REVIEW

doi:10.1038/nature12982

Circuit dynamics of adaptive and maladaptive behaviour

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The recent development of technologies for investigating specific components of intact biological systems has allowed elucidation of the neural circuitry underlying adaptive and maladaptive behaviours. Investigators are now able to observe and control, with high spatio–temporal resolution, structurally defined intact pathways along which electrical activity flows during and after the performance of complex behaviours. These investigations have revealed that control of projection-specific dynamics is well suited to modulating behavioural patterns that are relevant to a broad range of psychiatric diseases. Structural dynamics principles have emerged to provide diverse, unexpected and causal insights into the operation of intact and diseased nervous systems, linking form and function in the brain.

A long-sought goal in psychiatry is to understand the concrete mechanistic distinctions between adaptive, physiological behavioural states and psychiatric-disease-related behavioural states, which are symptomatically complex in a way that can seem inaccessible to physical interpretation or precise intervention. This situation stands in contrast to, for example, our mechanistic understanding of the equally diverse symptoms of heart failure (ranging from shortness of breath to swelling of extremities), which can be concretely explained and understood using a single physical formulation with robust causal explanatory power: reduced pumping action of the heart muscle. Unfortunately, such causal tissue-level understanding does not yet exist in psychiatry[1–3], despite pioneering genetic and pharmacological studies that have provided causal insight at the molecular level, and brain-wide observational techniques such as BOLD–fMRI (blood-oxygen-level-dependent functional–magnetic resonance imaging) and electroencephalography that have provided circuit-level correlates (but without causal information)[1,2,4].

Although neural connections are presumed to transmit information-rich signalling streams, long-range projections might be relevant to neuropsychiatric symptoms through neural dynamics (how brain activity is managed and coordinated over time and space), as significantly as through circuit coding (how detailed neural information is actually represented). Recent advances in neuroscience have revealed that surprisingly potent and specific control of complex behaviours arises from modulating circuit projection dynamics, even though we have very little causal knowledge of information coding or representation. This Review will consider the technological advances that allow this insight into the causal underpinnings of maladaptive behaviour, in the context of the structural dynamics findings that have been made accessible by the application of new technologies.

Clinical context

Deep-brain stimulation (DBS) with microelectrodes has set the stage for real-time causal intervention in neuropsychiatric conditions. Microelectrodes can be placed in anatomically well-defined locations for region-specific neuromodulation[5], and have delivered potent and specific elicitation or resolution of subsets of symptoms in disease states ranging from parkinsonism to depression[6–11]. However, DBS mechanisms and effects have been mysterious, partly because DBS electrode stimulation directly causes mixed patterns of excitation and inhibition in diverse local cells and in axons from distant sources that are passing through and may be unrelated to the function of the implanted region[5]. An interesting pattern is emerging from our clinical experience of psychiatric DBS; many of the most promising targets involve electrode contact placement in white matter (the long-range axonal connections that wire brain regions together) rather than in grey matter or cell-body-dense regions[6,8]. DBS targets that fit this pattern include subcallosal cingulate white matter for depression, the medial forebrain bundle for depression and anhedonia (the lack of enjoyment of normally rewarding experiences), the anterior commissure for depression, zona incerta (ZI) or subthalamic nucleus (STN)-proximal white matter tracts for depression and obsessive–compulsive disorder (OCD), ventral capsule white matter in OCD and depression, inferior thalamic peduncle white matter in OCD and depression, and even the vagus nerve axon bundle (including afferent fibres to the solitary tract nucleus) in depression[6]. However, the mechanistic implications of this pattern are unclear. The brief high-frequency pulses that are typically delivered through DBS electrodes may simply be better suited to stimulating axons than cell bodies[5,12], and as point sources, DBS electrodes will more efficiently control a tract of axons in a relatively small volume than directly control a larger region of cell bodies in grey matter[13].

Apart from these important technical considerations, long-range projection properties (whether adaptive or maladaptive) may represent suitable final common pathways for governing psychiatry-related behavioural states. Although structurally defined, these features of brain anatomy can place natural bounds on tissue dynamics, and can do so independently of neural coding per se. The excitability, myelination and conduction properties, valence (excitation, inhibition or modulation) and net synaptic strength of axonal connections that constitute a particular long-range projection in the brain (all physical quantities that can be set by genetics, development and plasticity) are suited to govern circuit-level dynamical properties that have previously been linked to psychiatric disease, such as excitation/inhibition (E/I) balance[14–17], synchronization of activity across and within brain regions[18–25], and extent of activity propagation through brain regions[26–28]. Until recently, testing the causal impact of specific long-range projections on behaviour was not possible — achievable only with a new experimental methodology.

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Technology

Stimulated, in part, by this clinical context, technologies that work with specificity at the level of circuit wiring in animal models have become the focus of much recent research interest in neuroscience. New approaches that allow interrogation of specific nervous system connections include projection-defined activity control, as well as projection activity mapping and structural mapping. The outcome of this technology development has been the ability to control and observe specific connections within the intact nervous system through precise circuit-level measurements and interventions.

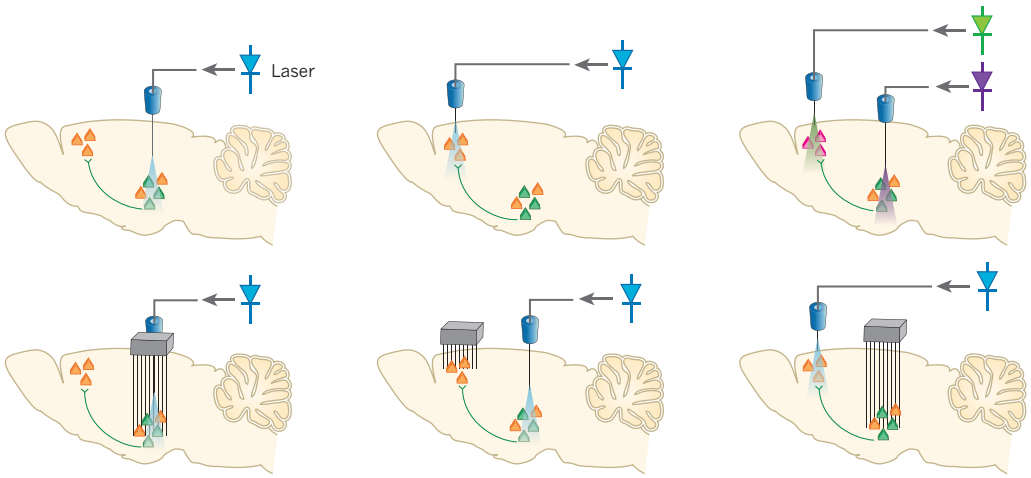
Achieving control over cells defined by specific connectivity is difficult, but essential to progress along the path towards the causal understanding of function and dysfunction of neural circuitry. Already, research in some invertebrate nervous systems (within which defined cells can be selectively controlled by microelectrodes) has demonstrated the importance of knowing the wiring patterns of the cells targeted for control, to achieve predictable influence over the complex and sensitive dynamical properties of even small networks of neurons[29]. However, in mammalian systems, until very recently it was not possible to control the high-speed dynamics of cells defined by wiring, because even when directed to fibre bundles or white matter, electrodes cannot select as the initial direct target a particular projection defined by cell-body origin and trajectory. Optogenetics[30,31] (Box 1), which allows researchers to have direct control over cells defined by projection pattern[4,32–34], was initially applied to brain disease in a study probing DBS mechanisms[13]. It was found that amelioration of parkinsonian symptoms could be most robustly achieved when the light-sensitive elements were chiefly afferent axons rather than local cell bodies[13,35]. Shortly after, optogenetic control of specific trajectory-defined projections in behaviour was achieved[36], unexpectedly revealing endogenous inhibition of anxiety-related states in the amygdala, in a study using gain- and loss-of-function interventions to targeted amygdala projections. Optogenetic approaches have since allowed projection-defined activity control in behaviours related to reward, motivation, depression, social interaction, compulsions, cognition and other domains of normal and maladaptive brain function (discussed later). Of note, projection targeting is only one application of optogenetics that is distinct from other approaches such as controlling distinct cell types within a region to assess the impact on physiology or behaviour, developing prosthetic or repair strategies, mapping detailed wiring patterns, and perturbing dynamics precisely during high-dimensional observation (recording or imaging) for population and state space analysis.

Controlling activity in cells defined by projection pattern would ideally be complemented by the observation of native activity and structure in these cells (Box 1). Indicators of neural activity could in principle be used to selectively record the activity of a projection *in vivo*, for example with genetically encoded calcium indicators (GECIs)[37], which can be introduced through focal injection of increasingly sophisticated targeting viral vectors[24,38–41]. Cells could be connectivity-defined for activity imaging if this transduction process were to occur in a wiring-dependent manner,

BOX 1

Typical projection-targeting experimental configurations

Opsin genes carried by viral vectors are focally injected into the brain. Top left, a projecting population has been defined by injection of a retrograde viral tool to deliver an opsin or recombinase[48,124] into the upper left cell population; to provide projection specificity of the control, light is delivered by a fibre-optic not to the same location but to a distinct focal region of upstream cells (typically about 1 mm^3 in size). Top middle, in a technically different approach[4,32–34,125], microbial opsins — ideally enhanced with membrane-trafficking motifs from mammalian channels[124,126] — are transported down axons, which become the photosensitive elements in downstream fibre-optic-targeted structures. In this case, a well-tolerated (chiefly non-retrograde) adeno-associated virus or lentiviral vector may be used, but weeks are needed for effective opsin transport. Inhibition of the projection can be achieved with an inhibitory opsin gene[36,92], encoding a chloride or proton pump, whereas stimulation of cells defined by the projection can be achieved with an excitatory opsin gene[34,123], encoding a cation channel (in the case of the latter, by using channelrhodopsins, antidromic action potential backpropagation from the site of light delivery may occur, which can confer the feature of fully recruiting cells defined as possessing the specified projection among other possible projections). Top right, recording with various modalities (activity photometry[122] is shown here with an additional readout optical fibre, or electrical recording as in bottom row) at any point of interest in the brain allows assessment of the circuit dynamical underpinnings of the behavioural changes observed or photo-tagging of cells defined by projection. Bottom left, high-speed multi-unit electrical recording; spikes corresponding to cells defined by genetically determined opsin expression are identified through sufficiently low-latency spikes after a light pulse. Bottom right, same as at left except cells are defined for photo-tagging by projection illumination rather than projection transduction. Adapted with permission from ref. 127.



and certain rabies- or herpes-virus-based tools (although these are limited in utility by toxicity to target cells) will transduce axon terminals at the projection location[42–45], in principle allowing the observation of activity in projection-defined cells. Although these upstream cells may be distributed over large distances, activity imaging is carried out focally, thereby spatially defining cells that give rise to a specific projection (Box 1).

Activity recording of projection-defined cells (although not of the projection itself) when animals are exhibiting a certain behaviour has been achieved using optogenetics itself as an identification tool to discriminate recorded cells[46–48] based on wiring phenotype. Through this approach, an excitatory opsin is conditionally expressed based on projection pattern by the introduction of the opsin-carrying axon-transducing virus (for example, rabies or herpes) into a terminal field of the projection. Then pulses of light delivered to the cell-body location may elicit the lowest-latency (directly excited) electrical spikes from cells bearing the projection of interest (Box 1); the characteristic waveforms of these fast-responding cells can be quantitatively defined so that subsequent multielectrode recording during behaviour will allow the recognition of spikes from these projection-defined cells[46–48]. Although the specificity of this 'tagging' strategy is not absolute unless spike latencies in the low-ms range can be achieved or (more likely) details of the local circuitry preclude fast indirect synaptic activation[33], this combination of optogenetics and multielectrode recording has allowed, in some settings, the real-time activity recording of cells defined by the projection target (which need not be identical to the distant activity of the projection termination itself) during mammalian behaviour.

Finally, to complement projection-based activity control and projection-based activity measurement, detailed observation of the brain-wide physical form of the projections in question will be important. Visualization of neural projections linked to causality information and molecular descriptors will deepen our understanding of the neural structural dynamics that underlie behaviour, although so far pioneering sectioning or ablative electron microscopy and array tomography methods[49–53] (in some cases with correlational information on activity patterns) are not readily linkable with causal information on the behavioural significance of the connections. Clearing chemicals such as Sca*l*e[54], BABB[55,56], SeeDB[57] and ClearT[58] allow degrees of brain transparency (although without molecular phenotyping because the resultant brain tissue remains largely impermeable to macromolecular labels such as antibodies). High-throughput projection-mapping[50,59] tools involve thin-sectioning and reconstruction by alignment, and, in an approach without sectioning, the electrochemical technology CLARITY allows the construction of crosslinked hydrogels from within tissue and subsequent electrophoretic removal of membrane lipids, thereby allowing the penetration of photons and macromolecular labels throughout the intact mammalian brain[60,61] (Fig. 1). All of these approaches can be linked to causal or activity information on the projections observed if registration of data sets is conducted; projection activity could be accessible not only with acute slice approaches[62] but also with *in vivo* behavioural methods because opsin or GECI probes can be integrated with, or include, cell-filling fluorescent labels that allow projection mapping by light microscopy after the completion of behavioural testing or activity imaging. If needed, light-microscopy-based projection maps can also be linked to ultrastructural information[60] to describe the synaptic properties and targets of the projection.

Circuit-level findings

Together, the technologies delineated have opened the door to determining and understanding the causal significance of defined projections in the control of behaviour. Here, I discuss behavioural findings that have emerged about the interrelationships between adaptive and maladaptive behaviour through experiments probing causal structural dynamics at cellular resolution and within the intact brain. For the purposes of emphasis on behavioural and clinical insight, I highlight the major psychiatric symptom domains relating to anxiety and depression. A complete summary is provided in Table 1.

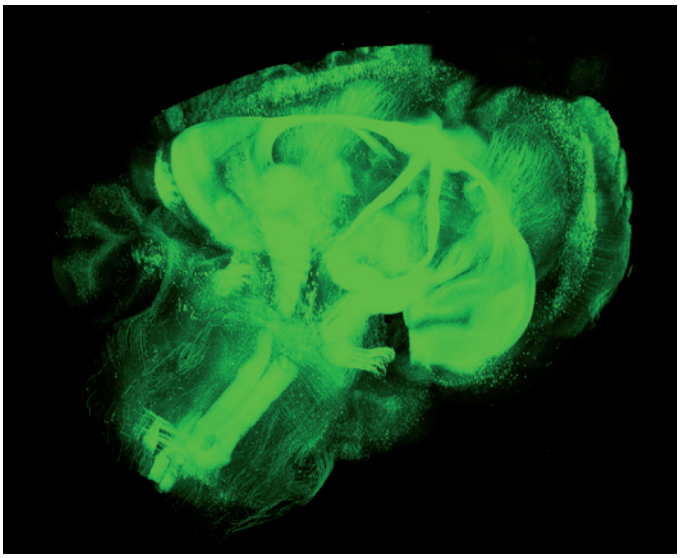


Figure 1 | Visualizing projections in the intact mammalian brain. The entire mouse brain has been processed with CLARITY[60,61] (with Thy-1 type projection neurons labelled in green); such brain wide structural data sets may be integrated with other data streams from the same animal, including activity records during behaviour (as obtained with GECIs), stable activity markers such as immediate-early genes, whole-mount molecular phenotyping, behavioural scores in the presence or absence of optogenetic or disease-model interventions, and opsin–fluorophore expression patterns. In general, whole-brain analysis is useful for interpretation; for example, use with multiple retrograde labels may help to determine whether individual cells include collaterals projecting to multiple sites, an important consideration in projection targeting. Adapted with permission from ref. 61.

Causal projection dynamics linked to anxiety

Anxiety disorders, which include generalized anxiety disorder, panic disorder, post-traumatic stress disorder and OCD, represent the most prevalent class of psychiatric condition. Building on earlier work that implicates structures such as the extended amygdala in fear and anxiety, and the striatum in OCD, recent studies have made headway in our understanding of the causal projection dynamics of anxiety-related symptoms. In an emerging theme, fundamentally different results were often obtained when projection-defined circuit elements were recruited, compared with the recruitment of region-defined cell-body populations.

In initial anxiety-related investigations[36], freely moving mice were assessed using the elevated-plus maze and the open-field test, which allow inference of apprehension in the absence of immediate threat. When the cell bodies of excitatory (CaMKIIα-expressing) neurons were optogenetically stimulated in the basolateral amygdala (BLA) (without regard to projection target), an immediate (and immediately reversible) anxiogenic effect was observed in which subjects began to avoid exposed areas of test arenas, without other changes in locomotor performance. Conversely, when these neurons were optogenetically inhibited, an anxiolytic effect was seen[36]; together, these results were consistent with classical anxiogenic models of the BLA.

However, more refined projection targeting revealed unanticipated anxiolytic circuitry embedded within this otherwise anxiogenic BLA circuit environment. When only a subpopulation of the CaMKIIα-expressing elements — namely lateral projections from the BLA towards the central nucleus of the amygdala (CeA) — were stimulated instead, a prominent anxiolytic effect was observed[36]. Conversely, inhibition of this projection elicited anxiogenesis[36]. This surprising arrangement (that required optogenetic projection targeting to resolve) is well-suited to providing diverse access nodes for swift and versatile endogenous modulation[2,63], and no doubt further anatomical and functional complexities will be elaborated. For example, studies of conditioned fear[64,65] have identified two functionally and genetically distinct types of unit in the centrolateral component of the CeA, and optogenetic stimulation of one of these

Table 1 | Projection-defined activity dynamics causally implicated in psychiatric-disease-related behaviour

Projection origin	Projection target	Elicited activity in projection	Behaviour	References
mPFC	LHb	Excitation	Passive/immobile (swim test)	76
	DRN	Excitation	Active/mobile (swim test)	76
		Inhibition	Passive/immobile (swim test)	76
	NAc	Excitation	Appetitive	93
BLA	CeL/CeA	Excitation	Anxiolytic (risk)	62
		Inhibition	Anxiogenic (risk)	62
	adBNST	Excitation	Anxiolytic (risk, respiratory)	68
		Inhibition	Anxiogenic (risk, respiratory)	68
	NAc	Excitation	Appetitive	92, 93
		Inhibition	Aversive	92
	vH	Excitation	Anxiogenic (risk)	121
		Inhibition	Anxiolytic (risk)	121
adBNST	LH	Excitation	Anxiolytic (risk)	68
	PB	Excitation	Anxiolytic (respiratory)	68
	VTA	Excitation	Appetitive	68
vBNST	VTA	Excitation (glutamatergic)	Aversive, anxiogenic	100
	VTA	Excitation (GABAergic)	Appetitive, anxiolytic	100
	LH	Excitation (GABAergic)	Increased food consumption	88
	LH	Inhibition (GABAergic)	Decreased food consumption	88
vH	NAc	Excitation	Appetitive	93
LDT	VTA	Excitation	Appetitive	97
LHb	VTA	Excitation	Aversive	97
EPN	LHb	Excitation	Aversive	99
VTA TH	NAc	Excitation	Appetitive	81
	mPFC	Excitation	Aversive	122
	LHb	Excitation (GABAergic-TH)	Appetitive	123
ARC AgRP	PVH	Excitation	Increased food consumption	87
vmOFC	VMS	Excitation (chronic: probably favouring potentiation of projection)	Increased grooming (wild-type mice)	70
LOFC	CMS	Excitation (acute: probably favouring inhibitory cell targets)	Reduced grooming (Sapap3-mutant OCD mouse model)	71

adBNST, anterodorsal bed nucleus of the stria terminalis; ARC AgRP, agouti-related peptide cells in arcuate nucleus; BLA, basolateral amygdala; CeA, central amygdala; CeL, centro-lateral amygdala; CMS, centromedial striatum; DRN, dorsal raphe nucleus; EPN, entopeduncular nucleus; LDT, laterodorsal tegmentum; LH, lateral hypothalamus; LHb, lateral habenula; LOFC, lateral orbitofrontal cortex; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PB, parabrachial nucleus; PVH, paraventricular hypothalamus; TH, tyrosine hydroxylase; vBNST, ventral BNST; vH, ventral hippocampus; vmOFC, ventromedial orbitofrontal cortex; VMS, ventromedial striatum; VTA, ventral tegmental area.

populations inhibited neurons projecting to periaqueductal grey[65]; this work illustrates that sufficiently precise genetic cell-type targeting (where feasible) can give rise to a kind of projection targeting, and together with earlier work has deepened our understanding of fear behaviour[66,67].

Although the initial anxiety study[36] focused on risk-avoidance behaviour (in the elevated-plus maze and the open-field test), the clinical anxious state is not characterized by risk-avoidance alone. Physiological phenomena such as respiratory-rate changes and aversive subjective sensations are an enormously important part of the clinical picture, contribute to morbidity and mortality and can be studied quantitatively in animal models. A subsequent paper[68] extended the projection targeting concept in anxiety to study assembly of the anxious state from multiple separable features. This study found that a long-range projection from the BLA to the bed nucleus of the stria terminalis (BNST) was unexpectedly able to favour anxiolysis, that within the BNST itself oppositional subnuclei favoured anxiogenesis or anxiolysis, and that distinct outgoing projections from the anxiolytic subnucleus (the anterodorsal BNST, adBNST) each recruited distinct features of anxiolysis[68] (Fig. 2). Surprisingly, the BNST projection to the lateral hypothalamus was found to recruit only a behavioural risk-avoidance feature, the projection to the ventral tegmental area (VTA) was found to recruit only an appetitive (positive-valence conditioning or rewarding) feature, and the projection to the brainstem parabrachial nucleus was found to recruit only a reduced respiratory-rate feature[68] (Fig. 2). Projection targeting allowed the determination of the causal nature of this feature separability, illustrating aspects of how complex behavioural states may be coordinately assembled and disassembled by projection dynamics. It is worth underscoring here that neural codes per se, in terms of the spiking representations of information, are not understood for any of these internal states or behavioural outputs, but relatively complex and specific behaviours can still be elicited and suppressed using projection targeting of circuit dynamics.

OCD highlights some of the challenges (and illustrates some of the solutions) for the study of disease-related symptoms in animal models. The disorder is characterized both by symptoms that are difficult to assess in experimental animals (recurring and intrusive thought patterns) and by symptoms with clear rodent correlates (compulsive behaviours that are poorly suppressed even when adverse consequences result). As with many psychiatric diseases, insight into the overall syndrome may be obtained in animal models by focusing on those symptom domains that can be recapitulated experimentally. For example, circuits involving the orbitofrontal cortex (OFC) and striatum have previously been correlated with clinical OCD[69], and two recent papers have now causally tested these circuits using projection targeting in mouse models of compulsive behaviour. One study[70] involved driving cells projecting from the ventromedial OFC to the ventromedial striatum (VMS), and tracked grooming behaviour (compulsive grooming bears some similarity to behaviours in the clinical condition). Repeated bursts of activation of this projection led to increased grooming over days, suggesting a mechanism by which pathological behavioural patterns subserved by that projection could become difficult to suppress. Another group began with a model of compulsive grooming (Sapap3 mutant mice) and targeted a projection between the lateral OFC and the centromedial striatum (CMS)[71]. Acutely driving this projection recruited feedforward inhibition in the CMS and gave rise to suppression of compulsive grooming. OFC is important for many behavioural and cognitive processes, including aspects of attention, reversal learning, risk responsiveness and

response inhibition[69,72]; together, these papers causally implicated specific projections from the OFC to the striatum in modulating compulsive grooming relevant to OCD.

Causal projection dynamics of depression-related behaviour

One of the core diagnostic criteria for major depressive disorder is the clinically defined symptom of hopelessness, a profound negativity about the future that can be behaviourally manifested as a predilection to discount the value of choices or effort in the present. Such a behavioural pattern can be pathological and give rise to severe morbidity (and mortality from suicide); however, when environmental conditions are adverse, a 'passive-coping' behavioural state — in which minimal energy is expended — could in fact be more adaptive than an active-coping state[63,73–76]. A recent report described the results of modulating dynamics in targeted projections arising from the prefrontal cortex, in the setting of a behavioural task (the automated forced swim test, FST) designed to detect temporally precise transitions between active and passive coping[76].

When cells with a projection between the medial prefrontal cortex (mPFC, an anterior forebrain structure involved in executive function and planning) and the dorsal raphe nucleus (DRN, a major locus of serotonergic neurons) were optogenetically stimulated during the automated FST, rats were found to shift towards an active-coping behavioural regime (swimming or climbing); the same fibre-optic-targeted intervention did not alter nonspecific locomotor activity assessed in the open field[76]. Surprisingly, the opposite finding was seen when cells defined by projections from the mPFC to the lateral habenula (LHb) were targeted; rats shifted toward the passive-coping (floating or immobile) regime, illustrating the importance in optogenetics (for true functional specificity) of not just cell-type targeting but also projection targeting allowed by the fibre-optic neural interface[40]. And when cells defined by projection from the mPFC to the BLA were optogenetically recruited, no behavioural effects in this test were seen[76]. It was also found that targeting corresponding focal cell-body regions (instead of defined projections) was not well-suited to elicitation of this specific class of behavioural effect. First, when DRN cells were driven optogenetically, but nonspecifically, increased active behaviour was seen in the FST but also in the open field, indicating elicitation of a less-specific behavioural state[76]. Second, when mPFC cells were generally recruited without specifying projection class, no transition between active and passive behavioural state was seen, probably partly because oppositional projections were recruited[76].

The converse of depression-related behavioural states (with maladaptively low value assigned to actions or available choices) would be states in which predictions are maladaptively positive. Manic and hypomanic behaviour may fall into this category in the form of the core criteria of increased goal-directed and risk-taking behaviour (along with certain character traits and behavioural patterns such as pathological gambling, especially those associated with dopamine (DA)-system modulation[77,78] in which perception of risk[79,80] is impaired and/or perceived likelihood of positive outcomes is inflated). In a transgenic rat line that allowed opsin expression only in tyrosine hydroxylase (TH)-expressing cells[81] in the VTA (that is, DA neurons), stimulation of these cells was recently found to favour the active-coping behavioural pattern in the automated FST without nonspecific locomotion effects in the open field[75]. Electrical recording in the anatomical target of one of these outgoing projections (the nucleus accumbens, NAc) from multiple single units during the automated FST revealed that modulating DA neuron dynamics altered key aspects of the neural representation of action in the NAc[75]. This particular projection (VTA–NAc) may be clinically relevant because recent work has suggested NAc-related projections are significant in treating patients with depression using DBS[10,82]. It will be interesting to investigate possible anatomical and causal linkages between the mPFC–DRN[76] and VTA–NAc[75] pathways.

The vegetative symptoms of psychiatric disease are those linked to basic physiological functions, including appetite, sleep–wake balance and sexual behaviour. These symptoms are individually variable during depressive episodes, but in aggregate are common, debilitating and constitute core criteria for diagnosis of major depression. The initial optogenetic control of behaviour in mammals was in fact conducted on sleep–wake behaviour through the hypocretin neurons of the lateral hypothalamus; certain patterns of activity delivered to these cells in mice were found to favour the transition from sleep to wake[40]. It was later found that driving noradrenaline neurons in the locus coeruleus (LC) exerted a rapid and profound effect on awakening[83,84], and by driving hypocretin neurons with an excitatory opsin while simultaneously inhibiting LC noradrenaline neurons with an inhibitory opsin, it was observed that the effect of hypocretin neurons on awakening[40,84,85] could be accounted for by projections to the LC. Early applications of optogenetics to another vegetative category, feeding behaviour, have included defining (and modulating) the reward value of feeding (an important step towards understanding how the reward value of feeding could become altered in the setting of psychiatric disease). Precisely tuned control over the value of nutrient consumption was achieved using optogenetic drive of DA neurons in the VTA, which shifted the value of one class of nutrient to be greater than the highly appetitive value of sucrose[86]. Later papers used optogenetic projection targeting to evoke strikingly excessive feeding behaviour with relevance to depression, eating disorders and obesity by driving agouti-related peptide (AgRP) neurons that project from the arcuate nucleus to the paraventricular hypothalamus (PVH)[87] or by driving the GABAergic projection from the BNST to the lateral hypothalamus[88].

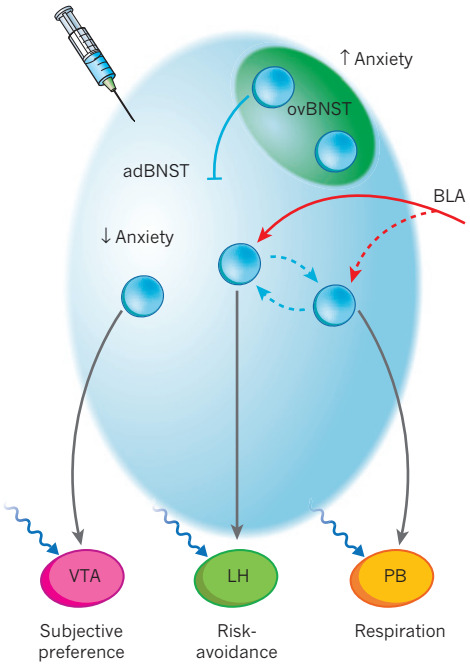


Figure 2 | Controlling projections in the intact mammalian brain. Behavioural state assembly and complexity can be assessed with projection targeting. Functional organization of the bed nucleus of the stria terminalis (BNST) circuitry is shown. The oval nucleus of the BNST (ovBNST) is anxiogenic, and the anterodorsal BNST (adBNST) is anxiolytic[68], as is the projection from the BLA (red arrows); the ovBNST may act to increase anxiety by inhibiting the adBNST or by independently influencing downstream structures. The adBNST projects to the ventral tegmental area (VTA), lateral hypothalamus (LH) and parabrachial nucleus (PB); each of these projections decreases a distinct feature of anxiety expression, and coordinated recruitment of these subpopulations may be implemented by recurrent circuitry in adBNST[68]. Projections are assessed by introduction of opsin (needle) into the adBNST, and independent targeting of fibre-optic interfaces depicted for each candidate downstream target (blue arrows). Here (as with medial prefrontal cortex projections and VTA projections) little mixed-site collateralization from single neurons is seen[68]. Adapted with permission from ref. 68.

Hedonic and anhedonic behaviours, beyond their relevance to feeding alone, are of substantial significance in psychiatric disease; anhedonia is a formal diagnostic criterion for depression, distinct from vegetative and motivational criteria. At the other end of the spectrum, excessive hedonic behaviour manifests in conditions relating to substance abuse and dependency, as well as in manic and hypomanic conditions. Dysfunction in responding to rewarding or appetitive stimuli can be studied in animals by determining to what extent behaviour can be positively conditioned by, or to what extent animals will work for, the stimuli in disease-like states[74]. Over the past five years, applications of optogenetic projection targeting under physiological conditions and in disease models have begun to illuminate the causal structural dynamics of hedonia and anhedonia.

An initial step came with the optogenetic drive of VTA DA neurons, to causally test whether different patterns of spikes in these defined cells could be appetitive for mammals[89]. In freely moving mice, high-frequency 'phasic' bursts of spikes delivered to VTA DA neurons were found to potently drive place-preference conditioning, and, interestingly, seemed preferable to mice compared with the same number of spikes delivered at a lower 'tonic' frequency[89] (highlighting the importance for optogenetics of eliciting precise temporal dynamics in the targeted cells). Although these initial experiments were carried out in *Th*-Cre transgenic mice to allow the targeting of VTA DA neurons with Cre-dependent opsin-expressing adeno-associated virus (AAV) vectors, in later work[81] rats were used as experimental subjects for robust assessment of the extent to which animals will work for a stimulus or reward. *Th*-Cre transgenic rats designed and created for this type of experiment were observed to execute many thousands of nose pokes per day (a test for experimental subject-initiated, motivated behaviour) to obtain optogenetically delivered phasic spike bursts in VTA DA neurons; furthermore, projection targeting revealed that rats will work for activity in VTA-DA cells that are even more precisely defined, namely the subset of these neurons projecting to the NAc[81]. It is worth noting that clinical DBS findings have pointed to the NAc in anhedonic symptoms of major depression[90,91].

Optogenetic projection targeting has identified a number of additional afferents to the NAc that also modulate reward-related behaviour or appetitive conditioning, in some cases connected to psychiatric-disease-related symptoms. Not only will animals work to receive stimulation of the defined BLA projection to the NAc, but also optogenetic inhibition of this projection creates a depressive-like phenotype of reduced motivation to obtain a normally appetitive sucrose solution[92]. Subsequent work has implicated excitatory afferents to the NAc from the ventral hippocampus (vH) and mPFC in promoting appetitive conditioning and reward-related behaviour[93]; dopamine type-1 (D1) receptors in NAc were implicated, and the vH pathway to the NAc was found to be potentiated by cocaine intake[93], consistent with the earlier finding that D1 receptors in the NAc are important for appetitive cocaine responses[94]. Additional cocaine-related projection targeting studies have illuminated the relevance of PFC–NAc pathways; inhibition of the projection from the PFC to the NAc blocked cocaine-seeking behaviour in rats[95], and specific inhibition of mPFC inputs to the NAc by optogenetic depotentiation elicited a reduction in cocaine-induced locomotor sensitization[96]. Together, these studies have elucidated fundamental aspects of causal structural dynamics that underlie abused-substance-related hedonic behaviours.

Recently, much important information has also come to light concerning the causal role of the projections into the VTA itself, which is relevant to hedonic and anhedonic behavioural states. An opsin-carrying rabies virus was delivered into the VTA; projection targeting was then achieved by delivering light to distinct upstream cell-body regions[97]. Conditioned place preference was seen when the projection from the laterodorsal tegmentum (LDT) to the VTA was driven (interestingly these projections synapse preferentially onto NAc-projecting outgoing VTA DA neurons)[97]. By contrast, conditioned aversion was observed when the projection to the VTA was driven from the LHb[97] — the same structure that supported a depressive-like passive-coping response in the FST when mPFC-to-LHb projections were driven[76]. In a complementary (and concordant) projection targeting set of results[98], LHb projections to the VTA were optically driven by transducing the LHb with a channelrhodopsin and illuminating the VTA; this intervention was also aversive (and in fact antagonized positive reinforcement)[98]. Interestingly, the aversive LHb projections to the VTA seem to favour, as synaptic targets, DA neurons that project, not to the NAc, but instead to the mPFC[97], perhaps outlining a passive–aversive loop of influence definable by optogenetic projection targeting, spanning the brain and schematized as mPFC–LHb–VTA–mPFC. There may be antagonism or competition for influence between appetitive and aversive projection networks across the brain, as the aversive LHb also seems to drive GABAergic cells in the rostromedial tegmental nucleus (RMTg) that in turn inhibit putatively behaviourally activating or appetitive VTA neurons projecting to the NAc (lateral shell region)[97].

Other influences beyond that of mPFC converge onto the LHb; for example, in rats, projection targeting was used to drive the glutamatergic entopeduncular nucleus to LHb projection[99], resulting in conditioned place aversion. The potential for complex regulation is substantial and, given the spatial overlap of the passive or aversive circuitry with behaviourally activating or appetitive influences at nearly every step (notably in the mPFC and VTA), no doubt both the anatomical precision of tract targeting and the type of behavioural challenge delivered will determine which competing pathway experimentally predominates. As an example of anatomical complexity, projections to the VTA can either promote or inhibit reward, depending on location and cell type of fibre origin in the anterodorsal or ventral BNST, respectively[68,100]. And as an example of behavioural history complexity, severe and acute social-defeat stressors seem to favour recruitment of an aversion-related DA cell population in VTA[101], in contrast to chronic mild stressors that favour recruitment of a behaviourally activating or appetitive DA VTA population[75]. Such complexity is expected in the mammalian brain; these experiments have provided only the first insights into the causal impact of defined spike patterns in specified cells and projections on mammalian hedonic behaviour.

Outlook

What is the significance of the observation that projection-specific dynamics operate naturally as control levers that are relevant to psychiatry? Careful interpretation is important here because a tested projection presumably does not subserve only the role implicated (Table 1), nor need all, or even most, of the fibres within the projecting tract contribute to the behavioural effects seen; of course, these considerations apply to any experimental intervention. The sign and direction of the net behavioural change captures only one resultant of the causal influence of the projection tested. Still, as already noted, in many cases a projection-specific dynamical modulation delivers more efficacious elicitation or correction of psychiatry-related adaptive or maladaptive behaviours compared with region-specific dynamical modulation (despite probably recruiting fewer targeted circuit elements). This pattern even extends beyond the psychiatric-disease-related domain; for example, recently, more precisely tuned behavioural changes were obtained through corticostriatal optogenetic projection targeting compared with direct stimulation in a challenging auditory behaviour task[48].

One interpretation is that greater experimental efficacy may be obtained through the directional and intersectional specificity (Box 1) inherent to AAV opsin transduction integrated with spatially separated fibre-optic-based projection targeting, which minimizes the generation of conflicting signals (such as push–pull on the same behaviour, as was observed in the mPFC FST). In addition, projection targeting allows direct control to only be exerted over cells that have long-range projections, without directly perturbing purely locally connected neurons that may be engaged in active circuit computations; although projecting cells can also be involved in computation and representation of information, these roles may be tied closely enough to the projection wiring itself that

delivery or inhibition of experimenter-provided spikes can be meaningful for modulating complex behaviours. But beyond such experimental considerations, the mammalian brain seems to be set up to allow natural behavioural state features to be tuned by projection dynamics (Fig. 2 and Table 1), and many psychiatric symptoms (such as the aversive or negative quality of the anxious state, which is dysregulated to the point of morbidity in anxiety disorders) may be extreme manifestations of these natural features. As already described, multiple-feature natural states (for anxiety, these can include respiratory and behavioural changes) can be managed from a centralized node (for example, the BNST) that assembles these components through outgoing projections[68] (Fig. 2). These projections may set the gain for execution of each feature, so that turning up or down the activity of these defined projections accesses a natural anatomically defined circuit signal for feature (or symptom) intensity.

This causal structural dynamics perspective contributes to our understanding of natural behaviour, but may also have clinical insight implications. Individual features of a behavioural state can be debilitating but not necessarily the overall state itself. For example, it may be appropriate to elevate both the respiratory rate and alertness to danger in a new environment, but only if the additional feature of subjective aversion is strongly recruited does a pathological state resembling clinical anxiety result. The clinical problem can be a feature of the state, not the state itself, and therefore identification of projection-specific dynamics that are relevant to disease has implications for our understanding of aversive, appetitive or maladaptive features that are more closely linked to clinically relevant morbidity than the overall behavioural state itself. Improved brain stimulation treatments[6,102] may eventually result from guiding focal interventions to brain locations where the identified symptom-related projection is most resolved from other projections; here integration of causal information derived from optogenetics may dovetail with patient-specific anatomical information from diffusion magnetic resonance imaging (dMRI)-based tract mapping and modelling[103]. Investigation of the synaptic plasticity rules governing optogenetically defined projections may also help us to elicit stable effects from brief interventions (which could be delivered through functional magnetic resonance imaging (fMRI)-guided trans-cranial magnetic stimulation (TMS)[104,105]). Molecular characterization of identified projections with techniques[61] for high-content identification of expressed transcripts and proteins may facilitate the development of more specific projection-informed and targeted drug therapies. And it will be interesting to explore whether separate efficacious white-matter DBS target locations for a given disorder[106] actually share a common projection component that might be identified by dMRI or CLARITY in human or animal settings[107–109]. Moreover, animal-model identification of causally important projections may guide seed placement for clinical dMRI mapping, even as dMRI maps linked to clinical symptoms in turn feed back to inform experimental target selection in animals and guide dynamical modelling.

In principle, modulation of connectivity properties can exert a highly sensitive and consequential influence (in certain models) over network dynamics and information flow[110–112], and simulated lesioning of locations (nodes or vertices in brain graphs) wired with different statistics (projection or edge properties) can give rise to different effects on network dynamics and connectivity[113]. However, as is well-recognized by graph theorists in neuroscience[114,115], it is not completely clear what biological scale these edges and vertices might most usefully represent, and in the course of generating hypotheses and valuable insights into network complexity and connectivity, current graph-theoretic approaches have considered spatial scales that range from cells to macroscopic subdivisions of the human brain[116]. Linking this field with causal projection-dynamics information arising from optogenetics may help to concretize the nature and scale of graph components and network measures, such as those describing node connectivity[113] (for example betweenness centrality). The physical understanding provided by projection-dynamic studies could also help us to interpret the effects of other circuit phenomena on psychiatric symptoms. For example, local E/I balance shifted by neurochemical influences or medications could alter recurrent feedback within a brain region such as the mPFC or extended amygdala (Box 1), and, in doing so, determine the extent of coordinated recruitment of behavioural state features or symptoms governed by specific outgoing projections[68].

Although broad avenues and opportunities exist for leveraging insights from causal structural dynamics findings, there are also limitations. Certainly, the challenge of projection complexity should be appreciated. Isolated tracts can control multiple behaviours, individual axons within a tract could differentially branch and affect multiple downstream regions in a symptom-relevant fashion, and individual axons could also deliver differentially complex and non-canonical combinations of neurotransmitters[117] that are potentially relevant to the diversity of symptoms seen in single discrete psychiatric diseases. From the perspective of psychiatric disease, it is also important to underscore that knowledge of causal projection-dynamics phenomena will be largely limited to those accessible with optogenetics and therefore will tend to exclude symptom domains that lack widely accepted animal models[74], extending to the delusions, hallucinations and disordered thinking of schizophrenia. These less-accessible symptom domains still probably involve dysfunctional projections or wiring[110,118–120] and are clinically known to cluster with other behavioural phenomena that are accessible in animal models (such as social withdrawal and stereotypies), which may therefore share related underlying circuit-dynamics manifestations; improved multidimensional and high-throughput behavioural quantification in the setting of simultaneous physiological readouts will facilitate detection of such unifying themes.

But even reliable and standard animal behavioural measures are subject to controversy and differences in interpretation. One opportunity in causal structural dynamics research going forward may therefore be to improve disease-model validation and to refine standard animal behavioural measures, partly through integration of projection targeting behavioural research with clinical projection research (such as recent large-scale human connectome efforts using dMRI, involving tractography data linked to symptoms). But in the end, no animal model will fully capture the ontogeny, pathophysiology and symptomatic complexity of human psychiatric disease. Therefore, it is useful to maintain attention not on diseases, but on restricted symptom domains. This approach is ideal for animal modelling, well-suited to the specificity of optogenetic projection targeting and well-aligned with the symptom-treatment approach of real-world clinical psychiatry.

Psychiatric symptom domains, involving dynamical and distributed performance changes without frank regional cell loss, may in this regard share certain principles with developmental and evolutionary changes in neural circuitry. For example, over time certain environments and situations may come to assume a different significance for an organism, and it will be important to adaptably recruit different features of physiology to create a behavioural state more optimized for this shifting significance — without requiring a fundamental redesign of the neural coding of behaviour or representation of the environment itself. This perspective suggests why certain classes of projections may be particularly plastic — or designed for variability — in a way that does not affect the neural code per se that corresponds to a motor plan or representation of sensory data, but rather the magnitude and valence of a behavioural state feature. This flexibility in projection functionality may in turn help to explain the prevalence of psychiatry-related structural dynamics in the mammalian population, representing hotspots for adaptive or maladaptive alteration in symptoms analogous to chromosomal hotspots for mutation relevant to cancer. Among other useful properties, projection dynamics may not only represent concrete physical manifestations of psychiatric symptoms, but may also help to contribute to modularity and flexibility of behavioural states during development and evolution. ■

Received 31 July; accepted 8 November 2013.

1. Akil, H. *et al*. The future of psychiatric research: genomes and neural circuits. *Science* **327,** 1580–1581 (2010).
2. Deisseroth, K. Optogenetics and psychiatry: applications, challenges, and

opportunities. *Biol. Psychiatry* **71,** 1030–1032 (2012).
3. Abbott, A. Novartis to shut brain research facility. *Nature* **480,** 161–162 (2011).
4. Deisseroth, K. Controlling the brain with light. *Sci. Am.* **303,** 48–55 (2010).
5. Maks, C. B., Butson, C. R., Walter, B. L., Vitek, J. L. & McIntyre, C. C. Deep brain stimulation activation volumes and their association with neurophysiological mapping and therapeutic outcomes. *J. Neurol. Neurosurg. Psychiatry* **80,** 659–666 (2009).
6. Holtzheimer, P. E. & Mayberg, H. S. Deep brain stimulation for psychiatric disorders. *Annu. Rev. Neurosci.* **34,** 289–307 (2011).
This is a recent comprehensive review of DBS targets and effects in psychiatry.
7. Oluigbo, C. O., Salma, A. & Rezai, A. R. Deep brain stimulation for neurological disorders. *IEEE Rev. Biomed. Eng.* **5,** 88–99 (2012).
8. Benabid, A. L. & Torres, N. New targets for DBS. *Parkinsonism Relat. Disord*. **18** (Suppl 1), 21–23 (2012).
9. Goodman, W. K. & Alterman, R. L. Deep brain stimulation for intractable psychiatric disorders. *Annu. Rev. Med.* **63,** 511–524 (2012).
10. Bewernick, B. H., Kayser, S., Sturm, V. & Schlaepfer, T. E. Long-term effects of nucleus accumbens deep brain stimulation in treatment-resistant depression: evidence for sustained efficacy. *Neuropsychopharmacology* **37,** 1975–1985 (2012).
11. Chan, D. T. *et al*. Complications of deep brain stimulation: a collective review. *Asian J. Surg.* **32,** 258–263 (2009).
12. Birdno, M. J. & Grill, W. M. Mechanisms of deep brain stimulation in movement disorders as revealed by changes in stimulus frequency. *Neurotherapeutics* **5,** 14–25 (2008).
13. Gradinaru, V., Mogri, M., Thompson, K. R., Henderson, J. M. & Deisseroth, K. Optical deconstruction of parkinsonian neural circuitry. *Science* **324,** 354–359 (2009).
This initial study of optogenetic projection control in modulating behaviour targeted afferents to the subthalamic nucleus that influence parkinsonian symptoms.
14. Andrews-Zwilling, Y. *et al*. Hilar GABAergic interneuron activity controls spatial learning and memory retrieval. *PLoS ONE* **7,** e40555 (2012).
15. Rubenstein, J. L. & Merzenich, M. M. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* **2,** 255–267 (2003).
16. Yizhar, O. *et al*. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **477,** 171–178 (2011).
17. Chao, H. T. *et al*. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* **468,** 263–269 (2010).
18. Akam, T., Oren, I., Mantoan, L., Ferenczi, E. & Kullmann, D. M. Oscillatory dynamics in the hippocampus support dentate gyrus–CA3 coupling. *Nature Neurosci.* **15,** 763–768 (2012).
19. Blumhagen, F. *et al*. Neuronal filtering of multiplexed odour representations. *Nature* **479,** 493–498 (2011).
20. Cardin, J. A. *et al*. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* **459,** 663–667 (2009).
21. Carlén, M. *et al*. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol. Psychiatry* **17,** 537–548 (2012).
22. Figee, M. *et al*. Deep brain stimulation restores frontostriatal network activity in obsessive-compulsive disorder. *Nature Neurosci.* **16,** 386–387 (2013).
23. Rho, Y. A., McIntosh, R. A. & Jirsa, V. K. Synchrony of two brain regions predicts the blood oxygen level dependent activity of a third. *Brain Connect.* **1,** 73–80 (2011).
24. Sohal, V. S., Zhang, F., Yizhar, O. & Deisseroth, K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* **459,** 698–702 (2009).
25. Tiesinga, P. H. & Sejnowski, T. J. Mechanisms for phase shifting in cortical networks and their role in communication through coherence. *Front. Hum. Neurosci.* **4,** 196 (2010).
26. Jirsa, V. K. Connectivity and dynamics of neural information processing. *Neuroinformatics* **2,** 183–204 (2004).
27. Stroh, A. *et al*. Making waves: initiation and propagation of corticothalamic Ca^{2+} waves *in vivo*. *Neuron* **77,** 1136–1150 (2013).
28. Airan, R. D. *et al*. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. *Science* **317,** 819–823 (2007).
29. Gutierrez, G. J., O'Leary, T. & Marder, E. Multiple mechanisms switch an electrically coupled, synaptically inhibited neuron between competing rhythmic oscillators. *Neuron* **77,** 845–858 (2013).
30. Deisseroth, K. Optogenetics. *Nature Methods* **8,** 26–29 (2011).
31. Packer, A. M., Roska, B. & Hausser, M. Targeting neurons and photons for optogenetics. *Nature Neurosci.* **16,** 805–815 (2013).
32. Fenno, L., Yizhar, O. & Deisseroth, K. The development and application of optogenetics. *Annu. Rev. Neurosci.* **34,** 389–412 (2011).
33. Yizhar, O., Fenno, L. E., Davidson, T. J., Mogri, M. & Deisseroth, K. Optogenetics in neural systems. *Neuron* **71,** 9–34 (2011).
34. Gradinaru, V. *et al*. Targeting and readout strategies for fast optical neural control *in vitro* and *in vivo*. *J. Neurosci.* **27,** 14231–14238 (2007).
35. Li, Q. *et al*. Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex. *Neuron* **76,** 1030–1041 (2012).
36. Tye, K. M. *et al*. Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature* **471,** 358–362 (2011).
This was the initial study targeting specific projections in behaviour; gain- or loss-of-function was delivered by optogenetic excitation or inhibition to a specific amygdala projection, with the resulting bidirectional expression of anxiety-related behaviours.
37. Akerboom, J. *et al*. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Front. Mol. Neurosci.* **6,** 2 (2013).
38. Kuhn, B., Ozden, I., Lampi, Y., Hasan, M. T. & Wang, S. S. An amplified promoter system for targeted expression of calcium indicator proteins in the cerebellar cortex. *Front. Neural Circuits* **6,** 49 (2012).
39. Saunders, A., Johnson, C. A. & Sabatini, B. L. Novel recombinant adeno-associated viruses for Cre activated and inactivated transgene expression in neurons. *Front. Neural Circuits* **6,** 47 (2012).
40. Adamantidis, A. R., Zhang, F., Aravanis, A. M., Deisseroth, K. & de Lecea, L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* **450,** 420–424 (2007).
41. Zhang, F. *et al*. Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nature Protocols* **5,** 439–456 (2010).
42. Osakada, F. *et al*. New rabies virus variants for monitoring and manipulating activity and gene expression in defined neural circuits. *Neuron* **71,** 617–631 (2011).
43. Antinone, S. E. & Smith, G. A. Retrograde axon transport of herpes simplex virus and pseudorabies virus: a live-cell comparative analysis. *J. Virol.* **84,** 1504–1512 (2010).
44. Miyamichi, K. *et al*. Cortical representations of olfactory input by trans-synaptic tracing. *Nature* **472,** 191–196 (2011).
45. Wickersham, I. R. et al. Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* **53,** 639–647 (2007).
References 44 and 45 describe rabies-based tools used to define and study afferent projections to cell populations *in vivo*.
46. Lima, S. Q., Hromadka, T., Znamenskiy, P. & Zador, A. M. PINP: a new method of tagging neuronal populations for identification during *in vivo* electrophysiological recording. *PLoS ONE* **4,** e6099 (2009).
47. Cohen, J. Y., Haesler, S., Vong, L., Lowell, B. B. & Uchida, N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature* **482,** 85–88 (2012).
48. Znamenskiy, P. & Zador, A. M. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. *Nature* **497,** 482–485 (2013).
49. Lichtman, J. W. & Denk, W. The big and the small: challenges of imaging the brain's circuits. *Science* **334,** 618–623 (2011).
50. Osten, P. & Margrie, T. W. Mapping brain circuitry with a light microscope. *Nature Methods* **10,** 515–523 (2013).
51. Bock, D.D. *et al*. Network anatomy and *in vivo* physiology of visual cortical neurons. *Nature* **471,** 177–182 (2011).
52. Briggman, K.L., Helmstaedter, M. & Denk, W. Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471,** 183–188 (2011).
53. Micheva, K. D. & Smith, S. J. Array tomography: a new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* **55,** 25–36 (2007).
54. Hama, H. *et al*. Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. *Nature Neurosci.* **14,** 1481–1488 (2011).
55. Dodt, H. U. *et al*. Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. *Nature Methods* **4,** 331–336 (2007).
56. Ertürk, A. *et al*. Three-dimensional imaging of solvent-cleared organs using 3DISCO. *Nature Protocols* **7,** 1983–1995 (2012).
57. Ke, M.-T., Fujimoto, S. & Imai, T. SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nature Neurosci.* **16,** 1154–1161 (2013).
58. Kuwajima, T. *et al*. ClearT: a detergent- and solvent-free clearing method for neuronal and non-neuronal tissue. *Development* **140,** 1364–1368 (2013).
59. Bohland, J. W. *et al*. A proposal for a coordinated effort for the determination of brainwide neuroanatomical connectivity in model organisms at a mesoscopic scale. *PLoS Comput. Biol*. **5,** e1000334 (2009).
60. Chung, K. *et al*. Structural and molecular interrogation of intact biological systems. *Nature* **497,** 332–337 (2013).
This article reports a chemical engineering method for visualizing and labelling projections in the intact brain.
61. Chung, K. & Deisseroth, K. CLARITY for mapping the nervous system. *Nature Methods* **10,** 508–513 (2013).
62. Regehr, W. G. & Tank, D. W. Selective fura-2 loading of presynaptic terminals and nerve cell processes by local perfusion in mammalian brain slice. *J. Neurosci. Methods* **37,** 111–119 (1991).
63. Tye, K. M. & Deisseroth, K. Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nature Rev. Neurosci.* **13,** 251–266 (2012).
64. Ciocchi, S. *et al*. Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* **468,** 277–282 (2010).
65. Haubensak, W. *et al*. Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* **468,** 270–276 (2010).
66. Iwata, J. & LeDoux, J. E. Dissociation of associative and nonassociative concomitants of classical fear conditioning in the freely behaving rat. *Behav. Neurosci.* **102,** 66–76 (1988).
67. Johansen, J. P. *et al*. Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. *Proc. Natl Acad. Sci. USA* **107,** 12692–12697 (2010).
68. Kim, S. Y. *et al*. Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature* **496,** 219–223 (2013).
This study reports the optogenetic decomposition of a behavioural state into component features by projection-targeting-based recruitment of separable anxiety-related features.

69. Fineberg, N. A. *et al*. Probing compulsive and impulsive behaviours, from animal models to endophenotypes: a narrative review. *Neuropsychopharmacology* **35,** 591–604 (2010).
70. Ahmari, S. E. *et al*. Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science* **340,** 1234–1239 (2013).
71. Burguière, E., Monteiro, P., Feng, G. & Graybiel, A. M. Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science* **340,** 1243–1246 (2013).
72. Stopper, C. M., Green, E. B. & Floresco, S. B. Selective involvement by the medial orbitofrontal cortex in biasing risky, but not impulsive, choice. *Cereb. Cortex* **24,** 154–162 (2014).
73. Krishnan, V. & Nestler, E. J. Animal models of depression: molecular perspectives. *Curr. Topics Behav. Neurosci.* **7,** 121–147 (2011).
74. Nestler, E. J. & Hyman, S. E. Animal models of neuropsychiatric disorders. *Nature Neurosci.* **13,** 1161–1169 (2010).
75. Tye, K. M. *et al*. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature* **493,** 537–541 (2013).
76. Warden, M. R. *et al*. A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature* **492,** 428–432 (2012).
This article describes optogenetic projection-targeting-based recruitment of prefrontal pathways favouring active-coping or passive-coping behavioural patterns relevant to depression.
77. Voon, V. *et al*. Dopamine agonists and risk: impulse control disorders in Parkinson's disease. *Brain* **134,** 1438–1446 (2011).
78. Young, J. W., van Enkhuizen, J., Winstanley, C. A. & Geyer, M. A. Increased risk-taking behavior in dopamine transporter knockdown mice: further support for a mouse model of mania. *J. Psychopharmacol.* **25,** 934–943 (2011).
79. Farrell, S. M., Tunbridge, E. M., Braeutigam, S. & Harrison, P. J. COMT Val^{158} Met genotype determines the direction of cognitive effects produced by catechol-*O*-methyltransferase inhibition. *Biol. Psychiatry* **71,** 538–544 (2012).
80. Foti, D. & Hajcak, G. Genetic variation in dopamine moderates neural response during reward anticipation and delivery: evidence from event-related potentials. *Psychophysiology* **49,** 617–626 (2012).
81. Witten, I. B. *et al*. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron* **72,** 721–733 (2011).
82. Bewernick, B. H. *et al*. Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression. *Biol. Psychiatry* **67,** 110–116 (2010).
83. Carter, M. E. & de Lecea, L. Optogenetic investigation of neural circuits *in vivo*. *Trends Mol. Med.* **17,** 197–206 (2011).
84. Carter, M. E. *et al*. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nature Neurosci.* **13,** 1526–1533 (2010).
85. Carter, M. E. *et al*. Mechanism for hypocretin-mediated sleep-to-wake transitions. *Proc. Natl Acad. Sci. USA* **109,** E2635–E2644 (2012).
86. Domingos, A. I. *et al*. Leptin regulates the reward value of nutrient. *Nature Neurosci.* **14,** 1562–1568 (2011).
87. Atasoy, D., Betley, J. N., Su, H. H. & Sternson, S. M. Deconstruction of a neural circuit for hunger. *Nature* **488,** 172–177 (2012).
88. Jennings, J. H., Rizzi, G., Stamatakis, A. M., Ung, R. L. & Stuber, G. D. The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. *Science* **341,** 1517–1521 (2013).
89. Tsai, H. C. *et al.* Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. *Science* **324,** 1080–1084 (2009).
90. Schlaepfer, T. E. *et al.* Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology* **33,** 368–377 (2008).
91. Grubert, C. *et al*. Neuropsychological safety of nucleus accumbens deep brain stimulation for major depression: effects of 12-month stimulation. *World J. Biol. Psychiatry* **12,** 516–527 (2011).
92. Stuber, G. D. *et al.* Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* **475,** 377–380 (2011).
Early projection-targeting study in mammalian appetitive and aversive conditioning, demonstrating bidirectional control of the BLA to nucleus accumbens projection using ChR2 and eNpHR3.0.
93. Britt, J. P. *et al*. Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. *Neuron* **76,** 790–803 (2012).
94. Lobo, M. K. *et al*. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* **330,** 385–390 (2010).
95. Stefanik, M. T. *et al*. Optogenetic inhibition of cocaine seeking in rats. *Addict. Biol.* **18,** 50–53 (2013).
96. Pascoli, V., Turiault, M. & Luscher, C. Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature* **481,** 71–75 (2012).
97. Lammel, S. *et al*. Input-specific control of reward and aversion in the ventral tegmental area. *Nature* **491,** 212–217 (2012).
This paper describes the behavioural control of appetitive and aversive conditioning by targeting distinct incoming projections (from LDT or LHb) into the VTA.
98. Stamatakis, A. M. & Stuber, G. D. Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. *Nature Neurosci.* **15,** 1105–1107 (2012).
99. Shabel, S. J., Proulx, C. D., Trias, A., Murphy, R. T. & Malinow, R. Input to the lateral habenula from the basal ganglia is excitatory, aversive, and suppressed by serotonin. *Neuron* **74,** 475–481 (2012).
100. Jennings, J. H. *et al*. Distinct extended amygdala circuits for divergent motivational states. *Nature* **496,** 224–228 (2013).
101. Chaudhury, D. *et al*. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* **493,** 532–536 (2013).
102. Lozano, A. M. & Lipsman, N. Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron* **77,** 406–424 (2013).
103. Deco, G., Senden, M. & Jirsa, V. How anatomy shapes dynamics: a semi-analytical study of the brain at rest by a simple spin model. *Front. Comput. Neurosci.* **6,** 68 (2012).
104. Li, X. *et al*. Using interleaved transcranial magnetic stimulation/functional magnetic resonance imaging (fMRI) and dynamic causal modeling to understand the discrete circuit specific changes of medications: lamotrigine and valproic acid changes in motor or prefrontal effective connectivity. *Psychiatry Res.* **194,** 141–148 (2011).
105. Chen, A. C. Causal interactions between front-parietal central executive and default-mode networks in humans. *Proc. Natl Acad. Sci. USA* **110,** 19944–19949 (2013).
106. Gutman, D. A., Holtzheimer, P. E., Behrens, T. E., Johansen-Berg, H. & Mayberg, H. S. A tractography analysis of two deep brain stimulation white matter targets for depression. *Biol. Psychiatry* **65,** 276–282 (2009).
107. Toga, A. W., Ambach, K., Quinn, B., Hutchin, M. & Burton, J. S. Postmortem anatomy from cryosectioned whole human brain. *J. Neurosci. Methods* **54,** 239–252 (1994).
108. Rauschning, W. Surface cryoplaning. A technique for clinical anatomical correlations. *Ups. J. Med. Sci.* **91,** 251–255 (1986).
109. Amunts, K. *et al*. BigBrain: an ultrahigh-resolution 3D human brain model. *Science* **340,** 1472–1475 (2013).
110. Cabral, J., Kringelbach, M. L. & Deco, G. Functional graph alterations in schizophrenia: a result from a global anatomic decoupling? *Pharmacopsychiatry* **45** (Suppl 1), 57–64 (2012).
111. Pinotsis, D. A., Hansen, E., Friston, K. J. & Jirsa, V. K. Anatomical connectivity and the resting state activity of large cortical networks. *Neuroimage* **65,** 127–138 (2013).
112. Sporns, O. The non-random brain: efficiency, economy, and complex dynamics. *Front. Comput. Neurosci.* **5,** 5 (2011).
113. Jirsa, V. K., Sporns, O., Breakspear, M., Deco, G. & McIntosh, A. R. Towards the virtual brain: network modeling of the intact and the damaged brain. *Arch. Ital. Biol.* **148,** 189–205 (2010).
114. Sporns, O. The human connectome: a complex network. *Ann. NY Acad. Sci.* **1224,** 109–125 (2011).
115. Bullmore, E. T. & Bassett, D. S. Brain graphs: graphical models of the human brain connectome. *Annu. Rev. Clin. Psychol.* **7,** 113–140 (2011).
116. Fornito, A. & Bullmore, E. T. Connectomic intermediate phenotypes for psychiatric disorders. *Front. Psychiatry* **3,** 32 (2012).
117. Tritsch, N. X., Ding, J. B. & Sabatini, B. L. Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature* **490,** 262–266 (2012).
118. Fitzsimmons, J., Kubicki, M. & Shenton, M. E. Review of functional and anatomical brain connectivity findings in schizophrenia. *Curr. Opin. Psychiatry* **26,** 172–187 (2013).
119. Lynall, M. E. *et al*. Functional connectivity and brain networks in schizophrenia. *J. Neurosci.* **30,** 9477–9487 (2010).
120. Maher, B. J. & LoTurco, J. J. Disrupted-in-schizophrenia (DISC1) functions presynaptically at glutamatergic synapses. *PLoS ONE* **7,** e34053 (2012).
121. Felix-Ortiz, A. C. *et al*. BLA to vHPC inputs modulate anxiety-related behaviors. *Neuron* **79,** 658–664 (2013).
122. Gunaydin, L. *et al*. Real-time optical measurement of projection activity: dynamics of genetically- and anatomically-defined neuronal afferents predict social behavior in freely moving mice. Program No. 872.04. 2013 *Neuroscience Meeting Planner*. (Society for Neuroscience, 2013).
123. Stamatakis, A. M. A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* **20,** 1039–1053 (2013).
124. Gradinaru, V. *et al*. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* **141,** 154–165 (2010).
125. Petreanu, L., Huber, D., Sobczyk, A. & Svoboda, K. Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nature Neurosci.* **10,** 663–668 (2007).
126. Mattis, J. *et al*. Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nature Methods* **9,** 159–172 (2012).
127. Zalocusky, K. & Deisseroth, K. Optogenetics in the behaving rat: integration of diverse new technologies in a vital animal model. *Optogenetics* **2013,** 1–17 (2013).

Acknowledgements I am deeply indebted to my patients over the years for their insight, perseverance and strength in working to convey the most complex and nearly inarticulatable inner thought processes and feelings associated with severe psychiatric disease. I am also grateful to my entire laboratory for support, as well as to V. Sohal, A. Schatzberg, H. Mayberg, R. Malenka, A. Etkin, L. Grosenick, A. Kreitzer, T. Insel, M. Warden, M. Zelikowsky and E. Ferenczi for comments and discussions over the years. K.D. has been supported by the Wiegers Family Fund, NARSAD, NIMH, NIDA, DARPA, the Keck Foundation, the McKnight Foundation, the Yu, Snyder and Woo Foundations, and the Gatsby Charitable Foundation.

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