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Pathological circuit function underlying addiction and anxiety disorders

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Current models of addiction and anxiety stem from the idea that aberrant function and remodeling of neural circuits cause the pathological behaviors. According to this hypothesis, a disease-defining experience (for example, drug reward or stress) would trigger specific forms of synaptic plasticity, which in susceptible subjects would become persistent and lead to the disease. While the notion of synaptic diseases has received much attention, no candidate disorder has been sufficiently investigated to yield new, rational therapies that could be tested in the clinic. Here we review the arguments in favor of abnormal neuronal plasticity underlying addiction and anxiety disorders, with a focus on the functional diversity of neurons that make up the circuits involved. We argue that future research must strive to obtain a comprehensive description of the relevant functional anatomy. This will allow identification of molecular mechanisms that govern the induction and expression of disease-relevant plasticity in identified neurons. To establish causality, one will have to test whether normalization of function can reverse pathological behavior. With these elements in hand, it will be possible to propose blueprints for manipulations to be tested in translational studies. The challenge is daunting, but new techniques, above all optogenetics, may enable decisive advances.

The clinical challenge

Anxiety disorders and addiction are often diagnosed in the same patient[1,2]. Both disorders remain clinically defined entities and escape even the latest generation (imaging) diagnostics because the underlying neuronal dysfunctions do not cause macroscopic changes or cell death.

The frequent comorbidity suggests an overlap in underlying neuronal mechanisms. Current models propose altered circuit function as a mechanism underlying these disorders (and other diseases not considered here, such as depression). Taken together, both conditions have in common a strong behavioral component and the absence of neuronal degeneration as an underlying cause. Note that this does not contradict volumetric imaging studies in addicted humans reporting gray matter alterations in various regions[3]. These changes likely represent structural remodeling downstream of the synaptic mechanisms or associated processes not causally related to the disease (for example, concomitant stroke in cocaine users, or alcohol or amphetamine neurotoxicity). Here we argue that a better understanding of the underlying circuit, cellular and synaptic mechanisms will enable new and efficient therapeutic approaches to anxiety disorders and addiction, which ultimately will greatly benefit patients.

On their own, anxiety disorders comprise a heterogeneous group of conditions, often apparent as a consequence of stress. Together, they are among the most prevalent of neuropsychiatric disorders, with a trend of increasing occurrence[4]. The etiology remains unknown but may include traumatic experiences along with genetic and developmental mechanisms. Moreover, as a consequence of their heterogeneity, the underlying pathophysiological processes are poorly understood and most likely involve a diverse set of mechanisms in various brain areas, ultimately converging on altered circuit function. Existing therapies, such as cognitive behavioral approaches or treatment with benzodiazepines or selective serotonin uptake inhibitors remain unspecific and are often of limited success.

Addiction is defined by compulsive drug use despite the negative consequences and by repeated relapse episodes[5]. While the prevalence of addiction over the last 20 years has remained constant, there has been an increase in the disease-associated cost[4]. Although the diagnostic criteria listed in the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* are useful in the clinic, they have contributed little to facilitating research into the underlying neural mechanisms. Theoretical and experimental approaches, by contrast, conceptualize addiction as a disease of hijacked decision-making[6], where preference is given to a very narrow range of behaviors despite financial, societal and legal cost. Relapse is a frequent and frustrating feature in patient management because it can occur even after prolonged periods of abstinence. It can be triggered by reminders or cues associated with previous drug use, stress or the accidental exposure to the drug[7]. Thus, drugs leave a trace in the brain that outlasts their actual presence and that may be ultimately responsible for the pathological behavior.

Addiction and anxiety disorders can be conceptualized as diseases that start with an unusually strong activation of circuits physiologically mediating emotions of opposing valence. As we will see, these circuits intersect in nuclei such as the amygdala and the ventral tegmental area (VTA), which traditionally have been associated with negative and positive valence, respectively. Reviewing the emerging literature calls this dichotomy into question and, rather, suggests that nuclei are hubs, where fear and reward circuitries intersect.

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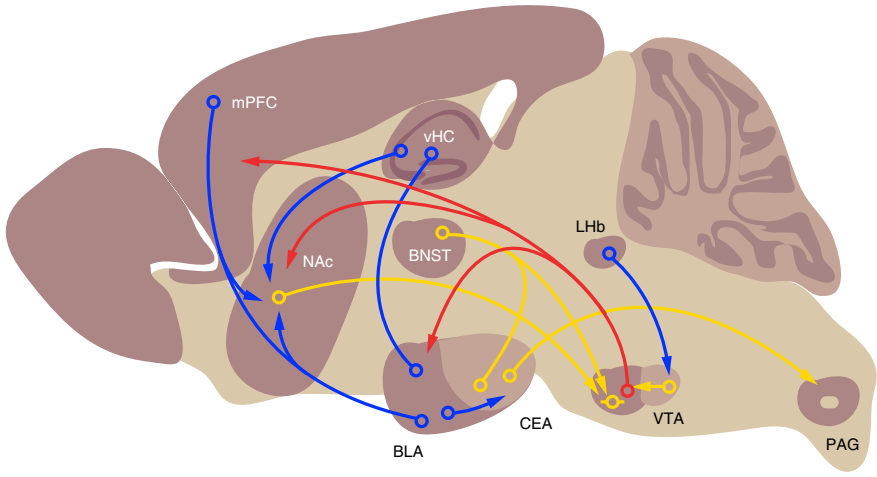

Figure 1 Long-range circuits involved in fear and reward perception. DA projections (red) originate in the VTA and project to the NAc, mPFC and BLA. Their main function is to modulate glutamate (blue) and GABA (gold) transmission. Glutamate projections from mPFC, BLA and ventral hippocampus (vHC) converge onto MSNs of the NAc and from the lateral habenula (LHb) onto the GABA neurons of the tail of the VTA. GABA transmission of MSNs as well as the BNST inhibits cells in the VTA, again preferentially GABA neurons, thus causing disinhibition. Many synaptic connections in this circuitry are subject to synaptic plasticity evoked by disease-defining experience.

The relevant circuits: the amygdalo-centric view

The delineation of neural circuits underlying pathological behavior in both conditions starts with a description of the functional anatomy (**Fig. 1**) and monitoring of neural activity in behaviorally relevant situations. In other words, the physiological circuits encoding emotional valence need to be characterized. Both fear and addiction circuitries have been extensively studied using various approaches, including neurotoxic lesions, pharmacological inactivation, tract tracing and electrophysiological techniques. The last, particularly when applied in the acute slice preparation, has also allowed characterization of synaptic transmission and its plasticity.

In the past, studies aimed at the neuronal basis of brain systems mediating fear and brain systems mediating reward have originated from independent lines of research[8]. Eventually, it became evident that brain areas such as the amygdala, the VTA, the medial prefrontal cortex (mPFC), the ventral hippocampus and the nucleus accumbens (NAc) both process emotions of opposing valence, thus controlling both aversively and appetitively motivated behaviors. This raises the question how output from a single nucleus can give rise to different behavioral outputs. Recent studies addressing the organization of these brain areas at the level of identified local circuits and output pathways have started to shed light on the basic principles underlying the processing of valence-specific and valence-neutral information and the generation of appropriate behaviors. The emerging evidence calls into question the traditional view that distinct valences and behaviors are mediated by dedicated brain areas (one nucleus, one function) but rather suggests a picture of functional neuronal networks in which local circuits dealing with different valences interact to transfer information across different brain areas (one circuit, one function). Therefore, investigations now aim to understand the functional diversity by applying cell type– and projection-specific manipulations.

The amygdala, a complex of subcortical nuclei located in the temporal lobe, has been identified as a key brain structure in many aspects of acquired and innate fear and anxiety-related behaviors[8–10]. The existing literature on anxiety using animal models predominantly refers to fear learning. While connections between fear learning and anxiety disorders remain complicated, the anatomical delineation of the relevant circuits has been very helpful. The basolateral complex of the amygdala (BLA), comprising the lateral (LA), basal (BA) and basomedial (BMA) nuclei, exhibits cortex-like cytoarchitecture, including about 80% glutamatergic spiny principal neurons and a functionally and anatomically heterogeneous population of GABA interneurons making up the remaining 20% (refs. 11,12). The LA is the main recipient of sensory afferents originating in auditory, visual and somatosensory cortex, as well as in the thalamus[13,14]. It is at these glutamatergic sensory input synapses onto LA principal neurons where activity-dependent associative synaptic plasticity is thought to be essential to the acquisition of a classically conditioned fear response. Although many different NMDA receptor (NMDAR)-dependent and NMDAR-independent forms of synaptic plasticity have been described in acute amygdala brain slices[15–19], the link to fear conditioning *in vivo* remain to be established[20,21]. The activity of BLA projection neurons is under the control of inhibitory interneurons. GABA inhibition has not only been shown to gate synaptic plasticity at sensory afferents but can also regulate the acquisition, specificity and extinction of conditioned fear[12,22]. Recently, it was found that conditioning to an auditory cue involves a stimulus-dependent shift in the excitation/inhibition ratio along the somatodendritic axis of BLA projection neurons: that is, distal dendrites become more disinhibited than the perisomatic region[23]. In particular, suppression of dendritic inhibition mediated by somatostatin interneurons appears to be a critical mechanism regulating the acquisition of fear memory in the BLA. In auditory cortex, a similar disinhibitory circuit involving layer 1 interneurons and parvalbumin basket cells in deeper layers can gate the acquisition of conditioned fear responses to complex auditory stimuli[24]. Whereas disinhibition in the BLA and auditory cortex enables the induction of associative plasticity, disinhibition in the hippocampus may block contextual fear conditioning. During the unconditioned aversive stimulus, hippocampal somatostatin interneurons inhibit dendrites of CA1 projection neurons, thus preventing plasticity and maintaining the existing representation of context[25]. In prefrontal cortex, disinhibition via parvalbumin basket cells synchronizes the activity of output neurons, thereby enhancing fear expression[26]. Together, these findings support a fundamental role for local inhibitory and disinhibitory circuits during distinct aspects of conditioned fear behavior. However, given that there are many types of interneuron, defined by their axo-dendritic morphology and their connectivity in the BLA, cortex and hippocampus[27–29], as well as their response to neuromodulatory systems, it will be important to identify their specific contributions to learned and innate defensive behaviors.

In addition to afferents from sensory cortices and thalamus, the BLA, in particular the BA, receives strong input from the ventral hippocampus and from the mPFC[13,30]. The BA is thereby in a position to integrate external and internal sensory, contextual and social cues in order to form, control and regulate the acquisition, expression and extinction of fear and anxiety-related behaviors[31,32]. Different subdivisions of the mPFC have opposite behavioral roles. Whereas activity in the prelimbic cortex contributes to sustained states of fear, activity and plasticity in the infralimbic cortex is required for the formation of long-term extinction memories[33]. Both the prelimbic and infralimbic

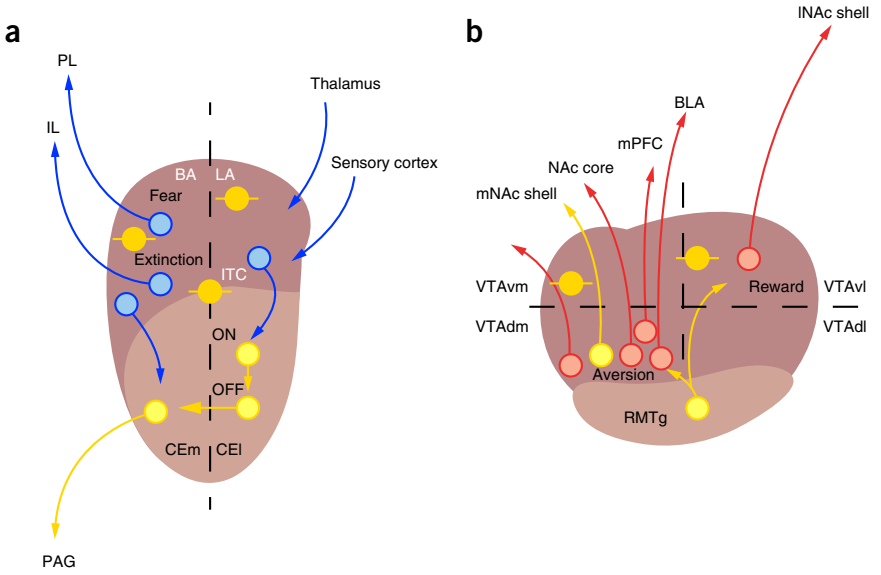

Figure 2 Emerging cell type–specific anatomy of the amygdala and the VTA. (**a**) The LA receives excitatory inputs (blue) from sensory cortex and thalamus. In the BA, glutamate neurons encoding fear or extinction project to the infralimbic (IL) and the prelimbic (PL) part of the mPFC, respectively. Neurons in these structures then connect to the CEA, where in the CEl a local disinhibitory circuit between ON and OFF cells has been described. The final output projects from the CEm to the PAG. Cells of the intercalated nucleus (ITC) are mainly GABAergic (gold). (**b**) DA neurons (red) of the dorsomedial part of the VTA project to medial NAc (mNAc), mPFC and BLA. Note the GABA projection neurons, which in the NAc control the activity of cholinergic interneurons. The DA neurons in the ventrolateral VTA (VTAvl) project to the lateral NAc (lNAc). Neurons in both parts are under the control of GABA interneurons, as well as the GABA neurons of the VTA tail (also called rostromedial tegmentum, RMTg). VTAdl, dorsolateral VTA; VTAdm, dorsomedial VTA; VTAvm, ventromedial VTA.

cortex send projections back to different targets in the amygdala, including the BA, and to distinct clusters of GABA neurons located between the BLA and the central nucleus (CEA). These so-called intercalated cells, possibly together with other inhibitory circuits, in turn directly or indirectly inhibit CEA output neurons[34].

Conversely, the BA also sends strong efferent projections to forebrain targets, including the mPFC and the ventral hippocampus[13,30]. Recent studies indicate that subpopulations of BA projection neurons (**Fig. 2a**) can target specific subdivisions of the CEA, the mPFC, the hippocampus, the bed nucleus of the stria terminalis (BNST) or the entorhinal cortex[35–39]. Such projection pathways can regulate specific behaviors, such as fear acquisition, fear extinction or anxiety-like behaviors. The reciprocal connectivity between the BLA and many of its targets in the forebrain suggests that these networks are organized in loops, at least from a macroscopic perspective. It remains to be investigated how such loops are organized at the level of identified cell types and circuits in order to reach a functional understanding of how the amygdala is embedded in a larger brain network controlling fear behavior.

Eventually, many aspects of conditioned fear responses, including motor, neuroendocrine and autonomic components, are driven by long-range projections from the CEA to targets located at various levels of the brainstem. In contrast to the BLA, the CEA is a striatum-like structure almost exclusively comprising GABA neurons resembling striatal medium spiny neurons (MSNs) forming precisely organized recurrent inhibitory circuits exhibiting much higher levels of spontaneous activity than in the BLA[40]. CEA output neurons, which are found predominantly in the medial subdivision (CEm) but also in the lateral subdivision (CEl), also release GABA, eliciting fear behavior when active[22]. The activity of CEA output neurons not only reflects excitatory input from BLA or other forebrain structures, including thalamus, hippocampus or insular cortex, but is also subject to disinhibitory gating by a population of spontaneously active GABAergic neurons located in CEl, so-called OFF neurons[41]. CEl_{OFF} neurons, which largely overlap with a genetically defined population of CEl neurons expressing protein kinase C (PKC)-δ and oxytocin receptors, are inhibited by the conditioned stimulus through inputs from PKCδ-negative CEl_{ON} neurons[42–44] and possibly from intercalated cells[34].

The effector circuits downstream of CEA output neurons are poorly understood. CEA output neurons, which are strongly regulated by neuropeptidergic inputs, contact several targets in the brainstem[45]. One important target involved in conditioned freezing or flight responses is the periaqueductal gray (PAG).

Like the amygdala, the PAG directly connects to the reward system, not only through ascending pathways conveying information about expected aversive events but also via projections to the mesocorticolimbic system, including the VTA[46]. The PAG might thus represent another important hub where interactions between circuits processing aversive and appetitive information occur, thereby regulating behavior in ambivalent conditions.

The relevant circuits: the mesolimbic view

It would be beyond the scope of the present review to provide a comprehensive list of all the studies that have contributed to delineating the connectivity of the mesocorticolimbic system (for review, see refs. 47,48). The system originates in the VTA (**Fig. 1**), where immunohistochemical quantification shows that about 65% of the neurons are positive for tyrosine hydroxylase, 30% are positive for glutamic acid decarboxylase (Gad) and 5% are positive for vesicular glutamate transporter, identifying them as dopamine (DA), GABA and glutamate neurons, respectively[49] (**Fig. 2b**). Some neurons express both vesicular glutamate transporter and dopamine transporter and co-release glutamate and DA when optogenetically stimulated. The functional significance of this observation, however, remains elusive. DA neurons project to the NAc with a medial-to-lateral mapping onto the medial NAc shell, the core and the lateral shell, as well as to the mPFC with little branching but considerable overlap in the terminal fields. GABA neurons target DA neurons locally (that is, serve as GABA interneurons) but also project to the NAc and mPFC. Many brain regions send excitatory afferents to the VTA, including a majority of brainstem nuclei (for example, laterodorsal tegmentum), the mPFC and the amygdala.

An essential prerequisite for the interpretation of *in vivo* characterization of neuronal activity is the unambiguous identification of the cell type under investigation. This is not trivial, because electrophysiological criteria (for example, spike width, firing frequency and adaptation) may not have the power to reliably classify individual neurons[50]. For example, an *in vivo* study recording neuronal activity in the VTA during a rewarding conditioning task corroborated three types of functional responses superficially reminiscent of the three groups described with

immunohistochemistry above[51]. The authors applied principal component analysis to reveal three unbiased hierarchical clusters, of which neurons of the first type were identified as DA neurons by optogenetic tagging. This is a powerful approach that consists of infecting the VTA with adeno-associated virus with an inverted channelrhodopsin2 (ChR2) construct flanked by double *loxP* sites in dopamine transporter–Cre mice such that recombination only occurs in DA neurons. If the waveforms of light-evoked action potentials match those of spontaneous spikes, a cell is identified as a DA neuron. All DA neurons responded to a cue predicting reward (a conditioned stimulus) or the reward itself with a phasic activation and to reward omission with inhibition, in line with the reward prediction error hypothesis[52]. In contrast, type II neurons displayed a sustained activation starting at the cue presentation and were not modulated by the reward itself. An exhaustive identification of all type II neurons was not achieved, but, conversely, all identified GABA neurons (identified by optogenetic tagging in vesicular GABA transporter–Cre mice) had the functional profile of type II neurons. The identification of type III neurons remains elusive. Interestingly, when the task was modified for aversive conditioning (for example, air puff), the diversity of responses was even larger. The aversive stimulus inhibited the majority of DA neurons, whereas some cells were strongly activated. Aversive stimuli, but not their conditioned predictors, also activated GABA neurons. In conclusion, DA neurons either behave as expected from the reward prediction error hypothesis, or they are strongly activated by aversion without detectable inhibition when an expected punishment is omitted.

This functional heterogeneity among DA neurons is intriguing, as the VTA was previously classified as a nucleus with a single, homogeneous functional output. *In vitro* observations have added credibility to this claim by revealing more subclasses. First, VTA DA neurons clearly differ from nigral DA neurons[53]. But even within the VTA, the amplitudes of the HCN (hyperpolarization-activated, cyclic nucleotide–gated; I_h)-mediated and D2 dopamine receptor (D2R)-evoked GIRK (G protein–coupled, inwardly rectifying potassium channel) currents, as well as the action potential trains elicited by small current injections, show variability[54]. While studies have lacked the specificity to classify individual neurons, substantial population differences have been revealed between rostrolateral (frequencies <10 Hz, large HCN and D2R-evoked currents) and posteromedial (frequencies >10 Hz, small or absent HCN and D2R-evoked currents) VTA neurons. However the most exciting observation in these studies was the segregation in the axonal projection of neurons that was obtained by carrying out recordings in neurons that had been labeled with retrogradely transported beads[55,56]. Rostrolateral DA neurons project to the lateral shell of the NAc, whereas posteromedial neurons separate into two streams (**Fig. 2b**). One bundle projects to the medial shell and core of the NAc, as well as the amygdala, while the other projects to the pre- and infralimbic cortex. It will be important to identify the conditions under which distinct subpopulations of DA neurons are activated and to determine the local and external circuit mechanisms controlling their activity. Combining *in vivo* and *in vitro* approaches has also demonstrated that VTA GABA neurons are heterogenenous[57]. Some function as interneurons: they locally inhibit DA neurons and cause aversion[58,59]. Others project to the NAc[60,61], where they selectively inhibit cholinergic interneurons and may aid in the processing of stimulus saliency[62]. As antidromically propagating action potentials elicited in the NAc terminals of VTA GABA neurons can inhibit VTA DA neurons, it is likely that some GABA neurons also have axonal bifurcations. Forcing cholinergic interneurons to pause in behaving mice enhances discrimination of a motivationally important stimulus that had been associated with an aversive outcome[63].

Observing emotional valence during behavior

Observations of neuronal activity during behavior thus suggest that subpopulations of VTA DA neurons exhibit valence-dependent coding[64]. An appealing hypothesis, albeit with only incomplete experimental support, is that these subpopulations may segregate with their projection. Those reaching the mPFC may be activated by salient but aversive stimuli, while NAc projecting neurons would be prototypical reward neurons. These findings are also in line with results of a recent study[65], which used optogenetic approaches to show that cholecystokinin signaling in the prelimbic PFC mediates anxiety- and depression-like behavioral responses to stress via distinct outputs to the amygdala and NAc, respectively. VTA GABA neurons are most likely activated by saliency independent of valence, causing the inhibition of some DA neurons and silencing cholinergic interneurons in the NAc, which may open a window for enhanced plasticity induction that permits associative learning.

Accumulating evidence demonstrates that amygdala circuits are important in reward conditioning and addictive behavior. In discriminative reward conditioning tasks, activity in the BLA is required not only for the acquisition of conditioned responses but also for reward devaluation[35,66]. Thus, similarly to their action in aversive conditioning settings, in which distinct amygdala output pathways oppositely regulate the extinction of conditioned fear responses[37,67], amygdala circuits appear to mediate opposite changes in conditioned stimulus value in appetitive tasks. *Ex vivo* experiments on slices from animals subjected to reward conditioning revealed synaptic changes at glutamatergic sensory afferents to LA principal neurons, very similar to those described after fear conditioning[35]. Whether this means that synaptic plasticity at the level of LA principal neurons is valence independent or whether there are distinct populations of LA neurons processing aversive and appetitive learning is an open question. Indeed, studies in monkeys and in rodents have identified cells exhibiting valence-specific plasticity in discriminative tasks[68]. The BLA sends a strong glutamatergic projection to the NAc. This projection is necessary for the acquisition of a discriminative appetitive response, but it is also involved in reward devaluation and cue-induced drug seeking behavior[66,69,70]. It remains to be seen whether distinct cellular substrates underlie these very different forms of learning.

In summary, using a combination of behavioral, anatomical and functional approaches, research over the past two decades has delineated the basic neuronal circuitry underlying reinforcement, fear conditioning and extinction. A more mechanistic understanding of the underlying physiological mechanisms has emerged from the dissection of the cellular (micro-) circuitry at the level of defined cell types. These studies have revealed that the acquisition, expression and extinction of conditioned fear behavior is regulated at multiple sites and by multiple mechanisms. Taken together, these studies suggest that the units that define the behavior are the circuits, and not the nuclei, which are merely the hubs where local circuits and long-range projections interact across larger networks. This may lead to a paradigm change in neurology and psychiatry from nucleus-dominated topological thinking to circuit-dominated interpretations of symptoms. In particular, nuclei of the mesolimbic system are sites of intersections of neural circuits with functional specialization; it is therefore not surprising that lesions lead to a wide range of behavioral changes.

Disease-associated synaptic plasticity

Analyzing synaptic transmission *ex vivo* has proven very powerful for the purpose of characterizing adaptive plasticity in response to addictive drugs. In brief, the experiments consist of exposing the animal to an addictive drug (either by injections administered by the experimenter or by self-administration) followed by an incubation

period that can vary from a few hours to several weeks. Once brain slices are prepared, synaptic transmission can be characterized using whole-cell patch-clamp techniques. Many labs have used this approach to characterize changes induced by exposure to addictive drugs[71–75]. (Surprisingly, the plasticity induced at synapses is not affected by the slice preparation, during which tissue is damaged, releasing the cytosolic content of many cells.) In the VTA, for example, measuring the degree of rectification (which entails the appearance of GluA2 subunit–lacking, calcium-permeable AMPA receptors (AMPARs)) and the AMPAR/NMDAR ratio (which entails decreased NMDA function due to the appearance of GluN3 subunit–containing NMDARs[76]) in DA neurons is sufficient to determine with high confidence whether the rodent has received an addictive drug or not[77]. Such drug-evoked synaptic plasticity appears within hours of the first injection of every addictive drug yet tested. Specifically, at excitatory afferents onto DA neurons of the VTA, drug-evoked synaptic plasticity appears after cocaine, morphine, nicotine, amphetamine, ethanol[72], benzodiazepines[78,79] and cannabis[80] exposure and persists days after the drug has been cleared from the brain. In other words, these *ex vivo* experiments have revealed an initial drug trace, which may underlie altered behavior. While these initial observations have sparked much interest, over the last 10 years much progress has been made understanding the molecular mechanisms involved in drug-evoked synaptic plasticity, and, equally importantly, optogenetic approaches have allowed researchers to pinpoint the synapses involved (that is, identify the specific afferents involved and determine the affected outputs). In line with the existence of subclasses discussed above, not all VTA DA neurons express drug-evoked synaptic plasticity. Combining optogenetic projection targeting and retrograde labeling of DA neurons, a seminal study showed that DA cells receiving their inputs from the laterodorsal tegmentum and projecting to the shell of the NAc express drug-evoked synaptic plasticity[55]. As described above, these are the DA neurons thought to encode the reward prediction error, whereas excitatory inputs onto DA neurons activated by aversive stimuli are unaltered following exposure to addictive drugs.

Unraveling the molecular expression mechanisms of drug-evoked synaptic plasticity (rectification and increased AMPAR/NMDAR ratio) has provided arguments for a functional metaplasticity[81]. In this model, changes at excitatory afferents of the VTA are merely permissive for more extended circuit alterations, which may be driven by subsequent doses of the addictive drug because the metaplasticity changes the rules for induction of activity- and experience-dependent plasticity. The dual redistribution of AMPARs and NMDARs determines the activity requirements for synaptic calcium entry[76]. Canonical AMPARs are calcium impermeable and canonical NMDARs only flux calcium when the cell is depolarized, a condition that is met when the pre- and the postsynaptic neurons are active at the same time. After drug exposure, by contrast, GluA2 lacking AMPARs flux calcium preferentially at hyperpolarized potentials and GluN3-containing NMDARs are poorly permeable to calcium ions even when depolarized. The optimal condition to induce activity-dependent plasticity in this condition therefore is the inhibition of the postsynaptic neurons while the presynaptic cell fires. In other words the rules of induction switch from classical Hebbian to 'anti-Hebbian'. Taken together these observations strongly suggest that drug-evoked synaptic plasticity in the VTA constitutes a switch that may enable additional adaptive changes elsewhere; for example, in the NAc. Addictive drugs obviously also directly target the striatum (for example, cocaine directly blocks the reuptake of DA at striatal terminals), but some forms of adaptive synaptic plasticity depend on initial synaptic changes in upstream structures. This was experimentally confirmed by varying the persistence of drug-evoked synaptic plasticity in the VTA while monitoring excitatory afferents onto NAc neurons[82]. After a week of daily cocaine injections, altered synaptic transmission can be detected more than a month later in neurons of the NAc. However, if drug-evoked synaptic plasticity in the VTA is rapidly reversed after each injection, synaptic transmission in the NAc is normal 1 month after withdrawal. Conversely, a single cocaine injection, which normally does not leave a trace that persists for more than 1 month, can lead to altered transmission in the NAc if cocaine-induced adaptation in the VTA is prevented from returning to baseline. With repetitive drug exposure—for example, after several days of self-administration followed by a month of withdrawal—robust synaptic changes are observed in the NAc. Interestingly, cocaine-evoked plasticity is selectively observed in NAc D1 dopamine receptor–expressing (D1R) MSNs[83]. In these neurons, afferents from the mPFC and the ventral hippocampus express contrasting forms of drug-evoked synaptic plasticity[84]. mPFC–to–D1R MSN transmission after withdrawal from cocaine self-administration is dominated by rectification of AMPA transmission[85], whereas the strong input to NAc D1R MSNs from the ventral hippocampus becomes even stronger through the insertion of GluA2-containing AMPARs[86]. In a model of incubation of craving, the glutamatergic input from the BLA to MSNs undergoes synaptic plasticity[87].

In models of anxiety disorders, the locus of cellular and synaptic changes underlying pathological fear and anxiety behavior has not been identified. This may reflect, in part, the facts that defensive behaviors related to fear and anxiety are regulated and controlled by a large, distributed network of interconnected brain areas and that only subsets of neurons and synapses are likely to be involved. Maladaptive plasticity in many of these brain areas could potentially lead to similar behavioral consequences. Several *ex vivo* studies have identified synaptic changes associated with the acquisition or extinction of classical fear conditioning. Such changes involve alterations in synaptic strength at sensory thalamic[19] or cortical afferents[17] to BLA principal neurons, BLA inputs onto intercalated cells[88] or onto defined subtypes of CEA neurons[44]. Moreover, recent evidence from mouse genetic models for psychiatric conditions associated with anxiety phenotypes has identified specific deficits in the function and plasticity of excitatory inputs to the amygdala and in the function of local inhibitory circuits[89–92]. In future studies, it will be important to address the cellular and synaptic specificity of disease-associated functional changes and to investigate whether such changes are cause or consequence in the pathophysiological processes mediating progression of anxiety disorders.

Causal link between synapses and behavior

The various forms of drug-evoked synaptic plasticity demonstrate that drugs cause a functional circuit remodeling, but this does not establish a causal role in the pathological behavior, such as compulsive drug use in addiction. By extension, it is possible that excessive fear may induce similar changes in circuits described above (**Table 1**)—for example, in those DA neurons of the VTA that are activated by aversive stimuli, within the local circuitry of amygdala or other brain areas, or in long-range projection pathways. Whether such synaptic or cellular changes in turn can causally contribute to the pathogenesis of anxiety disorders such as PTSD represents a question of great importance for future studies. Several approaches exist to strengthen such causal links, all aiming at selectively manipulating mechanisms of synaptic plasticity and with the goal of altering maladaptive behavior. A large body of literature, taking advantage of the understanding of molecular mechanisms of synaptic transmission, has used a myriad

Table 1 Comparison of circuit models for addiction and anxiety disorders

	Addiction	Anxiety disorders
Functional anatomy	DA projection to NAc and mPFC and amygdala LHb input, which through a relay in the RMTg inhibits DA neurons Inhibitory networks within VTA NAc is site of convergence	Amygdala circuitry: large-scale network reciprocally connected, including BLA, CEA, mPFC, vHC, NAc, PAG
Neuronal activity during behavior	Punishment, reward and saliency DA neurons Heterogeneous response of GABA neurons that not always mirrors DA neuron activity	Behavior-specific neurons in BLA, CEA, mPFC, etc.: e.g., fear neurons, extinction neurons Appetitive conditioning neurons and more
Manipulation to connect to behavior	Lever pressing for DA stimulation Dynamic CPP/A on DA and GABA neurons	Dominant negative interference with plasticity, genetic ablations of neurons, Arch or ChR2 manipulation of BLA neurons and specific projections to PL, IL, BNST, vHC, etc.
Disease-inducing manipulation	Excessive VTA DA neuron activation (pharmacology or optogenetics), genetic predisposition	Traumatic, uncontrollable stress; genetic models
Ex vivo synaptic trace	Drug-evoked synaptic plasticity: Excitatory transmission Inhibitory transmission	Fear conditioning/extinction induced changes at various synaptic inputs including excitatory and inhibitory circuits
Characterization of disease-related synaptic plasticity	VTA: GluA2-lacking AMPARs, GluN3-containing NMDARs NAc: pathway-specific plasticity	Disease-relevant synaptic or cellular changes remain elusive
Reversal strategies	NMDAR LTD mGluR LTD	Pharmacological and behavioral strategies promoting fear extinction or reversal of conditioning
Causal implication in disease-related behavior	For sensitization For cue-associated relapse	For appetitive conditioning For fear conditioning
Potential for translation	DBS, but where? Which stimulation parameters? mGluR1 pharmacology?	?

Arch, archaerhodopsin; CPP/A, conditioned place preference/aversion; IL, infralimbic cortex; LHb, lateral habenula; mGluR, metabotropic glutamate receptor; PL, prelimbic cortex; RMTg, rostromedial tegmentum; vHC, ventral hippocampus.

of genetically modified mice (and some rats) to test for drug-adaptive behaviors. However these approaches may be limited by confounding developmental adaptations observed in knockout mice, which can only partially be addressed by the advent of conditional or inducible genetic manipulations. Conversely, behavioral pharmacologists have delimited mechanisms involved in behavior, but these fall short of circuit and cell type specificity.

Optogenetic control of identified neurons has emerged as a powerful approach for delineating the circuits that may carry the activity ultimately causing the behavior. Moreover, optogenetic tools can be used to specifically target defined synaptic inputs, thereby allowing studies relating synaptic function and behavior in an unprecedented manner. A proof-of-principle study has linked a cocaine-evoked potentiation of excitatory afferents onto NAc D1R MSNs to behavioral sensitization[83]. This study started with an *ex vivo* characterization of excitatory transmission in the NAc after an injection protocol that leads to an enhanced (sensitized) locomotor response. In parallel, excitatory afferents to D1R MSNs, but not D2R MSNs were potentiated. In the next step, an NMDAR-dependent long-term depression (LTD)-inducing protocol was applied, which led to the normalization of transmission *ex vivo*. When this same protocol was applied *in vivo*, in mice in which ChR2 had been transfected into cortical neurons projecting to the NAc, the optogenetic depotentiation erased locomotor sensitization. More recently, another study used a similar approach to establish a causal link between another form of drug-evoked plasticity in the NAc and incubation of craving, a model of relapse[87]. This study implicates in particular the afferents from the BLA. Other components of the relapse behavior seem to be mediated by distinct forms of drug-evoked plasticity. For example, in cue-associated cocaine seeking, action-outcome encoding (that is, correct discrimination between the cocaine associated lever and a second, inactive lever) is mediated by plasticity at mPFC-to-NAc D1R MSNs (see also above), whereas the vigor of the seeking behavior relates to drug-evoked plasticity at the input from the ventral hippocampus to this same population of NAc neurons[93]. A caveat, however, seems in order. Optogenetic stimulation synchronously activates ensembles of neurons, which may drive behavior in a quite artificial fashion. Establishing strong causalities will thus require not only gain-of-function experiments with

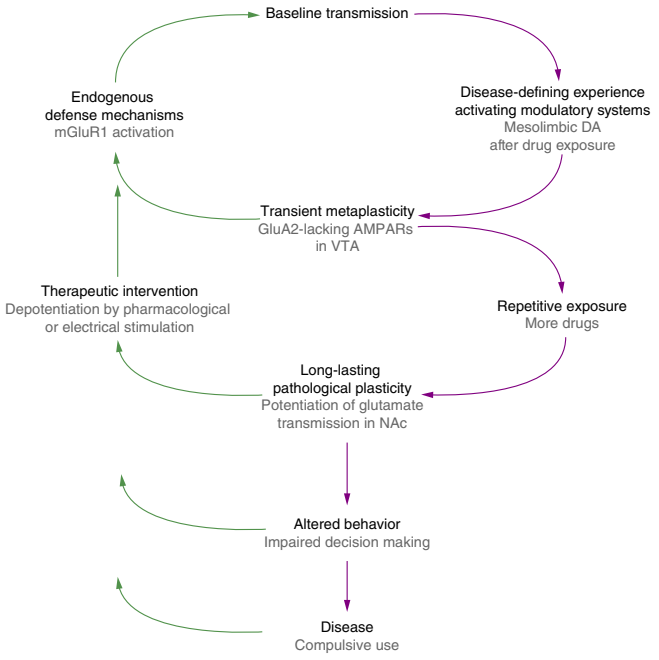

Figure 3 Schematics of proposed model. For explanation, see text. Specific examples for addiction are in gray. Violet arrows indicate processes that induce pathology, green those that restore normal function.

Table 2 Comparison of optogenetics and DBS

	Optogenetics	DBS
Indications	None	FDA approved: PD, essential tremor, dystonia, OCD[a] Experimental: depression, addiction, anxiety disorders
Stimulation protocols	With blue light, up to 20 Hz with ChR2, up to 100 Hz with ChETA	Typically 130 Hz, 60 μs; lower frequency possible
Technical challenge	Placement of fiber optics Viral transfection requiring Cre-*loxP* system Expense of surgical approach	Expense of surgical approach
Mechanism	Gating of ion channel expressed in selected neurons	Unclear; combined effect on neurons in target region (depolarization block) and passing axons. Cell type specificity not possible.
Persistence of effect	Either direct control of neuronal activity or protocols to induce or reverse plasticity; can last days to weeks.	Effects only observed during stimulation
Limitations for human application	Safety of viral vectors Promoter-based cell type specificity Persistence of effector expression Device implantation	Side effects due to nonspecific stimulation Lack of established targets for addiction and anxiety disorders

FDA, US Food and Drug Administration; OCD, obsessive-compulsive disorder; PD, Parkinson's disease.
[a]Severe OCD received a FDA humanitarian device exception in 2009.

stimulation protocols mimicking physiological activity, but also specific and temporally controlled inhibition.

Causal relationships between neuronal activity and defensive, fear-related behavior have mostly been addressed at the level of defined cell types or projection pathways. For example, optogenetic inhibition of LA principal neurons during fear conditioning impairs learning[23], whereas optogenetic stimulation of principal neurons can, at least in part, substitute for the unconditioned stimulus[94]. However, optogenetic stimulation, while inducing LTP at auditory inputs to the LA, cannot substitute for the unconditioned stimulus[95]. This suggests that conditioned stimulus–shock pairing produces additional synaptic modifications that are also required for fear conditioning. One such modification is the synaptic plasticity in the CEA[44]. Consistent with a role for activity-dependent synaptic plasticity in the LA in fear conditioning, dominant negative constructs that prevent induction of synaptic plasticity onto LA principal neurons also impair learning[19], and genetic ablation of LA neurons that were active during learning abolishes previously learned fear responses[96]. With regard to fear extinction, for example, it was recently demonstrated that altering the balance of activity between projections from the BA to the prelimbic or to the infralimbic cortex determines the robustness of extinction memories[37]. Using optogenetic means to specifically enhance or attenuate these pathways revealed that increased activity of the pathway from BA to infralimbic cortex promotes the formation of long-term extinction memories whereas enhancing activity of the pathway from BA to prelimbic cortex results in spontaneous recovery of conditioned fear responses. Likewise, recent studies found that optogenetic inhibition of BLA projections to the entorhinal cortex interferes with contextual fear conditioning[39] and that manipulations of long-range projections from the BLA to the hippocampus or to the BNST have anxiogenic or anxiolytic effects, respectively[36,38]. The BNST, in turn, not only connects to the PAG but also sends glutamatergic and GABAergic projections to the VTA, to the hypothalamus and to the parabrachial nucleus. Optogenetic modulation of distinct BNST efferent pathways has been found to have opposite and specific effects on anxiety behavior[97]. In particular, bidirectional modulation of anxiety behavior by optogenetic manipulation of glutamatergic versus GABAergic projections from BNST to VTA may indicate that activity of the mesocorticolimbic system is an important component of what constitutes a complex state of anxiety. In addition to projections of the amygdala or extended amygdala, a genetically defined projection from the lateral septum to the hypothalamus has recently been identified as regulating stress-induced persistent anxiety[98]. It remains an open question, however, whether pathological fear and anxiety are associated with altered activity in these pathways and, if this is the case, which synaptic and cellular mechanisms underlie dysregulated circuit activity.

Implications for emerging therapies

Because drug-evoked or stress-induced synaptic plasticity is in principle reversible, a model that builds on plasticity mechanisms driving circuit alterations and eventually pathological behavior may offer new translational perspectives (**Fig. 3**). The idea is straightforward: to design protocols that would restore baseline transmission and normalize behavior[99]. Many studies have now provided proof of principle that careful examination of the synaptic parameters can guide the design of a protocol that, when applied *in vivo*, can restore normal transmission. For example, if addictive drugs potentiate a synapse, depotentiation may be achieved by applying the appropriate depression protocol. This may be achieved with pharmacological manipulations that enable endogenous activity to efficiently reverse drug-evoked alterations. Behavioral models, proven efficacious in anxiety disorders, possibly combined with pharmacological treatments supporting or reversing specific forms of synaptic plasticity, may erase fear memories or attenuate drug craving. A particularly promising approach, suggested by optogenetic proof-of-principle studies, will be to apply stimulation protocols that would reverse drug-evoked or stress-induced synaptic plasticity in humans (**Table 2**). The challenge is to develop techniques and devices that would allow efficient and safe interventions in humans. Available methods approved for other indications include deep brain stimulation (DBS) and transcranial magnetic stimulation.

Conclusions

Above all, the disease models discussed here have advanced our mechanistic understanding of addiction and anxiety disorders. The demonstration that one can be sick because defined cell types or synapses change their properties may also apply to other diseases, such as depression, schizophrenia or autism spectrum disorders. It may also help to advance the understanding of diseases traditionally regarded as purely neurodegenerative, such as Alzheimer dementia, where the lack of correlation between neuronal death and clinical severity has sparked interest in alternative models.

The concept of synaptic disease also has implications for therapeutic strategies. The neural circuitry implicated in anxiety disorders has been very comprehensively described whereas adaptive plasticity at identified synapses has been more thoroughly examined in addiction. There is no doubt, then, that interactions between fields will enhance progress, which may have implications for the ability to treat addiction and anxiety disorders, and perhaps others.

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