refine our understanding of complex psychiatric disorders.

Gal Richter-Levin: There is a growing feeling in the field that current preclinical models of psychiatric disorders are not satisfactory. Thinking about ways to improve their predictivity requires the identification of the pitfalls of existing approaches, and the translation of those points into ways to improve the models.

Box 3 | **Recommendations and key points from the ANMI meeting**

- Novel therapeutic approaches are needed. We should not pursue improved selective serotonin- or noradrenaline-reuptake inhibitors (SSRIs/SNRIs), or the other classes of drugs that have reached their limits. The old idea that every disease has a neurotransmitter is defunct. The brain is extremely complex, but we can define some of this complexity at different levels: molecular, neurotransmitter, cellular, electrophysiological, behavioural and systems. The key is to find the crucial nodal points at each level and redirect the complexity, not reconstruct it.
- Revisions of the *Diagnostic and Statistical Manual of Mental Disorders* 4th Edition (DSM-IV) criteria will not be the answer. There is now sufficient knowledge on molecular, genetic, electrophysiological and imaging differences in patients with psychatric disorders, and these differences must be taken into account in any new classification. Co-morbidities are to be expected and these should also be taken into account.
- The nosography for disorders such as schizophrenia is almost impossible as all patients are different, and we should perform factor analyses of subsystems (for example, hallucinations, delusions and cognition) with brain biomarkers to look at these subsystems.
- In this respect, certain brain areas (such as the hippocampus, amygdala and frontal cortex) show distinct and reproducible changes in imaging and even histomorphological studies, which can be modelled in animals. Definition of interventions in animals and humans, using new models that are transposable between preclinical and clinical research, will be crucial for future drug discovery.
- Research into trophic factors, energy supply and intracellular signalling pathways seems to open promising new directions for research in psychiatric disorders.
- Neuroimaging studies have been essential for recent research into psychiatric disorders and clearly illustrate the role of the different brain areas. These studies can also be used to demonstrate changes in the volume of brain areas, which can be altered in psychiatric disorders and also by drugs (for example, by lithium in the treatment of bipolar disorders). Animal models can be re-engineered from this knowledge, although brain imaging in rodents remains problematic. Evaluation of drug effects on the temporal coordination of neuronal activity in intact animals could be valuable.
- Environmental problems linked to genetic susceptibility might coincide at specific points during development to provide the substrate for psychiatric disorders.
- Advances in the understanding of the effects of stress on neuronal plasticity might provide promising, revolutionary targets for drug therapy of pyschiatric disorders.
- Global clinical trials involving large diverse populations are unreliable. Drugs should be tested in populations of well-selected patients that can be characterized. Symptoms for schizophrenia and depression exist for testing, but the underlying mechanisms will cut across current categories. Well-engineered small trials are much more useful. However, there is a shortage of experimental scientists at the level of animal models and experimental clinical sciences.
- Industry–university–government partnerships are necessary to progress these endeavours.

Clearly, the lack of sufficient knowledge about the nature of the disorders to be modelled and the resultant lack of clear diagnosis of psychiatric disorders makes it difficult to develop adequate animal models. Furthermore, psychiatric diagnosis depends largely on personal interviews that are not possible in animal models. It is believed that most psychiatric disorders, such as schizophrenia and PTSD, involve high-level functional impairments. It is not clear that such high-level impairments can be mimicked in rodents or other experimental animals.

Another problem in animal-model research is the tendency to treat assumptions about the models as established facts. A good example is the selective serotonin reuptake inhibitors (SSRIs), which were developed under the assumption that the impairment in depression is largely that of serotonergic neuromodulation. There was no clear evidence for that when these drugs were developed. We now know that SSRIs are not always effective in depressive patients, although they are sometimes effective in a range of other psychiatric disorders such as obsessive compulsive disorder (OCD) and PTSD. Furthermore, despite their name, these drugs were found to have effects other than serotonin reuptake inhibition (for example, increased expression of glucocorticoid receptors). Nevertheless, much of the animal-model research into the neurobiological basis of depression remains focused on the 'serotonergic hypothesis'.

When attempting to translate the above into ways to improve preclinical models, it might be beneficial to first set up multidisciplinary teams that include psychiatrists, psychobiologists and neuroscientists, which will initially identify the most relevant symptoms of a disorder to be studied in a preclinical model. For example, in the case of PTSD, although rodents are not an ideal animal model to study the neural basis of higher cortical functional impairments, they are probably an excellent model system to study more basic, anxiety-related aspects of the disorder. For example, we were able to demonstrate that, similar to the suggested effect of childhood trauma on the ability to cope with stress in adulthood, rats that were exposed to juvenile stress were impaired in their ability to cope with stress in adulthood[54]. Re-exposing juvenile-stressed rats to stress in adulthood resulted in a long-term period of heightened anxiety, reminiscent of physiological symptoms of PTSD[55].

Second, a powerful approach would be to set up teams of psychobiologists, neuroscientists, biochemists and molecular biologists that could examine, within the framework of a single team, behavioural, electrophysiological, biochemical and molecular aspects of the model.

Typically, a single behavioural variable, assumed to best represent the outcome of a behavioural manipulation, is used in order to validate the efficacy of the manipulation and of the model. An array of tests, resembling the clinical approach to diagnosis, might be a more reliable way of identifying affected animals. Such an approach would also improve the possibility of taking into account individual differences, an issue often ignored in preclinical studies[56].

Question 5

How could patients be best defined to improve the likelihood of clinical success?

Yves Agid: A key area in clinical research in the field of psychiatry is the use of subtle semiological approaches, and neuroimaging essentially provides an index of the location of the brain dysfunction. Indeed, careful, detailed clinical examinations and interviews are extremely powerful for elucidating the mechanisms of brain neuronal dysfunctions, and for proposing a new nosography of psychiatric illnesses. This approach has the most heuristic value when it is closely connected with experimental physiology (in particular, in primates when convergent programmes are used in the field of behaviour, neuroanatomy and neurophysiology).

Helen Mayberg: Imaging-based diagnostic tests, although routinely used in the clinical management of many medical illnesses, have yet to be systematically tested for any psychiatric disorder. In ischaemic

Glossary

Deep brain stimulation
Continuous therapeutic electric stimulation of subcortical areas at high frequencies (130 Hz) using chronically implanted electrodes.

Neuronal plasticity
The capacity of the nervous system to modify its organization. Such changes can occur as a consequence of many events, including the normal development and maturation of the organism, the acquisition of new skills ('learning') in immature and mature organisms, and after damage to the nervous system.

Long-term potentiation (LTP)
The prolonged strengthening of synaptic communication, which is induced by patterned input and is thought to be involved in learning and memory formation.

Long-term depression (LTD)
An enduring weakening of synaptic strength that is thought to interact with long-term potentiation (LTP) in the cellular mechanisms of learning and memory in structures such as the hippocampus and cerebellum. Unlike LTP, which is produced by brief high-frequency stimulation, LTD can be produced by long-term, low-frequency stimulation.

T1- weighted structural brain scans
MRI scans can be acquired with various types of contrast. T1-weighted images are weighted according to the so-called spin-lattice relaxation time (T1) of the protons that give rise to the MRI signals; such images provide good contrast between grey and white matter.

Transcranial magnetic stimulation (TMS)
A technique that is used to induce a transient interruption of normal activity in a relatively restricted area of the brain. It is based on the generation of a strong magnetic field near the area of interest, which, if changed rapidly enough, will induce an electric field that is sufficient to stimulate neurons.

Vagus nerve stimulation
Vagus nerve stimulation uses a commercially available device for treating both refractory seizure disorders and treatment-resistant depression. The procedure involves the surgical implantation of a small pacemaker-like device into the left chest wall with a wire running under the skin leading to coils wrapped around the left vagus nerve. The device delivers continual electrical pulses, in 30 seconds-on and 5 minutes-off intervals. Stimulation intensity and frequency of these electrical pulses (that is, dose) can be adjusted by programming the device using a hand-held computer.

heart disease, for example, evaluation of the integrity and calibre of the coronary arteries combined with tests of myocardial function are crucial determinants of the intervention strategy initiated following the diagnosis of an acute myocardial infarction[57]. The decision by a cardiologist to treat medically or surgically is neither arbitrary nor conciliatory. Rather, it is based on objective measurements of the primary organ of interest considered in the context of other contributing risk factors, including genetics, co-morbid medical conditions (for example, hypertension, diabetes and hyperlipidaemia), life-style factors (smoking, diet and exercise) and past cardiac problems.

Despite the availability of many effective treatments to treat a major depressive episode, there are no comparable clinical or biological markers that identify which patients are likely to respond to a given treatment or explain why one treatment modality or class of medication is effective when another is not. Currently, the choice of treatment for major depressive disorder is based on: trial-and-error; the accident of the type of clinician treating the patient (that is, psychiatrist or psychologist), who chooses a treatment based on their training and ideology; or patient self-selection biases.

In prioritizing a role for direct measures of brain functioning in the development of new algorithms for clinical management of depressed patients, a systematic characterization of pre-treatment patterns predictive of unambiguous remission to standard treatments is a necessary first step. In the short-term, this approach aims to identify brain biomarkers that can predict which patients are likely (or unlikely) to remit to a given first-line intervention. Similarly, long-term approaches could also identify which patients are vulnerable to relapse/recurrence during continuation or maintenance treatments, and in combination with other data (for example, genetic studies and early abuse history) identify illness markers as well as markers of vulnerability/resilience.

It is envisioned that in the future, a psychiatrist making a decision to treat a patient with major depression will choose a pharmacological or psychotherapeutic intervention based on objective measures of brain function in the context of risk factors, including genetics, co-morbid conditions, psychosocial issues and past history. Similarly, identification of genetic- or imaging-based subtypes might provide more homogeneous patient cohorts for the systematic testing of novel treatments. The need for biomarkers that can identify such response subtypes, including treatment resistance, is growing increasingly important with the availability of new somatic strategies that include repetitive transcranial magnetic stimulation[58] (rTMS), vagus nerve stimulation[59] (VNS) and, most recently, deep brain stimulation (DBS) (FIG. 7)[60]. Identifying which patients are likely to respond (or not) to such treatments would be an important advancement, particularly given the invasive nature of some of these new interventions. Functional neuroimaging provides testable strategies towards these clinical goals[61].

Daniel Weinberger: I would suggest structuring clinical samples based on key genotypes that affect outcome and treatment response. So, for example, catechol-*O*-methyltransferase (COMT) has been shown to affect the cognitive response to neuroleptic drugs[62,63], and variants in the serotonin transporter gene have been shown to affect response to SSRIs. This is, of course, only a small start in identifying genes that will affect treatment response, but the variability in outcome, in side effects, and its response will probably be reduced by genetic stratification of the analyses.

Anthony Grace: It is becoming increasingly apparent that many of the major psychiatric disorders, and schizophrenia in particular, might reflect a common pathophysiology that arises from more than a single aetiology[64]. Moreover, studies using factor analysis by Liddle and others[65] have shown that patients with schizophrenia can be defined based on distinct classes of symptoms, such as cognitive disturbances, positive symptoms, negative symptoms and disorganizational state. It could be that each of these subclasses of symptoms has a distinct pathological origin[66]. If this is the case, then targeting major classes of symptoms individually within patients might be the most effective therapeutic strategy.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES

The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CDK5 | CK1 | CK2 | COMT | DARPP-32 | *EN* | *LMX1B* | *NURR1* | *PITX3* | PKA |
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
attention deficit hyperactivity disorder (ADHD) | bipolar disorder | Cushing disease| obsessive compulsive disorder (OCD) | Parkinson's disease | schizophrenia |

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SUPPLEMENTARY INFORMATION

See online articles: S1 (figure)/S2 (figure)/S3 (figure)
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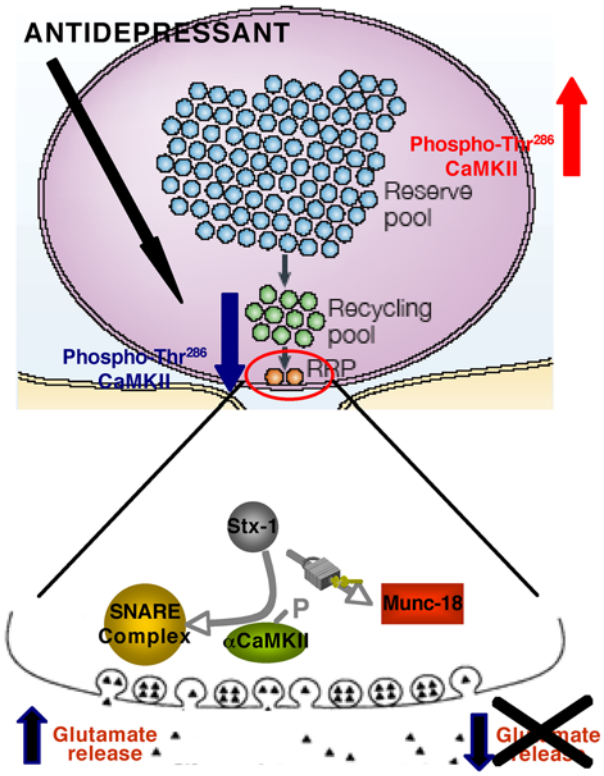
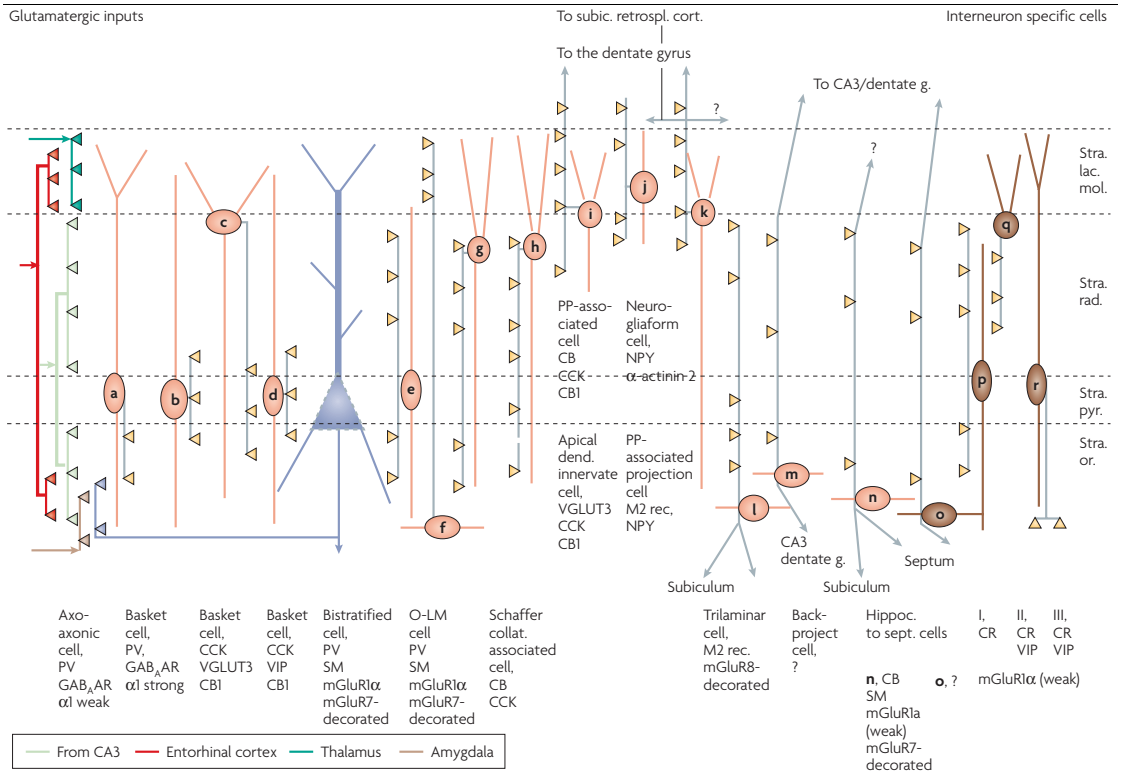
S1 | **Hypothesis on the molecular/functional changes induced by antidepressants in synaptic terminals.** Chronic (but not acute) treatment with different antidepressants (Selective inhibitors of serotonin reuptake, Selective inhibitors of norepinephrine reuptake, Tricyclic antidepressants) induces a redistribution of αCaM kinase II Thr^{286} phosphorylation within synaptic terminals, with a large phosphorylation increase in the total synaptic vesicle pool (representing the reserve pool) and a corresponding large decrease in synaptic membranes (containing the readily releasable pool associated to active zones) (Barbiero et al, Neuropsychopharmacology, in press). Syntaxin-1 (stx-1), one of three SNARE proteins forming the SNARE exocytotic complex binds to phospho-αCaM kinase II and this interaction promotes formation of the SNARE complex and neurotransmitter release[78] (Ohyama et al., 2002). The reduction of αCaM kinase II phosphorylation in synaptic membranes induced by antidepressants reduces the interaction syntaxin-1/αCaM kinase II, making syntaxin-1 more available for interaction with Munc-18. Munc-18 locks syntaxin-1 in a closed conformation that blocks the assembly of syntaxin-1 into the SNARE complex, resulting in a reduction of glutamate release[18].

Barbiero VS, Giambelli R, Musazzi L, Tiraboschi E, Tardito D, Perez J, Drago F, Racagni G, Popoli M. Chronic antidepressants induce redistribution and differential activation of αCaM kinase II between presynaptic compartments. *Neuropsychopharmacology* (in press).



S2 | **Basic layout of a cortical circuit in the hippocampus.** The figure shows the innervation of pyramidal cells by domain-selective GABAergic interneurons and interneurons by interneuron-specific GABAergic cells in the CA1 area of the hippocampus. The main lamina-specific glutamatergic inputs are indicated on the left. The somata and dendrites of interneurons innervating pyramidal cells are shown in orange, those innervating mainly or exclusively other interneurons are shown in brown. Axons are shown in light green and the main termination zone of GABAergic synapses are shown by yellow symbols. The proposed names of neurons, some of them abbreviated, are under each schematic cell and a minimal list of molecular cell markers is given, which in combination with the axonal patterns help the recognition and characterisation of each class. Note that one molecular cell marker can be expressed by several distinct cell types. Some cells are listed on the basis of limited data from only one study. Some additional cell types, which have not been reported in sufficient detail, are not indicated. Note the association of the output synapses of different sets of GABAergic cell types with the perisomatic region, and either the Schaffer collateral, commissural or the entorhinal pathway termination zones, respectively. Although the cells are best defined in the hippocampus, equivalent circuits are present throughout the cortical mantle. CB, calbindin; CR, calretinin; GABA, γ-aminobutyric acid; LM–PP, lacunosum–moleculare–perforant path; LM–R–PP, lacunosum–moleculare–radiatum–perforant path; M2, muscarinic receptor type 2; NPY, neuropeptide tyrosine; PV, parvalbumin; SM, somatostatin; VGLUT3, vesicular glutamate transporter 3; VIP, vasoactive intestinal polypeptide. Adapted with permission from REF. 77.



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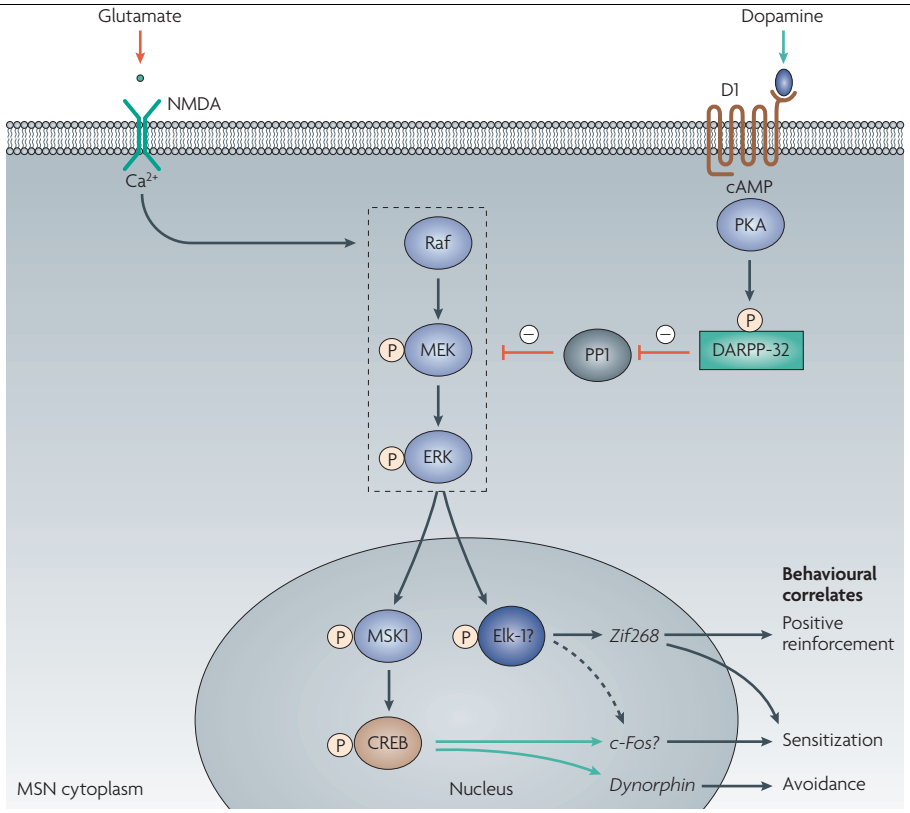
S3 | **Role of the ERK pathway in striatal neurons in response to drugs of abuse.** The ERK pathway is activated in striatal medium-size spiny neurons in response to cocaine and other drugs of abuse. This activation requires coincident stimulation of dopamine D_1 receptors and NMDA glutamate receptors. The crosstalk between these receptors involves the striatal-enriched protein phosphatase-1 inhibitor, DARPP-32. Among the targets of ERK, several nuclear proteins have been identified. The transcription factor CREB is regulated through activation of the kinase MSK1, whereas ELK1 is directly phosphorylated by ERK. MSK1 is essential for the induction of c-Fos and dynorphin but not of ZIF268/KROX24. In MSK1 knockout mice cocaine-induced locomotor sensitization is decreased, whereas conditioned place preference is facilitated. In contrast, in ZIF268/KROX24-deficient mice, sensitization is decreased and place preference is abolished. These results suggest that specific signalling pathways are limiting for specific behavioural responses. Although all signalling pathways are indicated as if taking place in the same cell, this is unlikely to be the case in reality. CREB, cAMP-response element binding protein; DARPP-32, dopamine- and cAMP-regulated phosphoprotein, M_r 32,000 Da; D_1, dopamine D_1 receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/ERK kinase; MSK1, mitogen- and stress-regulated kinase; MSN, medium spiny neuron; NMDA, *N*-methyl-D-aspartate; PKA, cAMP-dependent protein kinase; PP1, protein phosphatase 1.



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