Trends in Neurosciences

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Review

Circuit-Based Biomarkers for Mood and Anxiety Disorders

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Mood and anxiety disorders are complex heterogeneous syndromes that manifest in dysfunctions across multiple brain regions, cell types, and circuits. Biomarkers using brain-wide activity patterns in humans have proven useful in distinguishing between disorder subtypes and identifying effective treatments. In order to improve biomarker identification, it is crucial to understand the basic circuitry underpinning brain-wide activity patterns. Leveraging a large repertoire of techniques, animal studies have examined roles of specific cell types and circuits in driving maladaptive behavior. Recent advances in multiregion recording techniques, data-driven analysis approaches, and machine-learning-based behavioral analysis tools can further push the boundary of animal studies and bridge the gap with human studies, to assess how brain-wide activity patterns encode and drive emotional behavior. Together, these efforts will allow identifying more precise biomarkers to enhance diagnosis and treatment.

Highlights

Mood and anxiety disorders are complex neural-circuit-based conditions that arise from dysfunctions across multiple cell types, brain regions, and circuits.

Recent efforts in identifying circuit-based biomarkers using brain-wide activity patterns have shown promising results in stratifying disorder subtypes and identifying effective treatments for patients with mood and anxiety disorders.

Advances in multiregion recording techniques, unbiased data-driven analysis approaches, and machine-learning-based behavioral analysis tools now enable animal studies to effectively reverse translate biomarker-based approaches in human studies to understand how brain-wide activity patterns are altered in disease.

The combination of these tools with circuit-based manipulations can help researchers investigate the roles of specific cell types, regions, and circuits in emotional information processing, and how neural circuit dysfunctions manifest in maladaptive behavior.

Distributed Neural Circuits Underly Mood and Anxiety Disorders

Mood and anxiety disorders disrupt basic functions of individuals' lives, and are among the leading causes of disability [1]. In the USA, it is estimated that at a given timepoint, 10–20% of adults are impacted, and 20–30% of adults will experience a mood or anxiety disorder at some point in their lives (https://www.hcp.med.harvard.edu/ncs). However, most existing treatments are not effective [2,3]; largely due to a lack of clear understanding of disease etiology. The search for effective treatment is further complicated by the fact that mood and anxiety disorders are heterogeneous syndromes with various subtypes, where patients present diverse symptoms even for the same disorder, and often respond differently to the same treatment [4–7].

The Research Domain Criteria (RDoC) (Box 1), a research framework for mental disorders, recognizes the complexity in applying a symptom-based categorical approach in classifying disorders. Instead, the RDoC takes a dimensional approach that highlights the importance of investigating underlying neural circuits that may help differentiate and align with domains of function (e.g., fear and anxiety) [8]. The strategy acknowledges that mood and anxiety disorders are complex circuit-based conditions, resulting from dysfunction in distributed brain regions, neural connections, and cell types [9–11], and a neural-circuit-based approach may facilitate identification of novel treatment targets and detection of heterogeneity in patient population and treatment responses.

In recent years, considerable effort has been made to identify circuit-based biomarkers: biological signatures such as brain-wide neural activity patterns that reflect normal or pathological processes, or response to treatments [12,13]. Identification of biomarkers can facilitate diagnosis, categorize disease subtypes, and identify personalized effective treatment options for patients [14–16]. Based on their specific clinical applications, biomarkers can be classified into different categories (Box 2). An effective biomarker should be clinically relevant and detectable, dynamically reflect disease progression, and have high reproducibility and signal-to-noise ratio [17].

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Box 1. RDoC

RDoC is a research framework for mental disorders created by the National Institute of Mental Health that highlights the importance of neural circuit investigations in understanding different domains of function. It focuses on six domains of human functioning, each composed of behavioral elements (constructs) that are studied from typical to atypical functioning with different units of analysis, ranging from genes, molecules, cells, circuits, physiology, behavior, and self-report. Two domains of function focus on valence, which is the degree to which something is aversive (negative valence) or appetitive (positive valence) [124–127]. Instead of symptom-based categories, RDoC aims to guide research using biological, physiological, and behavioral measures and knowledge.

Domains of Function and Example Constructs

Negative valence systems: for example, acute threat (fear), potential threat (anxiety)

Positive valence systems: for example, reward responsiveness, reward learning

Cognitive systems: for example, attention, perception

Systems for social processes: for example, social communication

Arousal/regulatory systems: for example, arousal, circadian rhythms

Sensorimotor systems: for example, motor actions

One of the main challenges in identifying effective circuit-based biomarkers as diagnostic and treatment tools is an incomplete understanding of basic circuit abnormalities that drive distinct maladaptive behavioral states. Animal studies have proven crucial in these efforts, as they leverage a large repertoire of techniques to record and control genetically and anatomically defined cell types in real time. A reverse translational approach can inform these animal studies, using network-level information from human studies to guide rodent circuit-level investigation, allowing for refinement and optimization of strategies for treatment in humans.

Here, we highlight recent advances in network- and circuit-based investigations of mood- and anxiety-related behavior in rodents and humans. We then propose avenues to more closely link findings in rodents with human studies aimed at identifying circuit-based biomarkers for diagnostics, stratification, and ultimately, more rational designs for therapeutics.

Mood and Anxiety Circuits in Rodents: Cell Types, Projections, and Networks

Interacting Pathways and Circuits

With the help of a wide range of behavioral assays (Box 3), animal studies have identified many brain regions, cell types, and circuits that are important in mediating various aspects of mood- and anxiety-related behavior. Distinct components of a region (cell types and inputs/outputs) often encode different features of explored environments to generate appropriate behavioral outputs. Here, we highlight a few recent studies that have dissected circuits in a subset of candidate areas; specifically, the ventral hippocampus (vHPC), medial prefrontal cortex (mPFC),

Box 2. Classification of Biomarkers

Biomarkers can be classified into different categories based on their clinical applications [12]. These categories include:

- Diagnostic: biomarkers used to diagnose a disease
- Monitoring: biomarkers used to assess the status of a disease
- Pharmacodynamic/response: biomarkers that reflect changes in response due to a pharmacological agent
- Predictive: biomarkers predictive of treatment response
- Prognostic: biomarkers used to identify disease progression or recurrence
- Safety: biomarkers used to monitor adverse events
- Susceptibility/risk biomarkers: biomarkers used to identify individuals susceptible to a disease

Box 3. Animal Behavioral Assays in Assessing Mood and Anxiety Disorders

Animal studies need to rely on behavior as readout of animals' internal emotional state. Over the decades, many behavioral assays have been developed to assess mood- and anxiety-related behavior.

Depression-like behavior in animals includes social avoidance, anhedonia, **passive coping**, and learned helplessness, which parallel related behaviors in human patients [23,25,128]. Paradigms such as CSDS, sucrose preference test, tail suspension test, and inescapable shock procedure are designed to assess these symptoms. Resilience and susceptibility following CSDS are commonly defined by the degree of social interaction exhibited by the defeated mice in the social interaction test. Resilient mice exhibit greater degree of social interaction than susceptible mice.

Anxiety-related behavior is often studied using conflict-based approach and avoidance tasks, where animals' behavioral responses to anxiogenic stimuli are analyzed [22,24]. Paradigms including EPM, OFT, light–dark box, and novelty-suppressed feeding are based on the observation that rodents tend to avoid anxiogenic stimuli such as open bright spaces and novel food. Enhanced anxiety levels (anxiogenic phenotype) typically manifest in reduced open arm or area exploration in mazes. The degree of open area exploration can be modulated by drugs that alter anxiety levels in humans. Excessive grooming in rodents is another behavior that signals heightened anxiety.

Many of these tests are normally done in freely behaving animals but can be adapted to head-fixed experimental setups that are often needed to incorporate large population neural recording techniques such as two-photon imaging. One novel paradigm that is designed specifically to assess approach and avoidance behavior in a head-fixed animal is the virtual burrow assay [129].

Animals also show many physiological changes in these behavioral tasks, such as increase in levels of corticosterone [130], impaired immune functions [131], and metabolic and sleep disturbances [132,133]. More recently, facial responses in mice were also found to reflect internal emotional states [57].

Glossary

Anhedonia: reduced ability to feel pleasure. In rodents, sucrose preference test is commonly used to assess anhedonia, although it has limited clinical relevance in humans. Instead, subjective self-report questionnaires are more commonly used in humans.
Chronic social defeat stress: a commonly used rodent model of depression, where mice are subjected to daily defeat by aggressive mice (usually of a different strain, such as CD1). Defeated mice exhibit varying degrees of social avoidance, which is used to classify mice as resilient or susceptible to stress.
Functional magnetic resonance imaging: a noninvasive technique to measure brain activity by detecting changes associated with blood flow.
Intracranial electroencephalography: an electrophysiological monitoring technique that involves placing electrodes directly on the surface of the brain.
Local field potential: transient extracellular electrical signals from large number of surrounding neurons, used as a measure of brain activity.
Optogenetics: a technique that uses light to control neurons that have been genetically modified to expressive light-sensitive ion channels, allowing temporally and spatially precise circuit manipulations.
Passive coping: a type of helplessness behavior, such as immobility, commonly observed in rodents when forced to swim in a space with no escape.
Repetitive transcranial magnetic stimulation: a noninvasive brain stimulation technique that involves placing an electromagnetic coil over the scalp.
Resting-state functional connectivity: brain activity patterns at resting state in the absence of explicit tasks, usually assessed using fMRI.

orbitofrontal cortex, insular cortex (IC), amygdala, bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc), and ventral tegmental area (VTA). We focus on studies that have taken advantage of circuit-based interrogation techniques to understand how emotionally salient information is encoded (Figure 1). This is not an exhaustive analysis, as many other regions have been implicated in emotional regulation [18–21], and this topic have been covered in a number of excellent recent reviews [22–28]. In addition, it is likely that brain-wide recording methods may reveal other regions and circuits that play important roles in emotional informational processing.

vHPC

Recent studies in rodents have identified cell types and projections of the vHPC that modulate anxiety-related behavior. **Optogenetic** (see Glossary) modulation of vHPC and its inputs/outputs acutely impacts exploration of anxiogenic portions of the elevated plus maze (EPM) and/or open field test (OFT); classical tests of approach/avoidance conflict (Box 3).

vHPC neurons encode anxiety-related information, increasing their activity whenever mice explored open areas of mazes [29]. The increase is correlated with individual levels of avoidance, and cells that responded to anxiogenic environments do so reliably across tasks. In line with this, acute inhibition of vHPC reduces exploration in the EPM and OFT [29–31].

Similarly, inputs to the vHPC from the amygdala [32] and outputs from the vHPC to the mPFC and lateral hypothalamic area (LHA) are anxiogenic. vHPC–mPFC projection neurons in rats increase firing rates in the open arms of the EPM [33], and activation of this projection promotes anxiety [30] in a frequency-dependent fashion [34], consistent with enhanced vHPC–mPFC theta synchrony in anxiogenic environments [35]. Inhibition of vHPC–LHA projections in mice increases open arm exploration in the EPM, while modulation of vHPC–amygdala projections has no effect on anxiety, but modulates learned fear [29]. In contrast, outputs from the vHPC to lateral septum [30] and BNST [36] are anxiolytic in the EPM. Activation of the vHPC–BNST pathway may serve to interface the vHPC with the hypothalamic–pituitary–adrenal axis to decrease the release of stress hormones [37,38].

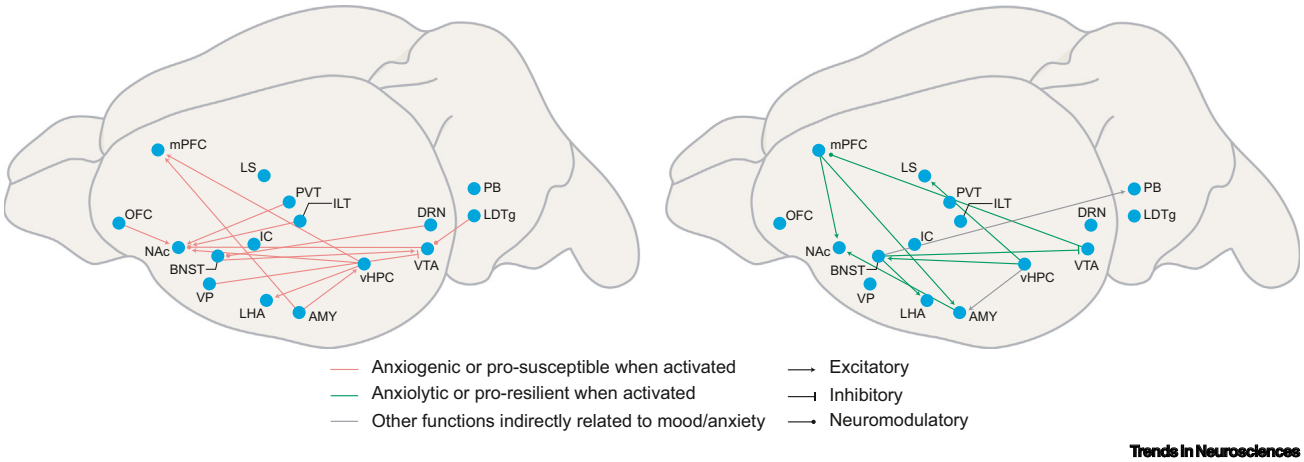

Figure 1. Simplified Schematic of Mood and Anxiety Networks from Recent Rodent Studies. Mood- and anxiety-related behavior and emotional states are mediated by local and long-range interactions across regions including medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), insular cortex (IC), ventral hippocampus (vHPC), amygdala (AMY), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), ventral pallidum (VP), lateral septum (LS), lateral hypothalamic area (LHA), paraventricular thalamic nucleus (PVT), intralaminar thalamus (ILT), dorsal raphe nucleus (DRN), ventral tegmental area (VTA), parabrachial nucleus (PB), and laterodorsal tegmentum (LDTg).

In mood-related behavior, mice susceptible to **chronic social defeat stress** (CSDS) show greater activity in the vHPC, in comparison to resilient mice [39]. Susceptibility is regulated by vHPC–NAc projections, as attenuation of this pathway increases resilience. Similarly, stress-induced **anhedonia** is associated with enhanced strength at the vHPC–NAc synapse, and depotentiation of this pathway reverses anhedonia [40]. However, the opposite effect has also been observed where optogenetic stimulation of vHPC–NAc projection induces conditioned place preference in mice, and chronic multimodal stress impairs LTP at the vHPC–NAc synapse [41]. The discrepancies may be partly due to different anatomical targeting and optogenetic stimulation protocols. Furthermore, adult neurogenesis in the vHPC is necessary for antidepressant treatment response in mice, with resilience to stress mediated via local circuit interactions and inhibition of dentate gyrus output [42–45].

Prefrontal, Orbitofrontal, and Insular Cortices

The mPFC and its inputs/outputs are known to be involved in anxiety- and mood-related behavior. In a study in mice, mPFC–vHPC theta synchrony was enhanced during high anxiety state [35], while mPFC–amygdala theta synchrony was enhanced when animals transitioned from dangerous to safe zones in OFT [46]. In the mPFC, vasoactive-intestinal-polypeptide-expressing interneurons are responsible for gating vHPC input, and inhibition increases open arm exploration in the EPM [47].

In mood-related behavior in mice, activation of the mPFC [48] and local parvalbumin-positive interneurons [49] are proresilient in CSDS and learned helplessness. Furthermore, PFC–amygdala coherence was negatively correlated with social interaction following CSDS, while enhanced PFC activation during exposure to an aggressor mouse predicted greater future susceptibility to CSDS [50]. Structurally, CSDS reduces mPFC myelination in mice [51], which is implicated in fear memory processing [52,53].

In the IC of mice, optogenetic inhibition of the posterior IC is anxiogenic [54], while inhibition of the anterior IC is anxiolytic in the EPM [55,56]. Posterior IC neurons also encode distinct emotional states, such as disgust and pleasure, that correlate with specific facial expressions [57]. In the orbitofrontal cortex, chronic inactivation of the region in rats reduces exploration in the OFT

[58], and excessive grooming, an obsessive–compulsive-disorder-like behavior in mice, can be elicited by repeated optogenetic activation of orbitofrontal–ventromedial striatal projections [59].

Amygdala and Extended Amygdala

The amygdala is composed of distinct cell types, projections, and subregions that are functionally heterogeneous. Its role in learned fear is well recognized and has been reviewed extensively [24,27]. In the basolateral amygdala (BLA) of mice, somata activation is anxiogenic in the EPM, while targeted activation of BLA projections to the central nucleus of the amygdala is anxiolytic [60]. The mPFC exerts top-down control over BLA activity and mPFC–BLA coherence is enhanced when mice transition from dangerous to safe environments in the OFT [46]. BLA projections back to the mPFC [61] and vHPC [32] are anxiogenic in the EPM, while BLA projections to the NAc are proresilient following CSDS [39]. Basomedial amygdalar neurons receive top-down control from the ventral mPFC that is anxiolytic [134]. In the basal amygdala, it has been reported that neurons do not encode global state of anxiety, but rather, two distinct populations of neurons show orthogonal patterns of activity during moment-to-moment changes in exploratory and nonexploratory defensive behaviors [63].

In the BNST, inhibition of oval BNST activity is anxiolytic, while the same manipulation in the anterodorsal BNST is anxiogenic in the EPM [62]. Moreover, three distinct subpopulations of neurons within the anterodorsal BNST project to the LHA, parabrachial nucleus, and VTA, and are responsible for modulating risk avoidance, respiratory rate, and positive valence conditioning, respectively. Furthermore, in a study in mice, a subpopulation of corticotropin-releasing factor neurons in the BNST were found to receive direct projections from serotonin-releasing cells in the dorsal raphe nucleus (DRN). These DRN–BNST projection neurons increase anxiety in the EPM by inhibiting anxiolytic outputs from the BNST to VTA and LHA [64]. BNST outputs to the VTA are also heterogeneous, and composed of anxiogenic glutamatergic neurons and anxiolytic GABAergic neurons in the EPM [65].

Mesolimbic Pathways

VTA dopamine neurons and their inputs/outputs also help control mood-related behaviors. Inhibition of VTA–NAc neurons induces resilience in mice following CSDS, while the opposite is observed following inhibition of VTA–mPFC neurons [66]. At the cellular level, susceptible mice show enhanced firing in VTA–NAc neurons accompanied by upregulation of hyperpolarization-activated current (I_h), while reduced firing rate is observed in VTA–mPFC neurons with no change in I_h current [66,67]. The VTA receives inputs from cholinergic neurons in the laterodorsal tegmentum that shows hyperactivity following CSDS in mice, and inhibition of this projection reduces social avoidance [68]. The VTA also receives input from ventral pallidum parvalbumin-positive interneurons, and inhibition of these neurons restores social interaction in susceptible mice [69].

In NAc, activation of medial spiny neurons (MSNs) enriched in dopamine receptor D1 (D1-MSNs) enhances social interaction in mice, while the opposite is observed following activation of D2-MSNs [70]. Similarly, increased baseline activity in D1-, but not D2-MSNs prior to defeat is predictive of resilience in mice [71]. Different inputs to the NAc also play distinct roles. Inputs from the vHPC [39] and intralaminar thalamus [72] are prosusceptible, while mPFC and amygdala inputs are proresilient [39]. Furthermore, activation of inputs from the paraventricular thalamic nucleus evokes aversion in real-time place preference tasks [73].

Global Anatomical and Functional Changes

These and many other studies highlight the idea that emotionally relevant information is encoded in a distributed network of cell types, circuits, and regions, and models for the study of mood- and

anxiety-related disorders show changes in distinct components of these networks. Recent studies have explicitly explored neuroanatomical and functional changes across multiple regions in rodents, similar to approaches often taken in human studies. For example, in mice, social avoidance following CSDS is negatively correlated with volumes of cingulate cortex and BNST, but positively correlated with volumes of the VTA and hippocampus CA3, among other regions [74]. Furthermore, interactions dominated by delta and beta oscillations from NAc to vHPC and VTA can predict future susceptibility to CSDS [16], indicating the utility of spatiotemporal dynamics as biomarkers to identify individuals vulnerable to depression. In addition to neural biomarkers, behavioral and immunological biomarkers can also help predict susceptibility to stress [75].

Whole-brain imaging techniques routinely applied in human studies, such as **functional magnetic resonance imaging** (fMRI), are also powerful tools in revealing brain-wide activity patterns in animals. For example, functional connectivity between the amygdala, HPC, and PFC is highly correlated with anxiety-like behavior in the OFT and EPM in mice [76]. Furthermore, acute stress blunts functional connectivity between the DRN and its outputs, and effects are reversed by antidepressant treatment, providing insights into the dynamics of the serotonin system [77]. Functional imaging also reveals that following CSDS, defeated mice show widespread activation across many regions including the PFC, BNST, vHPC, and NAc [78], which resonates with findings from studies using more focal recording techniques.

Human Studies: Identifying Activity-Based Biomarkers

While there are many studies aimed at understanding the network-level changes in mood/anxiety disorder in humans, we focus here on a few notable recent findings that have identified network biomarkers that fluctuate with mood/anxiety level, depression symptoms, or treatment response. These studies using multiregion recording tools identified regions and circuit-level biomarkers that can be further dissected using rodent models to determine relevant cell types and neural connections.

A recent study found that changes in HPC–amygdala interactions can predict subjects' worsening mood [15]. Specifically, analysis of multi-region **intracranial electroencephalography** (iEEG) has revealed that increased variance in HPC–amygdala coherence at the beta frequency range can predict worsening in mood in >60% of patients, more predictive than either region alone. These electrophysiological biomarkers are useful for not only capturing moment-by-moment mood fluctuations, but also revealing disease state and severity. For example, patients with post-traumatic stress disorder (PTSD) showed increased synchrony between frontal, temporal, and hippocampal regions at high gamma frequency band, relative to healthy controls, and high gamma in left hippocampus correlated strongly with PTSD symptom severity [79].

In addition to identifying temporal fluctuations and severity in symptoms, activity-based biomarkers can be particularly useful in stratifying disease subtypes and evaluating treatment effectiveness. In one recent study, fMRI was performed in depression patients to assess their **resting-state functional connectivity** patterns and identify biomarkers that could categorize disease subtypes [14]. Abnormal connectivity patterns in the limbic and frontostriatal networks correlate with different symptoms that categorize patients into subtypes. Such individual connectivity differences predicted treatment responsiveness to **repetitive transcranial magnetic stimulation** (rTMS) with 78% accuracy, which is greater than clinical symptoms alone. Similarly, prefrontal resting-state activity at the alpha frequency band predicts responsiveness to antidepressant treatment [80]. Low to moderate levels of functional connectivity between the right angular gyrus and other regions have also helped identify patients that may particularly benefit from antidepressant treatment [81]. In generalized anxiety disorder, treatment responsiveness can be

predicted by pretreatment activity in the anterior cingulate cortex and amygdala [82,83]. Similarly, patients with PTSD exhibit abnormal oscillatory activity in prefrontal, supramarginal, and interior parietal regions that correlates with working memory deficit. These abnormalities are eliminated after successful attention training to improve working memory, suggesting that this biomarker can help monitor and evaluate treatment effectiveness [84].

Some of the signatures of anxiety identified in humans are directly inspired by animal studies. For example, rodent vHPC is known to mediate anxiety-related behavior, often assessed using approach–avoidance tasks. Using a similar task in humans, activity in the anterior HPC (human homolog of rodent vHPC) increases with threat levels, and subjects with hippocampal lesions show reduced passive avoidance behavior [85], similar to rodent studies. Furthermore, HPC–mPFC theta synchrony in humans is positively correlated with threat memory processing in an anxiety-evoking context [86], as observed in animals [35].

As mood and anxiety disorders manifest from dysfunction across multiple brain regions, by exploring multiregion activity patterns, one can better understand neural correlates of symptoms, categorize disease subtypes, and identify effective treatments. Biomarkers can further assist the development of novel therapeutics, such as closed-loop stimulation paradigms that can identify biomarkers and rescue symptoms in real time [11,87]. However, to fully understand biomarkers and achieve their therapeutic potentials, reverse translation to animal studies is crucial.

The Gap

While there have been many advances in both human and animal studies, methodological differences have traditionally hindered close crosstalk between the two domains.

First, human and animal studies often assess brain activity at varying temporal and spatial scales and resolutions that make it difficult to translate the findings. Noninvasive imaging techniques used in human studies, such as fMRI, can capture multiregion activity, and thus, have been widely used in identifying multiregion interactions as biomarkers. However, the spatial and temporal resolutions of these techniques are often lower than those used in animal studies. Importantly, in regions where specific populations of neurons serve heterogeneous functions (e.g., BLA and BNST), the low spatial resolution used in noninvasive human studies limits the ability to understand the full functions of a brain region. More invasive techniques, such as iEEG, have greater temporal and spatial resolution, but they are conducted in individuals with electrode implants used to diagnose or treat neurological disorders, and accordingly, interpreting these recordings involves inherent caveats [15]. Recording techniques in animal studies, such as two-photon imaging, are often more easily controlled, and have higher temporal and spatial resolution. These methods can help explore the underlying cellular and circuit mechanisms and generate insights that in some cases, can be directly translated to humans. For example, intermittent theta burst stimulation, a technique largely based on rodent hippocampal electrophysiological studies, is a promising potential treatment for depression [88]. At the same time, the invasive techniques applied in animal studies are often limited to a simultaneous recording from up to a few brain regions, making it difficult to assess large-scale multiregion interactions.

Another difference worth highlighting is that human studies often use more complex behavioral tasks that capture a wider range of symptoms but are not easily translatable to animals. For example, depression patients often show bias toward overgeneralization when recalling positive memories in autobiographical memory recall [89], however, such task is not easily adaptable to animals. Instead, animal studies tend to focus on simple behavior, such as avoidance and approach, as indicators for an animal's internal emotional states. The relatively simplicity of

these behaviors allows researchers to investigate the underlying mechanisms in a more controlled manner, which in some cases facilitates their translation to humans. However, simple behaviors may not be sufficient to address the wide range of symptoms observed in patients that are often crucial in differentiating disease subtypes.

Bridging the Gap

Recent advances in neural recording and circuit-mapping techniques, behavioral assays, and analysis methods (Figure 2) provide a toolbox that can be leveraged to bridge the gap between animal and human research in mood and anxiety disorders (Box 4 and Figure 3).

Advances in Multiregion Recording and Projection-Mapping Techniques

Progress in recording techniques in animals can help reveal brain-wide neuronal activity patterns that encode behaviorally relevant variables, at high spatial and temporal resolution. These techniques help explore how multiregion interactions, as well as neural encoding of emotional information, may go awry in animal models for the study of mood and anxiety-related disorders. While it is often not feasible to achieve a similar level of recording resolution in humans, results from these animal studies can help identify specific neuron subtypes, circuits, and activity patterns underlying disease states that can guide further biomarker development in humans.

Advances in electrophysiological extracellular recording techniques have increased the number and stability of neurons recorded [90] (Figure 2A). Silicon probe technologies that traditionally

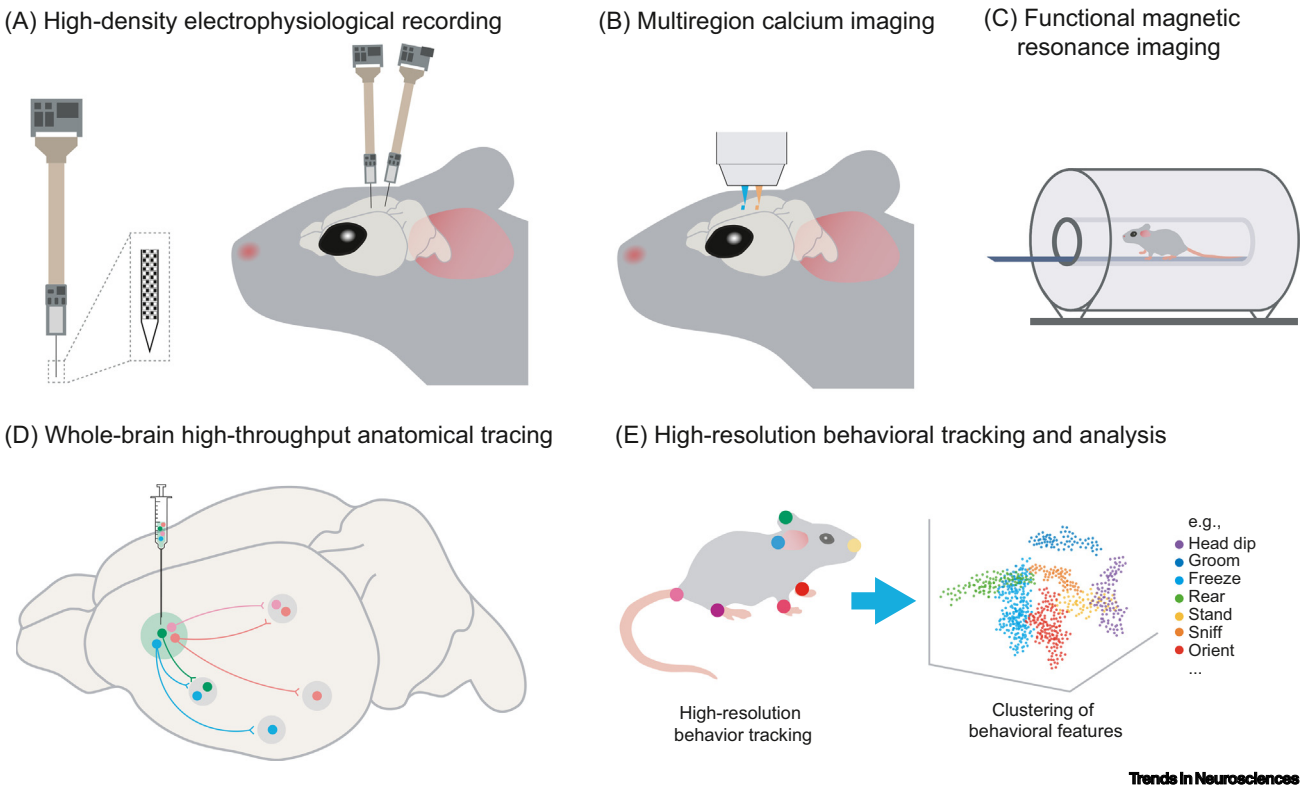

Figure 2. Advances in Techniques Applied in Rodent Research for Multiregion Recordings or Neural Circuit Mapping. (A) High-density extracellular electrophysiological recording (e.g., Neuropixels probes). (B) Multiregion calcium imaging (e.g., 1-photon, 2-photon, mesoscope, or fiber photometry). (C) Functional magnetic resonance imaging. (D) High-throughput anatomical tracing (e.g., BARseq). (E) High-resolution behavioral tracking and analysis (e.g., DeepLabCut). These approaches, either in isolation or by combined two or more of them, allow researchers to interrogate how multiregion neural activity patterns drive emotional behavior.

Box 4. Summary of Proposed Strategies

(i) Apply simultaneous multiregion recording techniques to identify brain-wide activity as biomarkers, similar to approach taken in human studies. These techniques allow capturing inter-region, population, and single-cell dynamics, and can be combined with anatomical mapping, cell-type- and projection-specific manipulations to gain mechanistic insight into how emotional information is encoded in the brain.

(ii) Reverse translate and expand the repertoire of behavioral paradigms used in animal studies to capture a larger range of functions (e.g., decision making). Conversely, human studies can take inspiration from some of the simpler behavioral assays used in animals where the circuit mechanisms have been thoroughly investigated.

(iii) Incorporate unbiased data-driven approaches to analyze recording and behavioral data, to uncover novel neural interactions that drive emotional behavior.

allow ≥16 recording sites on a single shank [91] have seen significant transformation over the years. For example, Neuropixels probes greatly increase the number of simultaneously recorded sites, to allow recording of activity from thousands of neurons across multiple different regions simultaneously [92], and can be adapted for chronic recording in freely behaving rodents [93]. Neuropixels probes are ideally suited to assess multiregion activity dynamics at both population and single-cell levels. Studies using these probes have revealed that even simple behavior engages complex brain-wide activity [94–96]. Other examples of high-density recording techniques include NeuroSeeker [97], polymer electrode arrays [98], and flexible mesh electronics that can achieve stable long-term recordings [99].

Multiregion optical imaging techniques have also been developed (Figure 2B). These techniques can image from cell-type- and projection-specific neuron populations, but standard techniques often have limited fields of view, and thus tend to focus on activity within a single region. Emerging techniques aim to challenge that limitation. For example, novel head-mounted microscopy (e.g., NINscope [100]) and two-photon imaging with expanded field of view (e.g., Trepan2P [101]) allow multiregion recordings. Widefield calcium imaging, while typically not having cellular resolution, can record mesoscale dynamics across multiple regions [102] and be used in freely

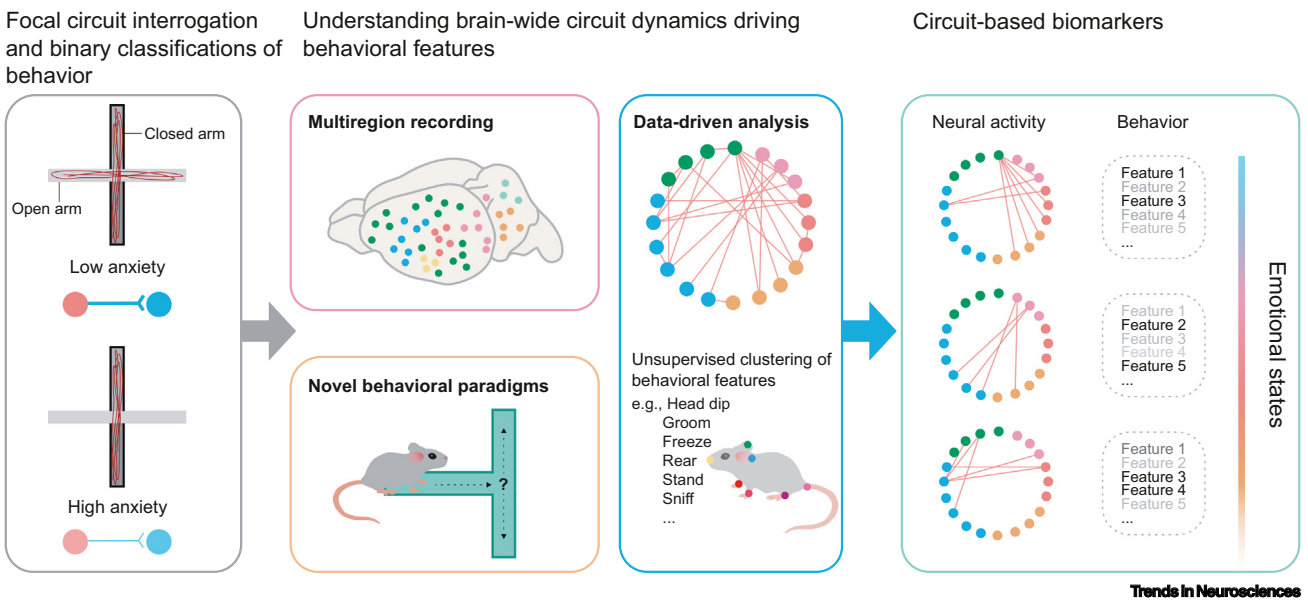

Figure 3. Toward Better Identification and Understanding of Circuit-Based Biomarkers for Mood and Anxiety. Moving beyond focal circuit interrogation and binary classifications of behavior, one can leverage recent advances in novel techniques to perform multiregion recordings, develop novel behavioral paradigms, and apply data-driven analyses to identify circuit-based biomarkers that differentiate between distinct emotional states.

behaving animals (e.g., cScope [103]). Two-photon imaging can be combined with widefield imaging to link local activity with global network dynamics [104]. Fiber photometry can also record from multiple regions by using multifiber patch cords [105].

Noninvasive imaging techniques, such as fMRI, can also be applied in animals (Figure 2C) and measure multiregion activity patterns that help validate whether the same circuits are impacted in animals as in humans, and provide ground for further reverse translation. For example, mice following chronic stress showed increased amygdala–PFC functional connectivity and white matter structural alterations in the cingulum, similar to those observed in human subjects [106]. Furthermore, imaging techniques can be combined with manipulations such as optogenetics to further investigate how specific projections and neuron subtypes drive multiregion activity patterns [77].

Understanding anatomical connectivity that drives circuit dynamics is key to developing therapeutic interventions. In comparison to conventional fluorescent or enzymatic tracers, recent advances have greatly enhanced the throughput and resolution in anatomical mapping (Figure 2D). For example, MAPseq [107] and BARseq [108] map brain-wide projection patterns of single neurons by labeling them with random RNA sequences ('barcodes'). The results can be related to gene expression and Cre-labeling patterns to help characterize projections of different cell types. Furthermore, projection tracing can be combined with single-cell RNA sequencing to understand how diversity in cell transcriptomic types governs connectivity patterns, physiology, and behavioral function [109–112]. Results could guide research into how specific genes and cell types influence disease predisposition and progression in humans, and facilitate novel therapeutic developments. Combining anatomical tracing with multiregion recordings and cell-type- and projection-specific manipulations can further elucidate the circuit mechanisms that facilitate neural interactions.

Novel Behavioral Tasks

In addition to deeper analysis of behavior in commonly used tasks to assess mood and anxiety-related behavior in rodents, it will be necessary to design novel behavioral tasks informed by human studies. One example is the area of decision making. In humans, decision-making performance can predict depressed state in major depressive disorder [113]. Furthermore, escape decisions to slow threats are linked to trait anxiety, and correlated with activity in vHPC, mPFC, amygdala, and insula [114]. Similarly, chronically stressed mice show impaired decision making in a cost–benefit conflict task [115], and biased action selection strategies toward habitual response in operant tasks [116]. Touch-screen-based decision-making tasks often used in humans have also been successfully reverse translated to rodents [117]. Further exploration of novel paradigms with trial-based behavioral designs, paired with high-density recording techniques, will provide unprecedented insights into how network-level activity patterns are impacted by distinct emotional states.

Unbiased Data-Driven Analysis Methods

Many recent studies have used unbiased data-driven machine-learning algorithms for analysis that have helped reveal unforeseen activity patterns that encode emotional states and drive behavior. Instead of a pure hypothesis-driven approach, these methods explore all activity patterns in the data to uncover potentially novel behaviorally relevant neural interactions that would not be easily predicted based on existing literature.

Both human and animal studies of mood and anxiety disorders have implemented such approaches to uncover novel behaviorally relevant interactions. For example, in order to identify multiregion interactions that significantly predict mood changes in humans, a recent study first applied unsupervised machine learning to determine networks of interactions between regions where rhythmic oscillations are most significantly correlated (termed intrinsic coherence

networks; ICNs) [15]. Then, supervised machine learning is used to determine how ICNs relate to mood fluctuations. These methods have revealed that a novel beta-frequency amygdala–HPC interaction that is highly conserved across subjects can predict worsening mood. Furthermore, analysis of spatiotemporal dynamics across eight regions pre- and post-CSDS in mice has identified significant **local field potential** (LFP) interactions that can predict and encode resilience and susceptibility [16]. Specifically, a discriminative cross-spectral factor analysis was first used to discover multiregion LFP patterns that change together over time (termed Electome Factors), taking into account features such as spectral power, synchrony, and phase directionality. A machine-learning classifier has been applied to determine how well the Electome Factors discriminate between different behavioral conditions (i.e., resilience vs susceptibility). Using this approach, specific Electome Factors can discriminate between susceptible and resilient mice, and predict future susceptibility pre-defeat.

The ultimate readout of neural activity is behavior, and in animal studies, that is the primary way to infer animals' internal emotional state. Thus, animal studies rely on a large repertoire of behavioral paradigms, where accurate quantification of behavior is crucial. Recent decades have seen the rise of machine-learning-based behavioral analysis tools that can automate body part tracking and apply unbiased analysis approaches to segment, classify, and quantify behavior, with the potential of unveiling unforeseen behavioral features (Figure 2E). These tools allow moving beyond binary classifications of behavior (e.g., open vs. closed arm time) to take a more in-depth look at the nuances in behavioral features and how they may be altered following manipulations (e.g., stress).

Combining such tools with neural recording methods enable high-resolution analysis of how neural activity is driving behavior. One recent successful example is DeepLabCut, a pose estimation toolbox that has become increasingly popular due to its ease of use and accuracy in automated tracking [118]. Toolkits such as B-SOID [119] and SimBA [120] have expanded on DeepLabCut to aid in identification of behavioral motifs. Other examples of behavioral tracking/classification algorithms include LocoMouse [121], LEAP [122], and MoSeq [123]. Facial movements [95] and expressions [57] have also been studied in greater detail, where facial expressions in mice are found to correlate with animals' responses to emotional stimuli and insular activity [57].

Such tools enable researchers to take a closer look, one could argue, into animals' internal emotional states. They not only help automate tracking, but also quantify moment-by-moment behavioral changes that allow for unbiased clustering of actions, leading to precise quantification and identification of distinct behavioral motifs and their transitioning probabilities. One intriguing possibility is that even when animals display similar overall behavior under binary classifications (e.g., time in open vs. closed arms), the nuanced behavioral features (that may occur in specific compartments of an apparatus, such as closed vs. open areas) may reveal differences in animals' internal states that could reflect distinct neural circuits and emotional information processing. If so, it may be worth exploring in humans whether high-resolution analysis of behavior can extract nuanced features that may reveal differences in underlying neural circuit dysfunctions in disorders, and even differentiate between subtypes of disorders. If so, high-resolution behavioral analysis combined with neural activity mapping may be a useful tool in establishing more accurate diagnostic criteria in patients.

Concluding Remarks

The development of innovative tools is pushing the boundary of animal research and is helping bridge the translation gap between animal model studies and those in humans. These techniques are allowing researchers to conduct high-resolution, cell-type-specific, multiregion recordings in

Outstanding Questions

What are the common versus distinct behavioral features between mood- and anxiety-related behavior? Are the common behavioral features related to the same underlying neural circuits and emotional information processing?

Can behavioral features and neural activity patterns at baseline predict future behavior and vulnerability to stress and anxiety?

How are external stimuli (e.g., stress) processed differently in individuals with varying levels of vulnerability to stress and anxiety? Can behavioral features be used to identify these differences?

How do input connectivity patterns to a region govern activation of subpopulations of neurons with distinct outputs and functions? How are large-scale connectivity patterns altered during chronic stress?

How do different subtypes of neurons within a region, such as D1- and D2-MSNs in the NAc, differentially modulate downstream activity?

Can single-cell transcriptomics inform neural connectivity and activity patterns, and vulnerability to mood and anxiety disorders?

Can acute manipulations in animal models (e.g., optogenetics) inform us about the underlying etiology of chronic disease state and potential treatment targets?

animals, to identify brain-wide activity patterns that reflect different internal emotional states, and examine how dysfunction in neural circuits manifest in maladaptive behavior (see Outstanding Questions). Together with studies in humans, progress in these areas paves the way for identifying more reliable and precise biomarkers that can enhance diagnosis, predict vulnerability to disease and response to treatment, and ultimately, help develop novel and effective therapies for the treatment of mood and anxiety disorders.

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