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Review

The emergence and influence of internal states

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SUMMARY

Animal behavior is shaped by a variety of "internal states"—partially hidden variables that profoundly shape perception, cognition, and action. The neural basis of internal states, such as fear, arousal, hunger, motivation, aggression, and many others, is a prominent focus of research efforts across animal phyla. Internal states can be inferred from changes in behavior, physiology, and neural dynamics and are characterized by properties such as pleiotropy, persistence, scalability, generalizability, and valence. To date, it remains unclear how internal states and their properties are generated by nervous systems. Here, we review recent progress, which has been driven by advances in behavioral quantification, cellular manipulations, and neural population recordings. We synthesize research implicating defined subsets of state-inducing cell types, widespread changes in neural activity, and neuromodulation in the formation and updating of internal states. In addition to highlighting the significance of these findings, our review advocates for new approaches to clarify the underpinnings of internal brain states across the animal kingdom.

INTRODUCTION

Nervous systems are in a constant state of flux, with rich internal dynamics that determine how brains respond to inputs and produce outputs. The hidden processes that underlie these dynamics can be described as "internal states" and include arousal, motivation, emotion, and varying homeostatic needs. Internal states allow us to integrate information about our external environment and internal physiological conditions into centralized brain states, which shape how sensory information is processed and orchestrate appropriate behavioral and physiological responses (Anderson, 2016; Bolles, 1967; Tinbergen, 1951).

Although internal states are difficult to observe directly, they can be inferred from observations of an animal's overt behavior and systemic physiology or from within the brain, such as by investigating neuronal dynamics or perturbing neural function. For instance, an animal's state of hunger can be determined based on caloric deficit and circulating hormones or its state of aggression inferred from observing attacks elicited by conspecifics. Likewise, several recent studies have discovered consistent changes in neuronal dynamics encompassing multiple cell types and brain systems concomitant to behavioral and/or physiological state changes (Gründemann et al., 2019; Lovett-Barron et al., 2020; Xu et al., 2020). A wide variety of animals—from jellyfish to humans—appear to organize their behavior in a state-like fashion, suggesting that the neural mechanisms that underlie the generation of internal brain states are evolutionarily ancient (Nath et al., 2017; Weissbourd et al., 2021). In humans, changes in state representation, switching, and timing are thought to occur in many psychiatric and neurological diseases. Here, our focus is on the study of experimentally tractable animal models, but the ubiquity of internal states across animal species suggests that general principles found in animals will hold relevance for understanding the human condition in health and disease.

Several recent technical advances have spurred remarkable progress in our ability to describe and investigate internal states in animal models. These include new and improved methods for tracking animal behavior, manipulating neurons, and analyzing population-level neural activity. Studies across a range of animal models now provide evidence that internal brain states can be controlled by the actions of small subsets of neurons but can influence activity across broad swaths of the brain, often in parallel. Across organisms, neuromodulators have been repeatedly identified as central elements in the generation of internal states, with a wide range of circuit organizations that deploy neuromodulators in distinct manners (Bargmann, 2012; Getting, 1989; Harris-Warrick and Marder, 1991; Marder, 2012; McGinley et al., 2015b).

Here, we begin by defining internal states, focusing on the features that characterize them. Next, we review the experimental approaches used to study internal states, the neural basis of internal states, and the central role that neuromodulation plays in the formation and function of internal states. Finally, we close by highlighting key emerging themes of internal state control

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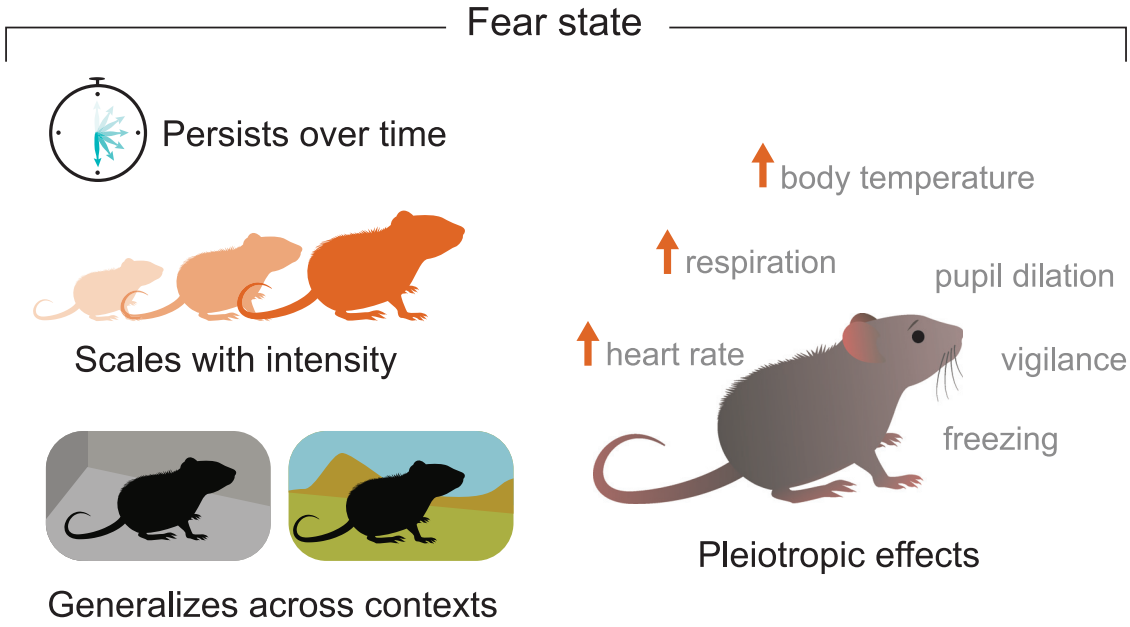

Figure 1. Features of an example internal state
Using fear in rodents as an example, we show how a central internal state can exhibit multiple features and influence a number of behavioral and physiological processes. Hallmark characteristics of an internal state, including persistence, scalability, and generalizability, are illustrated at left and pleiotropic effects associated with the state of fear are displayed on the right.

across species, including the ability of states to influence multiple circuits and cell types in parallel, the action of neuromodulators to mediate states in concert, the neural properties governing state transitions, and the persistence of states via recurrent dynamics. The principles discussed here derive from a large and diverse literature, growing out of psychology, neuroscience, cognitive science, biology, and ethology over many decades. As we cannot provide an exhaustive accounting of this work, we instead focus on specific principles that are common across organisms and highlight recent findings that have relevance for scientists currently studying internal states.

DEFINING INTERNAL STATES

Internal brain states can be defined from changes in physiology, behavior, and/or brain activity. We use the term "internal state" to refer to a state that can be independently controlled and that can occur simultaneously with other states within the same animal. For example, hunger and fear represent distinct internal states. The states that we discuss here all consist of changes in nervous system function that can be inferred from an animal's behavior (although such inference can be challenging, because states are not entirely overt; see below). In addition, some internal states involve changes in other parts of the body. For example, hunger involves changes in gut metabolism, hormone levels, and more. These interactions between the brain and the periphery can be bidirectional. We consider these peripheral changes to be important aspects of the state. We expect that the definition of "internal state" will become more precise as the field evolves, and we return to the complexities of this definition at the end of the review. In this review, we will start by discussing characteristic features of internal states, how they can be inferred from behavioral and physiological changes, and then their neuronal correlates.

Features of internal states

Internal states enable us to produce flexible and adaptive behavioral and physiological responses in a wide range of different settings. These internal states are stable enough to organize behaviors over long timescales and flexible enough to facilitate adaptive (or maladaptive) responses to different circumstances or changing environments. To be both flexible and stable, internal states often possess the following features: pleiotropy, persistence, scalability, generalizability, and valence (Figure 1) (Adolphs and Anderson, 2013; Anderson, 2016; Darwin, 1872; Tye, 2018). Pleiotropy refers to the feature that each state influences multiple aspects of behavior and physiology in parallel, such as body temperature, respiration, locomotion, sensory responsiveness, and more (Figure 1). Persistence describes the ability of internal states to produce behavioral and physiological responses that outlast the termination of the stimulus that initiated the response. We do not consider individual motor actions to be states, but persistent sequences of motor actions may be classified as states. Scalability indicates the ability of these responses to scale with the magnitude of the stimulus. Generalizability refers to the degree to which an internal state can produce responses to stimuli that are distinct from the original stimulus that elicited the response. Valence describes the positive or negative affect associated with that state. Taken together, the multifaceted and flexible nature of internal states provides evolutionary advantages for organisms across the animal kingdom.

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A prototypical internal state: Fear

The abovementioned properties of internal states can be conceptualized in the context of emotion and can be well illustrated using one of the most well-studied states in neuroscience and psychology—fear (Adolphs, 2008; Dukes et al., 2021; Fanselow, 2018; Fanselow and Pennington, 2018; Janak and Tye, 2015; LeDoux and Daw, 2018; LeDoux, 2017, 2020; LeDoux and Brown, 2017; Mobbs et al., 2019; Tovote et al., 2015; Tye and Deisseroth, 2012). For example, if you are afraid of flying on a plane, you might display a set of *pleiotropic* changes including an increase in heart rate, galvanic skin response, and feelings of anxiety, which *persist* well beyond the time in which you are exposed to the plane (stimulus). These neural and peripheral responses might *scale* with the strength of the stimulus such that they increase during turbulence, and they may *generalize* to other similar stimuli, such as helicopters or cars. The *valence* of this state is negative, causing you to avoid flying in a plane as much as possible.

In laboratory settings, the internal state of fear is often investigated using classical conditioning (Pavlov, 1927) in which an animal, often a rodent, is conditioned to fear a previously neutral cue (e.g., auditory tone) that, through training, comes to predict the occurrence of an aversive stimulus (e.g., foot shock). These classical conditioning paradigms allow for precise control over experimental parameters and their effects on fear. In both controlled, as well as more naturalistic settings, an animal may display a wide variety of fear-related behaviors—fleeing, freezing, and fighting—depending on the imminence of the threat and the shape of the environment (Fanselow, 2018; Fanselow et al., 2019; Fanselow and Lester, 1988; Perusini and Fanselow, 2015). These fear behaviors demonstrate hallmark characteristics of an internal state. For example, in rats and mice, freezing behavior *scales* with the magnitude of the foot shock (Fanselow and Bolles, 1979), *generalizes* to similar auditory cues, and can *persist* well beyond termination of the auditory stimulus (Quinn et al., 2002). These behavioral readouts correspond to physiological findings, which identify neurons that are active during fear conditioning and/or expression, *persist* in their activity beyond termination of a fear-eliciting stimulus, *generalize* their activity to similar stimuli, and *scale* the intensity of their activity depending on stimulus magnitude (e.g., Ciocchi et al., 2010; Haubensak et al., 2010).

Nevertheless, it is important to note that despite being heavily studied, fear represents one of the most hotly contested internal states, with many questions currently unanswered (see Mobbs et al., 2019 for a review of some of these issues). For example, what are the behavioral readouts that best capture the internal state of fear? How exactly is fear distinct from other similar states, such as anxiety? Do these states lie on the same continuum and thus collectively represent a larger internal state of defense? How does this internal state interact with prior experience? Finally, some have even argued that it may not be possible to truly study fear in nonhuman animals (LeDoux, 2020, 2021). Thus, although fear is a powerful, well-studied example of an internal state, fear also represents some of the challenges facing the field of internal states.

Although fear in rodents exemplifies many of the characteristics of an internal state—at both the behavioral and neurobiological level—examples of numerous behaviors influenced by internal states can be found in almost every species studied. In the sections below, we discuss a variety of internal states across different model organisms. Like many areas of biology searching for general principles, we believe that our understanding of internal states will benefit enormously from integrating results across multiple organisms and behavioral conditions (Jourjine and Hoekstra, 2021; Katz, 2016; Laurent, 2020; Yartsev, 2017).

EXPERIMENTAL APPROACHES TO STUDYING INTERNAL STATES

Investigating the neural basis of internal states requires the accurate inference of such states, extracted from measurements and manipulations of behavior, physiological parameters, and environmental context (Figure 2A). Here, we discuss different approaches for inducing and measuring internal states in a laboratory setting.

Experimentally inducing need states

Many studies rely on manipulating environmental or physiological variables to induce internal states. For instance, exposing animals to specific stimuli, environments, or physiological conditions has proven useful to induce binary global state changes; this includes induction of anxious states with threatening environments (Calhoon et al., 2018; Tovote et al., 2015), induction of hunger with food or nutrient deprivation (Livneh et al., 2020; Sayin et al., 2019; Vogt et al., 2021), and induction of thirst with water deprivation (Allen et al., 2019; Livneh et al., 2020; Zimmerman et al., 2017, Figure 2B). These studies often rely on single characteristic behaviors as a readout (approach versus avoidance, exploiting versus roaming, attack versus mounting), and the robustness of these need-state-induced behaviors allow for averaging results across individuals. Such approaches have been useful in identifying key characteristics of deprivation-induced need states, enabling the exploration of their neurobiological underpinnings (Sternson, 2013).

Inferring internal states from overt locomotor behavior

Locomotion represents a key observable variable from which internal states can be inferred. When observing locomotion over time, experimenters can classify epochs of fast-timescale actions into slower-timescale states distinguished by the probability and content of the animal's motion (Flavell et al., 2020; Ji et al., 2021; Marques et al., 2020; Poulet and Petersen, 2008; Figure 2C). Many organisms, including mammals, zebrafish, flies, and worms, display stable, global changes in behavioral patterns such as switches between active and inactive locomotor states. Active states, characterized by longer movement trajectories, include exploration and roaming. Inactive states, characterized by little or short locomotor bouts, include idling, dwelling, or exploiting (Flavell et al., 2013; Ji et al., 2021; Marques et al., 2020). These global patterns have been shown to also exist in more complex organisms, such as rodents (Gründemann et al., 2019). Similar state-dependent switches in active versus passive behaviors have been described in the contexts of active sensing versus quiescence (Poulet and Petersen,

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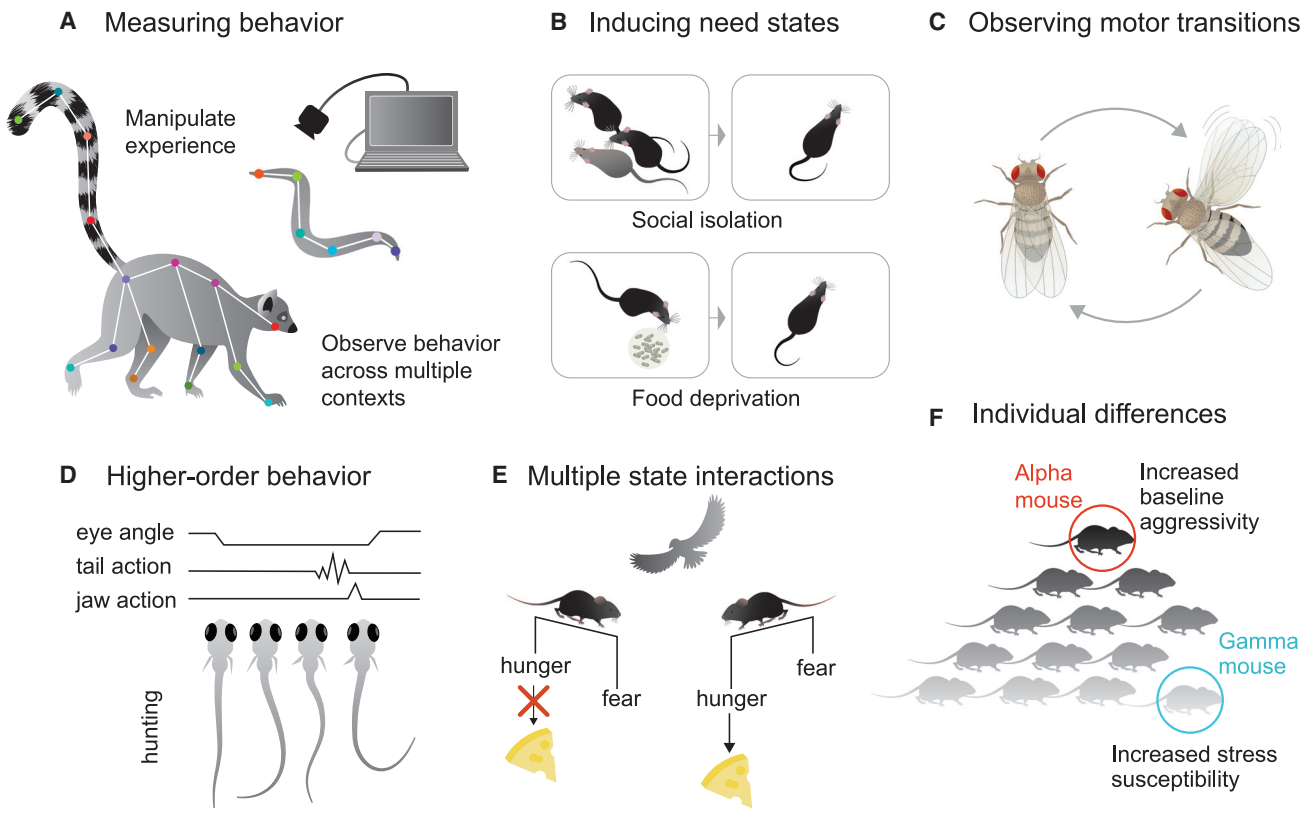

Figure 2. Approaches to infer the presence of internal states from observable behavior
(A) Measuring overt behavior by tracking animal movement (examples: keypoint-based pose tracking in lemurs and nematodes).
(B) Inducing need states through environmental control (examples: social or caloric deprivation in rodents).
(C) Inferring internal state from transitions in observable movements (example: fly wing extension during courtship).
(D) Inferring states from the co-occurrence of multiple behavioral features (example: hunting states of larval zebrafish).
(E) Multiple states can interact with one another (example: a hungry rodent may show less fear when foraging under predation).
(F) State expression can vary across individuals (example: a rodent's position in a social hierarchy influences their aggressivity and response to stress).

2008), running versus resting (Keller et al., 2012), or high versus low arousal (Rodriguez-Romaguera et al., 2020).

Measuring such bimodal changes in "state" can be achieved by tracking entire animals in space (Flavell et al., 2020; Ji et al., 2021; Marques et al., 2020) and measuring course locomotion parameters or spatial coverage. Movement can also be characterized in a more detailed manner, by tracking the position of the body and limbs over time to classify states; these studies are enabled by a recent proliferation of methods for tracking body posture (Box 1). States can also be inferred from their effects on the performance of repeatable motor behaviors with trial-like structures. For instance, the response rate and reaction time to sensory stimuli can be used to infer arousal or alertness across species (Harris and Thiele, 2011; Lovett-Barron et al., 2017; Maimon, 2011; McGinley et al., 2015b; Moore and Zirnsak, 2017; Musall et al., 2019).

Inferring internal states from higher-order behavior

Beyond classifying states from coarse locomotor behavior, recent studies have also focused on extracting more complex behavioral patterns to describe internal states. Although methods to track animal behavior are increasingly powerful (see Box 1), it remains challenging to analyze and understand the high-dimensional behavioral data arising from these tools (Berman, 2018; Datta et al., 2019). Toward this goal, machine learning (ML) has become key. For example, from the kinematic features extracted over long timescales, ML algorithms are able to extract and classify behavioral patterns and sequences, their variation across time and individuals, and their perturbation by drugs and disease models.

One such ML approach is Motion Mapper (Berman et al., 2014), which identifies behavioral modules by low-dimensional embedding and clustering. Recent evidence testing different unsupervised approaches for behavioral mapping and clustering argues that keeping the data in as many dimensions as possible for clustering is preferable (Todd et al., 2017). Other techniques use intuitive behavior annotation by the experimenter, which allows supervised ML algorithms to quantify these behaviors (e.g., Janelia Automatic Animal Behavior Annotator [JAABA]; Kabra et al., 2013). Another approach that has also been successful is to measure multiple behavioral parameters and infer underlying state(s) using probabilistic approaches. For instance, hidden Markov models (HMMs) have been employed to infer behavioral states in many organisms (Calhoun et al., 2019; Cermak et al., 2020; Marques et al., 2020). However, these techniques rely on

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Box 1. Methods for computational analysis of animal behavior

There has been a recent proliferation of techniques aimed at providing high-throughput, automated behavioral tracking and classification. These advances in behavioral analyses have been especially aided by the expansion of computational tools. Particularly, recent technological advances in machine vision and machine learning have revolutionized the capacities to automatically track, classify, and decode animal behavior. Artificial deep neuronal networks are a rich addition to the field of behavioral assessment and may be the foundation of a totally new field of computational neuroethology (Datta et al., 2019). Recently developed methods to measure animal behavior in different species include Stytra (Štih et al., 2019), TRex (Walter and Couzin, 2021), Ctrax (Branson et al., 2009), JAABA (Kabra et al., 2013), Optimouse (Ben-Shaul, 2017), LEAP (Pereira et al., 2019), DeepLabCut (Mathis et al., 2018), DeepEthogram (Bohnslav et al., 2021), DeepPoseKit (Graving et al., 2019), DANNCE (Dunn et al., 2021), MARS (Segalin et al., 2021), or a 3D virtual mouse (Bolaños et al., 2021). These methods allow for tracking everything from body parts to multi-action behavioral motifs. Details of these novel approaches can be found in a number of authoritative reviews published recently (Datta et al., 2019; Mathis and Mathis, 2020; Pereira et al., 2020).

variables that are quantified and identified by the experimenter as being state relevant.

Making use of the temporal sequence of behavioral actions over time has been a particularly powerful approach to infer internal states (Figure 2D; Berman et al., 2016; Luxem et al., 2020; Wiltschko et al., 2015; York et al., 2021). For example, two recent studies using this approach were able to classify the behavioral sequences that comprise the larval zebrafish's hunting behavior from specific eye and tail movements in the context of available prey (Johnson et al., 2020; Mearns et al., 2020). Another such technique, motion sequencing (Mo-seq; Wiltschko et al., 2015), is an ethologically inspired behavioral analysis method. In a recent landmark study, Wiltschko et al. (Wiltschko et al., 2020) automatically and effectively deconstructed behavioral differences and similarities elicited by a panel of neuroactive and psychoactive drugs in mice. Mo-seq was able to distinguish the behavioral changes elicited by the drugs, which each elicit movement reductions through different mechanisms, such as distinguishing catalepsy and sedation, and are often confused in traditional behavioral assays. Mo-seq was even able to predict drug dosage. These studies reveal that temporal sequence-based approaches can capture spontaneous transitions between diverse internal states across highly variable and diverse datasets.

Approaches for considering the co-existence and interactions of internal states

Despite the advances discussed above, one complication is that animals can be under the influence of multiple states at once. For instance, individuals may exist in one coherent state that integrates or selects from multiple internal needs and outside stimuli. For example, individuals may be influenced by diverse physiological and affective need states in parallel, such as thirst, hunger, fear, social isolation, and environmental conditions (availability of food and social or predator encounters). These needs and contextual changes elicit drives that compete or may be mutually reinforcing depending on the context (Duistermars et al., 2018; Eiselt et al., 2021; Thornquist and Crickmore, 2020; Figure 2E). Together, these parameters may result in integrated and complex internal states, which manifest as behavioral switches when one drive overcomes another or may serve to generate entirely unique behavior patterns. Indeed, recent work has highlighted the overlap between distinct states such as hunger and thirst (Eiselt et al., 2021; Gong et al., 2020). Interestingly, the lateral hypothalamus (LH) of the mouse has been found to be a key hub in organizing behavioral switches in response to multiple diverse internal states (Nieh et al., 2016), emphasizing the complex interactions between different need and motivational states.

To further understand the dynamics and organization of multiple internal states, such as whether they are organized hierarchically or in parallel, it may become necessary to study animal behavior over longer timescales in naturalistic settings, where animals are exposed to multiple needs and stimuli (Burnett et al., 2019; Burnett et al., 2016; Thornquist and Crickmore, 2020). For instance, can multiple states stably coexist or do brains exist in a unitary state that is a combination of multiple lower-level states? Are some states more likely to "win" control over behavior compared with other states? Such questions highlight the field's long-standing interest in understanding distinct need states and how they sit in a hierarchy, with each basic need emerging once a central need is met (Maslow, 1943). In turn, these questions generate new ones—what are the rules governing the hierarchy of state control over behavior? Do different states adhere to different rules? Further experiments are required to address these interesting questions.

Studying individuals to address the subjectivity of internal states

A particular challenge in studying internal states arises from individuality. Past experiences, social hierarchies, contextual factors, genetic background, and hormonal influences may determine the "personality" of individual animals and strongly shape how each individual reacts in common circumstances. Results from worms (Stern et al., 2017), flies (Honegger and de Bivort, 2018), zebrafish (Pantoja et al., 2016, 2020), and mice (Forkosh et al., 2019) argue that the neuronal underpinnings of internal states may best be addressed by studying individuals in detail (Figure 2F).

As an example of how detailed and individualized behavioral readouts may help the study of internal states, a recent study found evidence that facial expressions might represent innate and sensitive reflections of the subjective emotion state of individual mice (Dolensek et al., 2020). Employing machine vision and ML algorithms, Dolensek et al. categorized mouse facial expressions objectively and quantitatively at millisecond

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timescales. Notably, the authors demonstrated that the facial expressions revealed individual variability in intensity, value, and persistence of subjective emotion states (Anderson and Adolphs, 2014). Furthermore, other recent studies have found that a large fraction of the brain's activity can be explained by movement variables read out from the face or the body (Musall et al., 2019; Steinmetz et al., 2019; Stringer et al., 2019). These results highlight how powerful each individual's idiosyncratic behavior is in driving brain-wide activity changes, independent of task or stimulus involvement. This emphasizes the challenges of summarizing data across multiple animals without the ability to control for these variables.

In a powerful example of how prior experience can shape individual differences and contribute to variability in internal states, Remedios et al. (2017) found that exposure to social experience results in a shift in both a mouse's subsequent behavior and neuronal ensemble activity in the ventromedial hypothalamus (VMH). More specifically, naive male mice with no prior sexual experience demonstrate a lack of aggression toward male conspecifics, which correlates with an overlap in the neural ensembles that represent male versus female conspecifics. As males are exposed to repeated social experience, aggressive behavior emerges, coupled with a separation in the neuronal ensembles that represent male versus female conspecifics. Interestingly, this shift to aggressive behavior and separable male/female ensembles in the VMH varies across mice, highlighting that the neural populations driving aggression are subject to plasticity and sensitive to additional factors controlling individual differences.

Taken together, these findings collectively argue that experiences, as well as changes in bodily condition or physiological need, exert powerful influences on the neuronal machinery from which internal states emerge. Consequently, the internal states evoked by the same set of influences may differ depending on an individual's history and current contextual standing. An important question for future research will be to ask how endocrine, genetic, plasticity, and potentially further mechanisms may drive individual differences in internal state. It will be crucial to have individualized readouts of internal states at hand to tackle this important question.

Approaches toward improved state definitions

As mentioned above, internal states induce pleiotropic effects, impacting multiple behaviors and physiological parameters in parallel. Thus, to improve and refine the description and detection of changes in internal states, integrated multidimensional analyses including behavioral but also physiological measurements may be key. The available measures, and ease of using them, vary depending on the species being studied. For instance, the transparent larval zebrafish may be useful for videography of the body (heartbeat, muscle tone, blood flow, and respiratory movements) but less useful for testing circulating hormones (limited volume of blood to test). Larger animals, in contrast, can allow for chronically inserted devices that monitor metabolism and systemic physiology.

Future improvements in the methods to classify behaviors and internal states will likely involve making more measurements—simultaneous posture recording, physiological measures, and descriptions of the sensory environment and individual animal history. Importantly, ensuring that tools for collecting and integrating such multimodal information are "user friendly" will be critical in their widespread use, an essential component for the field's understanding of a given internal state. These approaches can provide more rigorous definitions of states that have already been extensively studied (arousal, fear, and hunger) and may also reveal currently unknown "states" that explain trends in behavior but do not yet have a clear label. For instance, recent studies have identified previously unrecognized connections between neural dynamics and metabolic state (Tingley et al., 2021). Ultimately, states may be best described directly from the brain itself. We next discuss common signatures of internal states across the brains of different species.

THE NEURAL BASIS OF INTERNAL STATES

Internal states have the capacity to influence multiple aspects of sensation, cognition, action, and systemic physiology. Here, we discuss recent work highlighting how distinct populations of neurons can generate different internal states and the influence of such states on the rest of the nervous system.

A neuronal population code of behavioral states

Several recent studies across different species and brain regions have highlighted that the behavioral state of an animal can be predicted and thus readout from the activity dynamics of neuronal populations that either span brain-wide networks or dominate single brain regions. For example, a study in the rodent basolateral amygdala found that two distinct neuronal populations of principle neurons predicted the switches between exploratory versus nonexploratory defensive states (Gründemann et al., 2019). Similarly, networks of neurons encoding exploitation versus exploration states have been identified in fish (Marques et al., 2020) and worms (Ji et al., 2021). Interestingly, behavioral states can be decoded with high accuracy from the combinatorial activity of diverse molecularly defined cell types but not from the activity of single cell types (Lovett-Barron et al., 2020; Xu et al., 2020). These and similar findings highlight that internal states are represented in neuronal population dynamics that recruit neurons across multiple different cell types, brain regions, and neuromodulatory systems.

Small subsets of neurons can drive state transitions

As described above, internal states are represented in combinatorial and complex activity dynamics of entire neuronal populations. Nevertheless, the use of methods to precisely activate neurons (Luo et al., 2018) has revealed that even small subsets of neurons can drive persistent brain states with influence over a variety of behavioral features in multiple different species. Dramatic examples abound in the study of rodent behavior, where optogenetic or chemogenetic activation of genetically and anatomically defined subsets of neurons can evoke specific behaviors and associated brain states (Anderson, 2016; Sternson, 2013; Yizhar et al., 2011). This includes the induction of behaviors associated with hunger upon stimulation of Agouti-related peptide (AGRP) neurons in the arcuate nucleus of the hypothalamus (Aponte et al., 2011; Chen et al., 2016; Krashes et al.,

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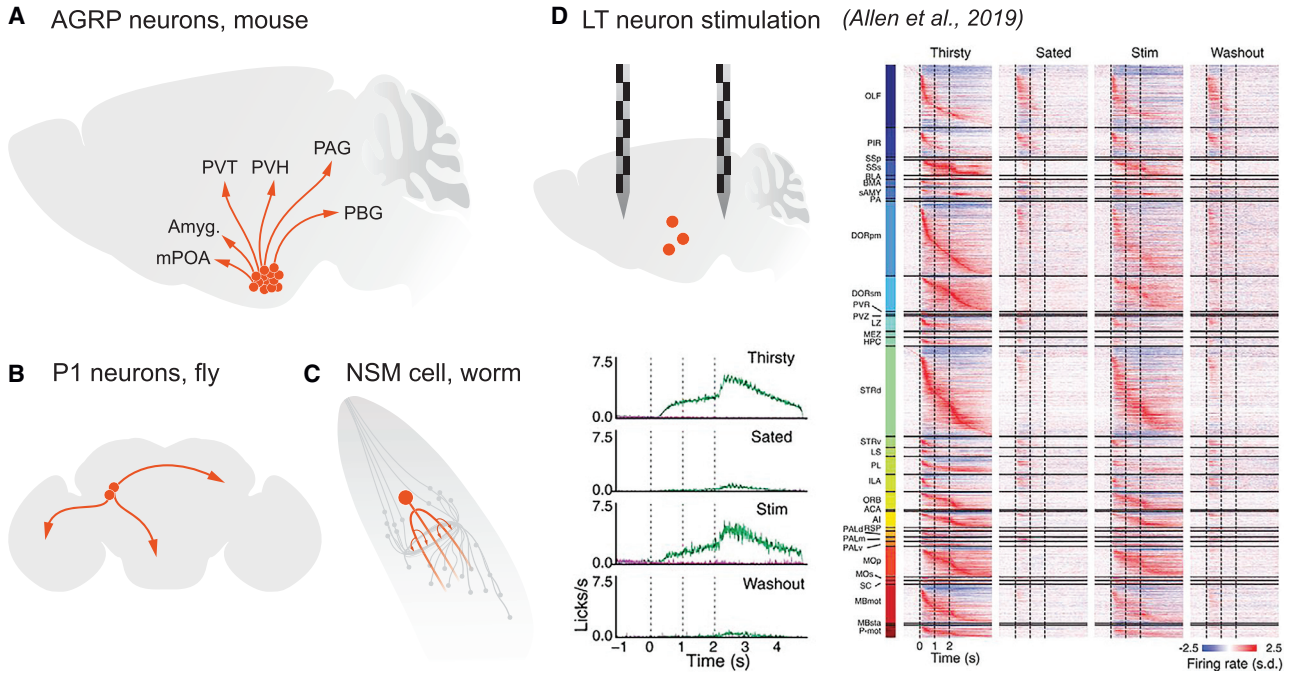

Figure 3. Collateralized projections and brain-wide influence of state-inducing neurons

(A) Schematic of projections from AGRP+ hunger-promoting neurons (red) in the arcuate nucleus of the mouse hypothalamus.

(B) Schematic of projections from P1 social arousal-promoting neurons (red) in the fly.

(C) Schematic of projections from the serotonergic NSM neuron (red) that promotes dwelling states in the nematode.

(D) Stimulating thirst-promoting neurons in the lamina terminals recapitulates the effects of natural thirst on behavior (bottom left) and neural populations recorded in multiple brain regions (right; from Allen et al., 2019).

2011), thirst-related behavior with stimulating neurons in the lamina terminalis (Allen et al., 2017a; Augustine et al., 2018; Leib et al., 2017; Oka et al., 2015), or aggressive behaviors with stimulation of neurons in the VMH (Falkner et al., 2016; Lee et al., 2014; Lin et al., 2011), among many other examples.

These experiments have revealed some important shared features of diverse state-inducing neural populations: brief activation of these cells drives persistent states, and these cells project to multiple brain regions to induce different aspects of the core brain state (Figure 3A). For instance, activation of hunger-associated AGRP neurons induces an aversive motivational state (Berrios et al., 2021; Betley et al., 2015), promoting mice to eat food when available (Aponte et al., 2011; Krashes et al., 2011). Feeding is driven by AGRP neuron projections to the paraventricular hypothalamus (PVH), LH, paraventricular thalamus (PVT), and bed nucleus of the stria terminalis (BNST) (Atasoy et al., 2012; Betley et al., 2013; Horio and Liberles, 2021) but also primes mice to eat more later through its projection to the PVH (Chen et al., 2019; Chen et al., 2016; Jikomes et al., 2016), increases attention to visual and olfactory food cues through projections to the PVT (Horio and Liberles, 2021; Livneh et al., 2017, 2020), suppresses fear and aggressive behavior through projections to the medial amygdala (Padilla-Coreano et al., 2016), and inhibits inflammatory nociception and the effects of appetite suppressants through projections to the parabrachial nucleus (PBN) (Alhadeff et al., 2018; Essner et al., 2017). Similarly, activation of thirst-associated neurons in the medial preoptic nucleus (MPON) that project to the PVT, PVH, or LH induces drinking behavior when water is present and induce a negative motivational drive (Allen et al., 2017a; Leib et al., 2017), in addition to increasing blood pressure through the hypothalamic projections (Leib et al., 2017). Furthermore, stimulation of defensive neurons in the dorsomedial subregion of the ventromedial hypothalamus (VMHdm) can produce defensive behaviors through projections to the anterior hypothalamus and midbrain (Wang et al., 2015), inhibit mounting behaviors and ultrasonic vocalizations through projections to the medial preoptic area (MPOA) (Karigo et al., 2021), drive biting through outputs to the periaqueductal gray (PAG) (Falkner et al., 2020), and possess a number of other output projections (Lo et al., 2019). These features allow a small set of neurons to influence a diversity of behavioral outcomes through specialized projections, a collateralization that is also present in the control of arousal (Poe et al., 2020), anxiety (Kim et al., 2013), and parenting (Kohl et al., 2018) in rodent brains.

The projections of putative state-control neurons are particularly well studied in rodents, but these principles have been found across multiple model systems, where stimulation of small sets of neurons with broad projections can influence internal states (Figures 3B and 3C). In the compact *C. elegans* nervous system, the activation of one or few neurons can induce state transitions, including the initiation of roaming and dwelling by pigment-dispersing factor (PDF)- and serotonin-releasing neurons, respectively (Churgin et al., 2017; Flavell et al., 2013;

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Box 2. Challenges and caveats for the manipulation of state-triggering neurons

Optogenetic, chemogenetic, and thermogenetic techniques can allow for targeted manipulation of state-promoting neurons, but these approaches may not reproduce the natural dynamics of these cells recorded in vivo. Although some molecularly defined subpopulations of neurons show concerted neural activity that can be reasonably approximated with optogenetic perturbations (i.e., mouse AGRP neurons; Betley et al., 2015; Chen et al., 2015; Mandelblat-Cerf et al., 2015), other populations show complex dynamics within a molecularly defined subpopulation (i.e., mouse VMHvl neurons; Falkner et al., 2014; Karigo et al., 2021; Remedios et al., 2017). In addition, state-triggering neurons may fluctuate on various timescales, from slow tracking of homeostatic features (Sternson, 2013; Zimmerman et al., 2017) to faster activity of arousal-associated neurons, which can track bias in behavioral (i.e., reaction time) and physiological (i.e., pupil diameter) measures (Maimon, 2011; McCormick et al., 2020; McGinley et al., 2015b). Manipulating the activity of neurons across fast and slow timescales, although accounting for their potentially different effects (Hong et al., 2018; Otchy et al., 2015; Wolff and Ölveczky, 2018), remains a challenge. In addition, many neurons with state-related activity may not necessarily be able to evoke the same state upon stimulation (Lovett-Barron et al., 2017).

With these caveats in mind, we should be critical about whether or not artificial activation appears to trigger seemingly "normal" behavioral manifestations of internal states. Are many manipulations sufficiently natural enough or constrained by the properties of downstream circuits to remain within the relevant neural population space (Jazayeri and Afraz, 2017; Wolff and Ölveczky, 2018)? Are conventional manipulations of neuromodulatory cell types routinely achieving saturating effects on downstream populations (Coddington and Dudman, 2018)? Are our measurements too coarse to discern the difference between natural- and unnatural-triggered states (e.g., measuring effects through neuron spike rates, overt behavior, or cortical EEG, for example) and would more nuanced measurements resolve these distinctions (e.g., measuring effects through ionic conductance, context-dependent ethograms, or manifold of population dynamics)?

In general, a better capacity to precisely match and perturb aspects of natural activity should reveal which components of neural dynamics are important or dispensable for the initiation, persistence, and multiplexing of internal states.

Ji et al., 2021), and the induction of low arousal/sleep states by peptidergic neurons (Nath et al., 2016; Turek et al., 2013, 2016). In *Drosophila*, aggression can be induced by activation of tachykinin-expressing neurons (Asahina et al., 2014), and threat displays are evoked by a small subset of anterior inferior protocerebrum neurons (Duistermars et al., 2018). A set of male-specific P1 neurons evokes a persistent internal state of social arousal, which enhances either aggression or courtship behaviors depending on context (Anderson, 2016; Bath et al., 2014; Clowney et al., 2015; Hindmarsh Sten et al., 2021; Inagaki et al., 2014a; Jung et al., 2020; von Philipsborn et al., 2011; Zhang et al., 2016); analogous neurons in female *Drosophila* have also been found to promote persistent behavior (Deutsch et al., 2020).

Although these activation studies are informative, it is important to consider the natural dynamics of state-triggering neurons as well, which may contribute to internal states in a dynamic regime not explored by artificial stimulation (Jazayeri and Afraz, 2017; Wolff and Ölveczky, 2018; Box 2).

Internal states influence neurons across the brain

Although internal states can be initiated by small subsets of neurons, their broad effects on behavior and systemic physiology suggest that states can have wide-ranging influence over the nervous system. Across model systems, internal states have been found to influence broad swaths of the brain—findings made possible through the application of optical and electrical techniques for large-scale cellular-level recording of neurons across multiple brain regions in behaving animals (Ahrens and Engert, 2015; Engel and Steinmetz, 2019; Lin et al., 2022; Urai et al., 2022).

One class of internal state that has been studied extensively is a state of arousal associated with movement, where awake animals' transition between periods of overt movement and/or enhanced alertness and periods of relative quiescence. In *C. elegans*, motor activity drives a large number of neurons across the head ganglia (Hallinen et al., 2021; Ji et al., 2021; Nguyen et al., 2016), whereas extended quiescence broadly suppresses activity (Nichols et al., 2017). In *Drosophila*, locomotion or tethered flight increases the activity of neurons across multiple brain regions (Aimon et al., 2019; Mann et al., 2021) including identified neurons with roles in visual processing (Chiappe et al., 2010; Hindmarsh Sten et al., 2021; Kim et al., 2015, 2017a; Maimon et al., 2010; Strother et al., 2018; Suver et al., 2012) and motor control (Ache et al., 2019). During zebrafish swimming, whole-brain imaging has revealed broad engagement of neurons across the forebrain, midbrain, and hindbrain (Ahrens et al., 2012; Chen et al., 2018; Dunn et al., 2016; Lovett-Barron et al., 2020; Naumann et al., 2016), with widespread suppression of neurons during quiescence (Andalman et al., 2019; Mu et al., 2019). In behaving mice, locomotion and/or movement of the face or limbs influences the activity of neurons across multiple regions of dorsal neocortex (Allen et al., 2017b; Kauvar et al., 2020; Makino et al., 2017; Niell and Stryker, 2010) and subcortical areas (Musall et al., 2019; Steinmetz et al., 2019; Stringer et al., 2019), even including the axons of retinal ganglion cells (Liang et al., 2020; Schröder et al., 2020). Overall, an animal's brain displays dramatic and widespread neural activity changes during movement versus quiescence.

Despite the convenience of measuring locomotion alone, states of high arousal can occur without overt movements of the limbs or face (Lovett-Barron et al., 2017; McGinley et al., 2015a; Reimer et al., 2014; Vinck et al., 2015). Therefore, it remains to be seen whether the neural dynamics in a rapidly moving animal reflect the internal state of the animal (McGinley et al., 2015b), efference copy-like feedback of motor actions

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(Ji et al., 2021; Kim et al., 2015, 2017a; Schneider et al., 2014), or a combination thereof (Liu and Dan, 2019; McGinley et al., 2015b; Reimer et al., 2014; Vinck et al., 2015). In cases where large populations of neurons could be recorded simultaneously, these locomotion/arousal-associated behavioral states are characterized by the evolution of a low-dimensional population state (Ahrens et al., 2012; Ji et al., 2021; Kato et al., 2015; Mu et al., 2019; Stringer et al., 2019). Whether such states appear at the cellular level in larger primate brains remains presently unknown, but there is evidence for broadly synchronized brain regions in humans (Fox et al., 2005; Raichle, 2015).

In addition to locomotion-related arousal, need states such as hunger and thirst are also shown to modulate large-scale neural activity. Hunger influences multiple aspects of *Drosophila* behavior (Kim et al., 2017c), through modulation of olfactory neurons (Ko et al., 2015; Root et al., 2011), gustatory neurons (Inagaki et al., 2014b), motor-control neurons (Jourjine et al., 2016; Yu et al., 2016), and other central brain populations (Inagaki et al., 2012; Krashes et al., 2009; Park et al., 2016; Tsao et al., 2018; Yapici et al., 2016). In zebrafish larvae, food restriction biases fish toward hunting behavior (Johnson et al., 2020), with hunger increasing the activity of serotonergic neurons in the raphe (Filosa et al., 2016) and caudal hypothalamus (Wee et al., 2019b), potentially by sensitizing visually responsive neurons in the optic tectum (Filosa et al., 2016; Yokogawa et al., 2012). In mice, hunger can influence cue-evoked activity in association cortices, amygdala, and brainstem (Burgess et al., 2016; Calhoon et al., 2018; Gong et al., 2020; Livneh et al., 2017, 2020; Lutas et al., 2019).

One particularly informative study (Allen et al., 2019) examined the impact of thirst state on a mouse's performance in a water-motivated behavioral task. Using large-scale electrophysiological recordings from populations of neurons across dozens of brain regions, the authors found that the state of thirst was widely encoded as a low-dimensional population state. This state influences both spontaneous and cue-evoked neural activity—largely increasing the rates and durations of task-responsive neurons (Figure 3D). Notably, thirst-related dynamics across multiple brain regions—but not all—were reinstated by optogenetic activation of dehydration-sensitive neurons in the subfornical organ. This suggests that both natural and optogenetic induction of an internal state can influence the activity of neurons throughout the brain, but subtle differences in the set of influenced brain regions distinguish between the two conditions. Whether natural or optogenetically evoked thirst states produce comparable subjective experiences for the animal or are capable of modulating the same set of behaviors is presently unclear.

As techniques for large-scale recording in freely moving animals advance (Cong et al., 2017; Grover et al., 2020; Ji et al., 2021; Juavinett et al., 2019; Kim et al., 2017b; Nguyen et al., 2016; Steinmetz et al., 2021), we expect that investigators will find that other internal states also exert a brain-wide influence, including those that evolve over longer timescales (Hrvatin et al., 2020; Stern et al., 2017) or whose classification is more complex, including parental behavior (Carcea et al., 2021; Kohl et al., 2018; Marlin et al., 2015; Wu et al., 2014), emotional regulation (Anderson and Adolphs, 2014; Dolensek et al., 2020), and the multiple effects of social deprivation (Anneser et al., 2020; Matthews et al., 2016; Tunbak et al., 2020; Zelikowsky et al., 2018).

It remains to be seen whether such brain-wide concerted activity patterns are important for the execution of state-dependent behavior or are a mere consequence of shared activity across recurrently connected circuits that span multiple brain regions. This could be tested in future studies by independently manipulating state-dependent population activity in different brain regions and measuring the effects on state-dependent behaviors and activity in other regions. To understand these mechanisms, better knowledge of how the cellular actions of neuromodulators collectively produce global brain state-dynamics is needed.

A CENTRAL ROLE FOR NEUROMODULATION

Perhaps the largest unifying factor identified in the control of distinct internal states and their impact on behavior is the role of neuromodulators (Bargmann, 2012; Bargmann and Marder, 2013; Flavell et al., 2013; Harris-Warrick and Marder, 1991; Kennedy et al., 2014; Marder, 2012; Nusbaum and Blitz, 2012; Taghert and Nitabach, 2012; Zelikowsky et al., 2018).

Neuromodulators occupy an ideal position with respect to the control of internal states—they modulate synaptic and cellular function over long timescales because of their impact on biochemical signaling and ion channel function, they can titrate their effects via magnitude of modulator release, and they can act locally as well as send far-reaching diffuse signals across multiple brain regions (van den Pol, 2012). This makes them prime candidates for the flexible, scalable, and persistent control of behavior—key requirements for an internal state.

Foundational principles discovered in reduced invertebrate circuits

Although much of this review focuses on the nervous systems of animals amenable to behavioral study of internal states, it is important to recognize that much of our understanding of neuromodulation derives from the study of invertebrate circuits in reduced preparations—including the stomatogastric ganglion of crustaceans, the swimming central pattern generator of the mollusk, the motor system of the leech, the abdominal and buccal ganglia of the sea slug *Aplysia*, and others (Bargmann, 2012; Bargmann and Marder, 2013; Getting, 1989; Harris-Warrick and Marder, 1991; Kristan and Calabrese, 1976; Marder, 2002, 2012; Marder and Calabrese, 1996; Marder and Thirumalai, 2002; Nusbaum and Blitz, 2012; Taghert and Nitabach, 2012). The experimental access of these circuits, often exhibiting complex and flexible rhythmic dynamics *in vitro*, enable detailed electrophysiological and biochemical analysis of functioning neural networks across states of experimentally induced modulation.

Pioneering studies using these preparations have established that neuromodulators are capable of switching functional networks between different modes of population activity (Dickinson et al., 1990; Eisen and Marder, 1984; Getting, 1989; Getting and Dekin, 1985; Nusbaum and Beenhakker, 2002; Nusbaum et al., 2001; Powell et al., 2021), through extrinsic and local sources of neuromodulation (Katz, 1998; Katz and Frost, 1995, 1996;

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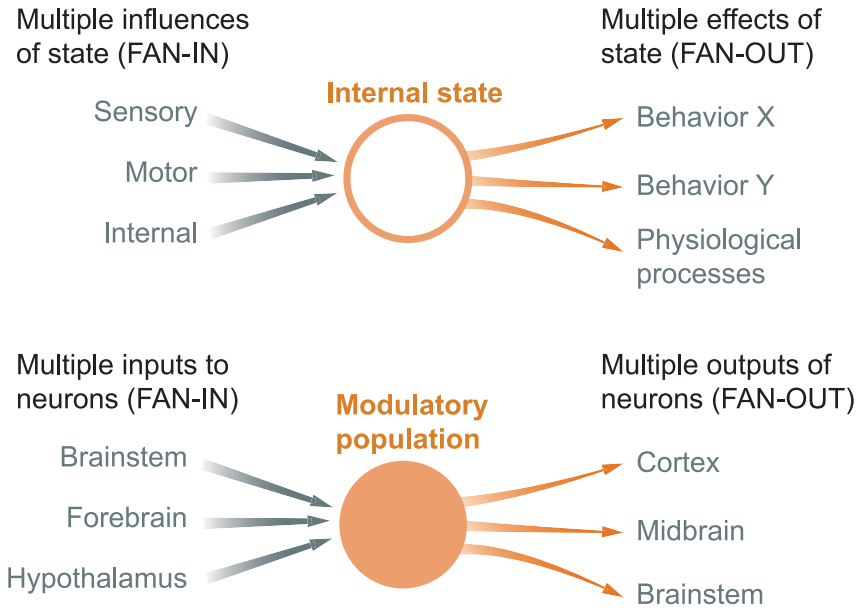

Figure 4. Fan-in and fan-out organization of internal states and neuromodulatory neurons
Top: internal states are influenced by the integration of multiple sensory, motor, and internal factors and themselves influence multiple behaviors and physiological processes. Bottom: similarly, many state-inducing neuromodulatory cell types integrate inputs from multiple brain regions and send outputs to multiple downstream regions.

Katz et al., 1994) that act upon membrane excitability and synaptic transmission (Katz et al., 1994; Martin et al., 1997; Nadim and Bucher, 2014). These neuromodulators exert their effects on multiple neurons and networks in parallel (Brezina, 2010; Harris-Warrick and Johnson, 2010; Harris-Warrick and Marder, 1991; Marder, 2012; Schwarz et al., 1980; Taghert and Nitabach, 2012), and each neuron or synapse is subject to modulation by multiple sources, often with converging effects on common intracellular signaling pathways and ionic conductances (Flamm et al., 1987; Hempel et al., 1996; Kintos et al., 2016; Swensen and Marder, 2000, 2001).

Although we cannot fully discuss the breadth and influence of this literature here, we would like to emphasize how its influence has greatly shaped subsequent work on state-dependent behavior and neuromodulation in larger animals. As we will discuss in the remainder of this section, these pioneering studies identified themes that are present across small and large circuits alike and raise still unanswered questions about how to interpret the complexity and behavioral significance of heavily modulated networks (Getting, 1989; Marder, 2012).

Neuromodulatory systems possess a fan-in/fan-out organization

Most ascending neuromodulatory systems display a characteristic organization in which a relatively small group of neuromodulator-producing neurons receives diverse synaptic inputs and sends diffuse projections to many brain regions (Figure 4; Ren et al., 2018; Saper et al., 2010; Weissbourd et al., 2014). This gives rise to a "fan-in" organization where signals converge onto the neuromodulator-producing neurons and a "fan-out" organization in which the modulators impact many downstream brain regions. This fan-out organization of neuromodulatory systems is observed at the anatomical level in diverse organisms (Figure 5A). For example, in *C. elegans*, the serotonergic neurosecretory motor neuron (NSM) releases serotonin at nonsynaptic neurosecretory terminals that are apposed to the nerve ring—the main neuropil of the worm's brain (Nelson and Colón-Ramos, 2013). In zebrafish, oxytocin neurons project from the hypothalamus to influence multiple regions across the forebrain, midbrain, brainstem, and spinal cord (Herget et al., 2017; Lovett-Barron et al., 2020; Wee et al., 2019a). In mice, multiple monoaminergic neuron types project across the brain (Ren et al., 2019; Schwarz et al., 2015). These are just a few of many examples. This overall organization likely allows neuromodulatory systems to encode the brain states by integrating multiple inputs and exert coordinated control by broadly influencing multiple brain regions simultaneously.

A notable alternative to this organization is local processing distributed across multiple sites, controlled by single (Zelikowsky et al., 2018, see "theme 1" below) or multiple neuropeptide systems. Such distributed effects could be far more prominent than is currently appreciated, driven by widespread expression of neuropeptides and receptors, which has been observed in *C. elegans* (Taylor et al., 2021) and in mammalian striatum (Castro and Bruchas, 2019) and neocortex (Smith et al., 2019). See theme 1 below for more on this topic.

Volume transmission allows neuromodulatory systems to signal diffusely and over long timescales

Another feature of neuromodulatory systems that may endow them with a specialized ability to control internal states is their action through volume transmission. Decades ago, electron microscopy studies of neurons that release biogenic amines, such as dopamine, serotonin, and norepinephrine, revealed that these cells often display putative active zones at nonsynaptic varicosities along their axons (Calas et al., 1974; Descarries and Mechawar, 2000; Descarries et al., 1996). These observations, which have also been made for dense core vesicle release sites in neuropeptide-releasing neurons, suggest that these transmitters can be released extrasynaptically (Oti et al., 2021; Persoon et al., 2018; van de Bospoort et al., 2012). In the case of neuropeptides, release from dendrites has even been observed (Ludwig and Leng, 2006). Many of these transmitters also function at classical synapses and the degree to which they act via synaptic versus extrasynaptic volume transmission varies by brain region (Moukhles et al., 1997). In invertebrate systems, extrasynaptic release sites for amines and neuropeptides are also widely observed (White et al., 1986). In addition, these transmitters can be released into circulating fluid, which allows them to act

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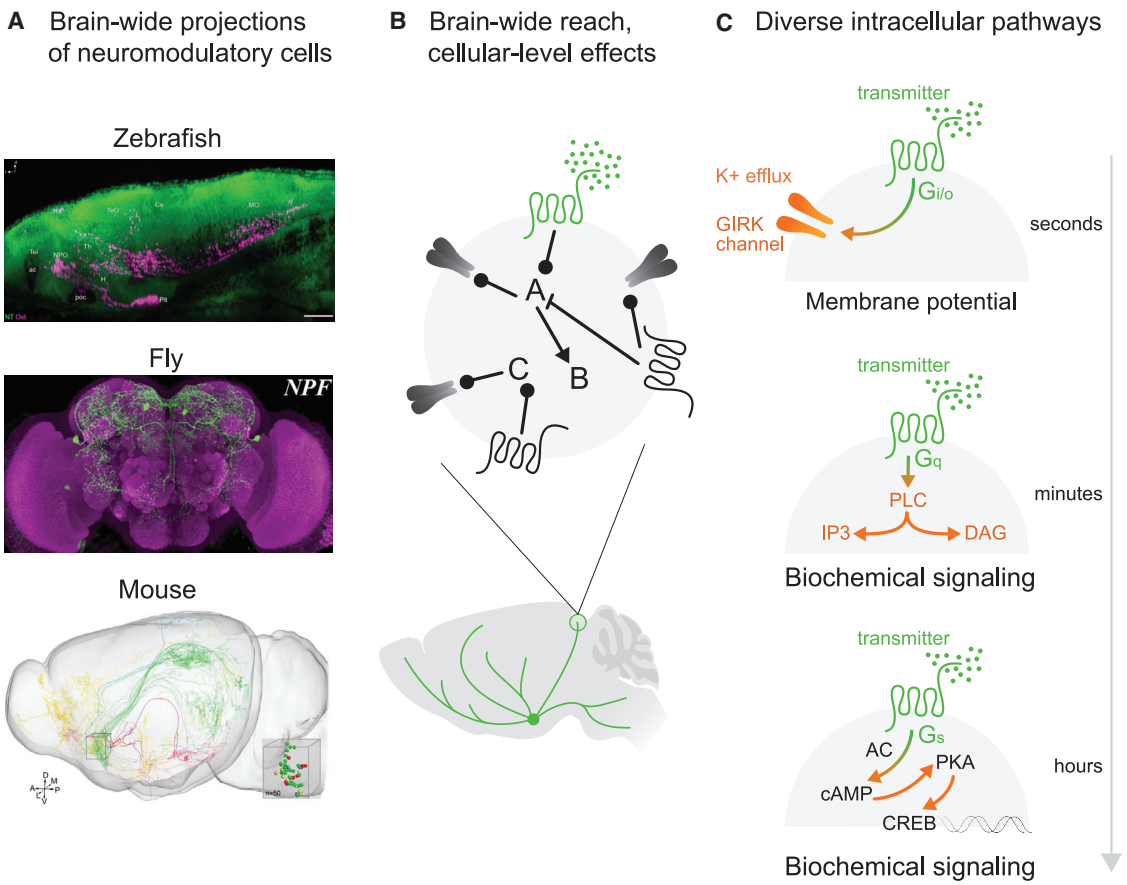

Figure 5. The broad reach and diverse cellular effects of neuromodulators

(A) Examples of broadly projecting neuromodulatory neurons in larval zebrafish (Herget et al., 2017), adult fly (Deng et al., 2019), and mouse (Li et al., 2018).

(B) Neuromodulation can target neurons across the spatial extent of the brain but, within target regions, acts at the scale of intracellular signaling.

(C) Schematics of various neuromodulatory signaling mechanisms in neurons, from rapid (top) to persistent (bottom).

as neurohormones (Kravitz, 2000; Reiter et al., 2014; White et al., 1986).

Extrasynaptic release of neuromodulators could allow these transmitters to diffuse and persist in brain tissue, which might allow for long timescale modulation of target cells. Indeed, the receptors and transporters for these transmitters are commonly localized microns or tens of microns away from active zones (Callado and Stamford, 2000; Liu et al., 2021). Measurements of extracellular amines and neuropeptides, via voltammetry and newer fluorescent sensors (Sabatini and Tian, 2020), support the view that neuromodulators persist in extracellular space for hundreds of milliseconds to many seconds (Bunin and Wightman, 1998; Callado and Stamford, 2000; Park et al., 2011). Work in this area has been most extensive for dopamine, and although recent results support the idea that dopamine can act through volume transmission, the presence of dopamine at levels sufficient to activate its receptors likely only occurs over a micron away from an active zone during synchronous release from multiple nearby active zones (Beyene et al., 2019; Jan et al., 1979; Liu et al., 2021). Estimates of neuropeptide diffusion based on photo uncaging suggest potentially longer-range diffusion (Banghart and Sabatini, 2012). Further studies using recently developed neuromodulator sensors will more precisely clarify these dynamics, which may be critical to internal state control.

Neuromodulators stably alter neuronal excitability to control persistent internal states

In addition to slow diffusion of the ligand, the long timescale action of neuromodulators is also thought to be due the fact that amines and neuropeptides primarily act through metabotropic receptors, which activate biochemical signaling pathways that remain active after receptor activation (Figures 5B and 5C). The activation of these pathways can modulate cellular excitability and a variety of other cellular processes. As described above, the effects of metabotropic signaling on neuronal activity have perhaps been best characterized in the stomatogastric ganglia of crustaceans, where metabotropic pathways converge onto a number of different currents to modulate neuronal excitability. However, classical neurotransmitters can also act through metabotropic receptors, for example, mGluRs, and neuromodulators can sometimes act via ionotropic receptors (Ringstad et al., 2009; Thompson and Lummis, 2006); hence, this feature does not fully distinguish the neuromodulatory systems from other neurotransmitters.

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Nevertheless, neuromodulator-dependent activation of metabotropic signaling has been directly linked to the generation of internal states.

Related to persistent internal states, neuromodulator-induced activation of metabotropic signaling is known to regulate persistent neural activity in many systems. For example, in the presence of a muscarinic agonist, current injection into mammalian layer V entorhinal neurons elicits a remarkably stable increase in firing rate that can occur in a graded manner (Egorov et al., 2002). In the presence of serotonin, spinal motoneurons display bistable activity (Hounsgaard and Kiehn, 1989). In *Drosophila*, dopamine acting through the Dop1R2 receptor and downstream potassium channels can stably alter the excitability of the dorsal fan-shaped body neurons to control sleep (Pimentel et al., 2016). In the striatum, dopamine persistently elevates the excitability of D1 receptor-expressing striatal projection neurons (Lahiri and Bevan, 2020). Indeed, metabotropic regulation of firing modes appears to be a common property of neurons (Derjean et al., 2003). *In vivo* electrophysiological studies of thalamic and cortical contributions to arousal states also support a role for neuromodulatory systems in eliciting stable activity (McCormick, 1992; McCormick and Prince, 1986; Pape and McCormick, 1989; Steriade et al., 1993). Behavioral state-correlated activation of cholinergic and noradrenergic axons in cortex is associated with sustained depolarizations in pyramidal cells (Goard and Dan, 2009; Meir et al., 2018; Pinto et al., 2013; Polack et al., 2013). Overall, these studies provide evidence that neuromodulatory control of persistent neural activity contributes to the generation of internal states.

Neuromodulators stably alter biochemical signaling to control persistent internal states

Studies linking neuromodulator-induced biochemical signaling to internal states have been most extensive for the cAMP-PKA pathway. Fluorescent sensors of cAMP levels and PKA activation have revealed persistent increases in cAMP levels and downstream signaling with kinetics on the order of tens of seconds to minutes in freely moving flies (Thornquist et al., 2021) and mice (Lee et al., 2019; Zhang et al., 2021). These kinetics have been tied to internal state generation in several organisms.

One example is the set of Corazonin neurons in *Drosophila*, a small group of neurons controlling the animal's drive to copulate. Graded accumulation of cAMP in these neurons over minutes during successive activity bouts can trigger a synchronous burst of network activity, or eruption, that changes the motivational state of the fly such that its copulation drive is reduced (Thornquist et al., 2021). Optogenetic elevation of cAMP levels in Corazonin neurons can elicit this state transition. Another example is from the zebrafish brainstem, where stable accumulation of evidence also occurs downstream of alpha-1B adrenergic receptors in radial glia, where noradrenaline release during successive futile actions stably increases glial calcium levels to elicit a transition to a passive behavioral state (Mu et al., 2019). Long-lasting activation of astrocytic signaling in mammalian circuits has also been linked to stable states of neural activity (Deemyad et al., 2018), suggesting that this may be a recurring mechanism for stable accumulation of persistent activity. Finally, a recent study of mating drive in male mice showed that stable increases in cAMP occur in MPOA neurons after transient hypothalamic dopamine release activated by a social encounter with a female (Zhang et al., 2021). This then triggers a stable state of motivation to mate, whose kinetics match cAMP kinetics in MPOA neurons. Together, these studies highlight how the timescale of biochemical signaling is closely linked to the persistence of internal states.

Other stable neuronal signaling pathways also contribute to behavioral state generation. Activation of the calcium-dependent protein kinase CaMKII in *Drosophila* Corazonin neurons delays a motivational state change that terminates copulation until 5–7 min after copulation begins (Thornquist et al., 2020). Interestingly, previous work has shown that CaMKII activation initially requires elevated calcium levels, but the activation of the 12-subunit CaMKII holoenzyme can be sustained in a calcium-independent manner through autophosphorylation of adjacent subunits, allowing for stable, minutes-long activation of the enzyme (Lisman et al., 2012; Miller and Kennedy, 1986). Sustained activation of CaMKII in Corazonin neurons detected through fluorescent reporter imaging was shown to have a causal effect on the timing of the motivational state transition of the fly. This work demonstrates how stable biochemical pathways within neurons can influence network activity and internal states.

Gene expression changes across internal states

Although stable, activity-induced changes in gene expression are essential for lasting behavioral changes during long-term memory and circadian timing (Dubowy and Sehgal, 2017; Yap and Greenberg, 2018), the role of dynamic gene expression in persistent internal states is less well studied. However, changes in gene expression have been notably detected across feeding states. For example, feeding state-dependent changes in neuromodulator (Entchev et al., 2015) and chemoreceptor (Sengupta, 2013) expression in *C. elegans* have been linked to satiety-related behavioral changes. Similarly, food deprivation alters the expression of hundreds of genes in AGRP neurons of the hypothalamus (Henry et al., 2015). Gene expression changes in LH are even associated with the onset of obesity over days (Rossi et al., 2019).

Gene expression changes have also been linked to other motivational drives, for example, the drive to copulate in *Drosophila*. Abstinence from copulation elicits an increase in activation of the neural activity-dependent transcription factor cAMP response element-binding protein (CREB) in a group of neurons that form a recurrent loop (Zhang et al., 2019). The stable expression of a CREB-induced potassium channel then influences mating behavior for hours to days after animals have mated and CREB activation has subsided. Given that activity-dependent transcription is a ubiquitous feature of neuronal gene expression and that it can reflect historical patterns of neural activity in a surprisingly precise manner (Brigidi et al., 2019), it may play a similar role in the control of other drive states. Given that these activity-dependent pathways are also known to regulate structural plasticity, future work may be aimed at examining whether internal states are accompanied by structural changes in neural circuits. Overall, the links between neuromodulator-induced biochemical signaling and internal state generation are now becoming

apparent, but our understanding of this relationship is still in its infancy.

EMERGING THEMES OF INTERNAL STATE CONTROL ACROSS SPECIES

Despite substantial variability among internal states within an organism and across different organisms, there exists a striking commonality in how some of these states are organized in the brain. Indeed, recent studies have identified several examples of common neural mechanisms that contribute to internal state control.

Theme 1: Internal states influence multiple circuits and cell types in parallel

Although the predominant view of internal states favors a "hub and spoke" type of "fan-out" mechanism (highlighted above), there is evidence for the control of internal states in a more distributed, parallel action manner. Here, we highlight a few key examples.

Above, we highlighted how neuromodulators can act locally within a given brain region to exert control over behavior. However, there is growing evidence that neuromodulators can exert their state-like control over behavior in a distributed manner across numerous brain regions simultaneously. For example, Zelikowsky and colleagues identified a role for the neuropeptide tachykinin 2 (Tac2) in the control of an internal brain state produced by prolonged social isolation stress (Zelikowsky et al., 2018). Using a multiplex approach employing a variety of loss-of-function techniques and testing multiple behaviors, the authors discovered that Tac2 signaling is necessary and sufficient for the effects of social isolation to produce enhanced aggression, persistent fear, and acute fear responses. Importantly, the authors found that each isolation-altered behavior was independently controlled by Tac2 signaling in distinct brain regions This "web-like" distributed, local circuit organization has also been shown to control additional states and systems.

One prominent example is the role of the neuropeptide PDF in the control of circadian rhythms. Indeed, PDF has been shown to coordinate the phase and amplitude of circadian rhythms through its action on separate populations of cells across the fly brain (Lin et al., 2004). Importantly, PDF operates in a distributed manner across the fly brain, providing unified and organized control over circadian rhythms in flies despite the unique effects that PDF exerts in a region-specific manner (Taghert and Nitabach, 2012). Local, distributed neuromodulation has also been recently studied in the context of rodent fear behavior, where disinhibitory interneurons in several neocortical regions have been found to be excited by local and afferent sources of the neuropeptide gastrin-releasing peptide (GRP) (Melzer et al., 2021). In the auditory cortex, GRP receptor signaling facilitates auditory fear conditioning, and the role of GRP signaling in other regions remains to be investigated.

Collectively, these studies highlight the potential biological benefit of a dispersed internal state, wherein separate behaviors can be controlled via distinct brain regions yet remain in concert with each other through overarching control by a single neuropeptide system. Although it is highly likely that in such examples additional signaling molecules are coreleased along with these neuropeptides (see theme 2 below), the ability of a single neuropeptide to exert large-scale effects across the brain and behavior is nevertheless striking.

Recent work has also shown that single neuromodulators are capable of controlling distinct internal states in different contexts. For example, although Tac2 has been implicated in the control of the state produced by prolonged social isolation (see above), work by Andero and colleagues has also identified a role for Tac2 signaling in the CeA in the fear state produced by exposure to footshock (Andero et al., 2014, 2016). Similarly, although PDF has been implicated in the regulation of circadian rhythms (see above), additional work by Flavell and colleagues using genetic screens, quantitative behavioral analyses, and optogenetics also identified a role for PDF in the control of roaming behavior in worms (Flavell et al., 2013). This pattern of neuropeptidergic "multipurposing" can be found in the identification of oxytocin in pair-wise bonding (Donaldson and Young, 2008; Froemke and Young, 2021; Insel and Young, 2001) but also maternal behavior (Marlin et al., 2015), fear (Pisansky et al., 2017), and other states. Finally, in a series of seminal studies, Galanin^{+} neurons in the MPOA were identified in the control of parental behavior in both males and females (Kohl et al., 2018; Kohl and Dulac, 2018; Wu et al., 2014), whereas Galanin^{+} neurons in the ventrolateral preotic area have been found to promote sleep and heat loss (Kroeger et al., 2018).

Overall, these examples highlight diversity in function and internal state control for single neuropeptides operating across the brain to control a single state, as well as the ability of a single neuropeptide to be "repurposed" to serve in the formation of multiple internal states. This diversity can range across brain regions and even species. Importantly, although it is tempting to assign one-to-one pairings between individual neuromodulators and internal states, this appears to be an oversimplification. In particular, neuromodulatory repurposing further reinforces the notion that neuromodulators—with their physiological properties, brain-wide networks, region specificity, and slow-release, persistent signaling properties—are ideal candidates for the control of internal states and their effects on behavior.

Theme 2: Neuromodulators act in concert

Many of the studies discussed in this review highlight the functional role of individual cell types and neuromodulatory transmitters, suggesting that each of these neuromodulatory systems plays a unique role in whatever state or behavior was examined. This is unlikely to be the case. One of the most salient lessons from the study of small invertebrate circuits is that neurons and synapses are modulated by multiple substances (Getting, 1989; Harris-Warrick and Marder, 1991; Marder, 2012), and their interactions produce emergent effects that are not easily predicted from the actions of one modulator alone (Flamm et al., 1987; Hempel et al., 1996; Kintos et al., 2016; Swensen and Marder, 2000, 2001).

Why this discrepancy between the small-circuit literature and more recent studies of neuromodulatory systems? A possible reason may be the bias of common laboratory techniques. Modern studies of neuromodulation often use genetic model systems, such as those discussed extensively here (worms, flies, fish, and mice), whose power comes from the specificity they afford: the ability to study a single genetically or anatomically

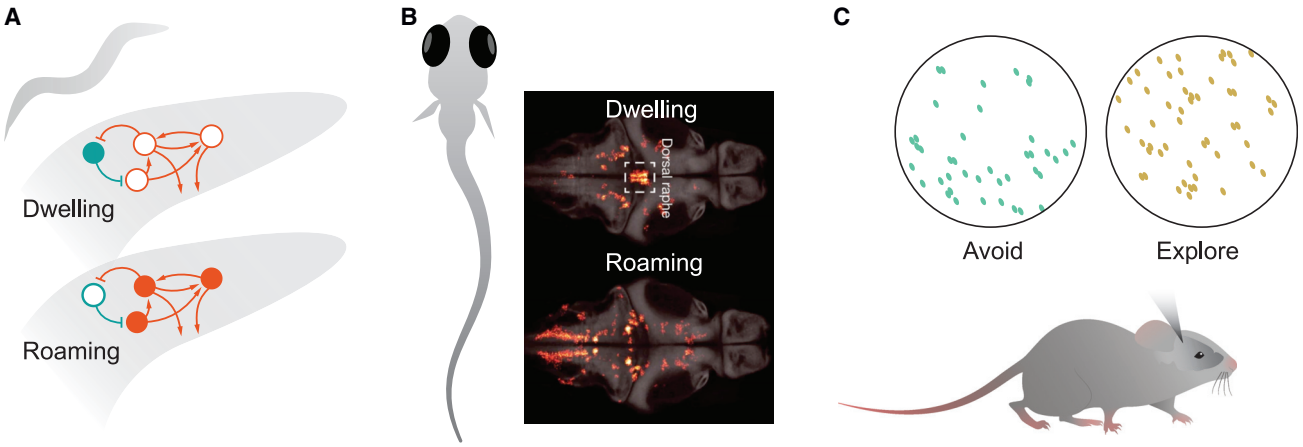

Figure 6. Opposing brain states engage mutually exclusive neural populations
(A) Roaming and dwelling states in *C. elegans* are supported by opposing sets of neurons that mutually inhibit each other (Ji et al., 2021).
(B) Separate brain-wide populations regulate roaming versus dwelling states in hunting larval zebrafish (Marques et al., 2020).
(C) Exploration versus anxiety engage different populations of neurons in the mouse amygdala (Gründemann et al., 2019).

defined cell type or analyze the actions of specific transmitters and receptors (Luo et al., 2018; Sabatini and Tian, 2020). In contrast, classical studies in small invertebrate circuits primarily used bath-applied neuromodulatory transmitters and hormones, allowing for the study of multiple transmitter actions.

We have reason to believe, however, that an accounting for ubiquitous comodulation will become more prominent in genetic model systems as well. For instance, in rodents, single-cell RNA sequencing has emphasized the fact that each cell expresses a large number of neuromodulatory receptors (Campbell et al., 2017; Henry et al., 2015; Kim et al., 2019; Moffitt et al., 2018; Saunders et al., 2018; Smith et al., 2019), and viral strategies allow investigators to control multiple independent cell types in the same animal (Luo et al., 2018). Furthermore, recent studies combining live functional imaging with *post hoc* registration to multiple gene expression markers (Bugeon et al., 2021; Lovett-Barron et al., 2017, 2020; von Buchholtz et al., 2021; Xu et al., 2020) provides the opportunity to image multiple genetically defined cell types at once. In larval zebrafish, this approach has demonstrated that multiple neuromodulatory cell types are coactive during states of heightened alertness (Lovett-Barron et al., 2017), and many hypothalamic neuropeptide-producing cell types are coactive across various homeostatic threats (Lovett-Barron et al., 2020).

We believe that an appreciation of comodulation will move the field away from the perspective of studying neural circuits as "labeled lines"—an approach so useful in the understanding of sensory systems and reflexes—and toward an understanding of modulated circuits as an emergent state produced by multiple interacting neuromodulatory effects (Getting, 1989; Harris-Warrick and Marder, 1991; Marder, 2012).

Theme 3: State transitions engage mutually exclusive neural populations

One common mechanism in the neural encoding of global brain states is the switching between largely mutually exclusive populations of neurons that encode opposing states. This is observed across species and brain states, including well-studied examples of sleep-state switching in mammals (Saper et al., 2010; Weber and Dan, 2016), zebrafish (Oikonomou and Prober, 2017), and invertebrates (Shafer and Keene, 2021) as well as mutually exclusive populations of neurons encoding hunger states in the zebrafish hypothalamus (Wee et al., 2019b) and distinct populations that encode separable internal states of social engagement in the mouse (Karigo et al., 2021).

The distinction between roaming and dwelling has been studied across species, where distinct neural populations produce these opposing states: exploration of large spaces in search of resources ("roaming") versus exploiting local resources by staying in place ("dwelling"). In freely moving *C. elegans*, the roaming-inducing neuropeptide PDF and dwelling-inducing monoamine serotonin (Flavell et al., 2013) recruit distinct populations of neurons that are active in a mutually exclusive manner to promote each behavior (Ji et al., 2021; Figure 6A). Of note, the neurons that generate these opposing neuromodulators mutually inhibit one another to generate this two-state system. Similarly, brain-wide imaging in freely swimming zebrafish larvae (Kim et al., 2017b) also revealed a pattern of mutually exclusive populations across the midbrain, diencephalon, and brainstem that encode long-lasting roaming and dwelling states during hunting behavior, as well as neurons that signal the transition from roaming/exploration to dwelling/feeding (Marques et al., 2020; Figure 6B). As in *C. elegans*, serotonergic neurons were implicated in initiating dwelling states. Finally, population imaging in the mouse amygdala revealed that across behavioral contexts, mutually exclusive populations of neurons encode general states of roaming-like exploratory movement and dwelling-like defensive behaviors (Gründemann et al., 2019; Figure 6C).

Together, these studies indicate that mutually exclusive internal states can be encoded in the opposing activity of neuronal populations. However, these "flip-flop" dynamics may not generalize to internal states that exhibit continuous variation or interactions with other states that are not mutually exclusive.

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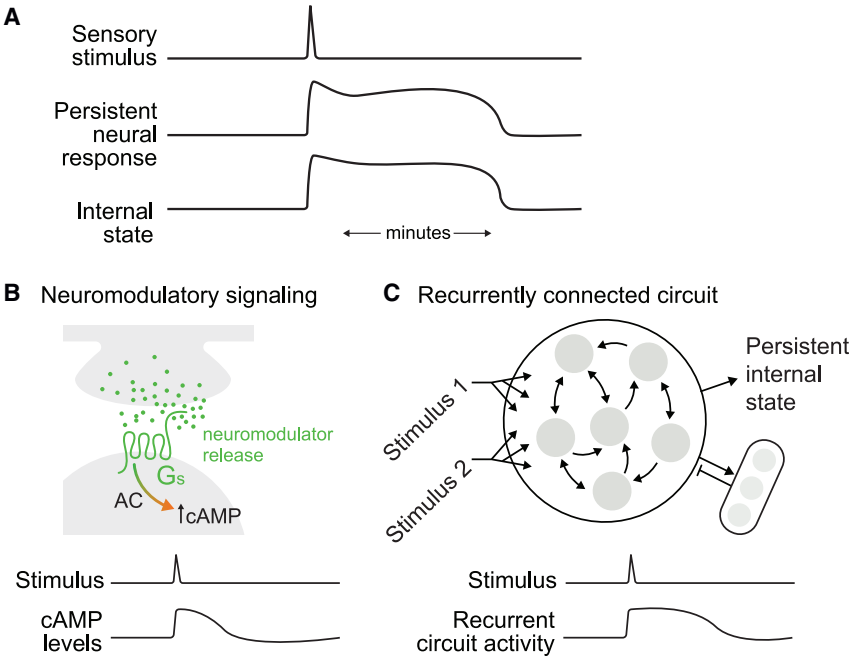

Figure 7. Multiple mechanisms can support the persistence of internal states

(A) Schematics of persistent neural and behavioral responses to transient sensory stimuli.

(B) One potential mechanism for generating neuronal persistence is slowly evolving biochemical signaling within neurons, which has been demonstrated to control the persistence of internal states in flies and mammals (Zhang et al., 2019, 2021; Thornquist et al., 2021).

(C) Another potential mechanism is recurrent excitation among interconnected neurons, as has been recently demonstrated to maintain persistent defensive behaviors in flies and rodents (Jung et al., 2020; Kennedy et al., 2020).

The population dynamics and switching mechanisms underlying these states are not yet well explored.

Theme 4: State persistence through recurrent dynamics

It has long been recognized that neural circuits with recurrent excitation might be able to generate stable neural responses to transient inputs (Joshua and Lisberger, 2015). For example, transient motor signals that move the position of the eye are received by a recurrently connected neural integrator circuit whose activity is persistently altered to maintain the position of the eye (Aksay et al., 2007; Miri et al., 2011). Recent work has now highlighted the importance of recurrent excitation for the generation of persistent internal states.

Studies of a neural circuit that controls behavioral states in female *Drosophila* provide new evidence that recurrent excitation is important for the generation of internal states. Activation of pC1 neurons in female flies elicits increased female receptivity to males and increased shoving and chasing, even several minutes after the optogenetic stimulus has terminated (Deutsch et al., 2020). Distinct subsets of pC1 neurons control female receptivity versus shoving and chasing behaviors. Interestingly, a brain-wide imaging approach revealed that activation of the pC1d/e neurons that control shoving and chasing induced persistent activity in many downstream brain regions, in addition to pC1 neurons themselves. A connectomic analysis showed that pC1 neurons are part of a recurrently connected neural circuit, with prominent reciprocal connections to aIPg-b and aIPg-c cells, which are also interconnected with one another. As all of these cell types are excitatory (Schretter et al., 2020), this suggests that pC1 is a functionally important node in a recurrently connected circuit that elicits a persistent behavioral state.

In male *Drosophila*, activation of a stable, recurrently active circuit also underlies behavioral state generation. Activation of the P1 interneurons elicits a minutes-long internal state that consists of elevated courtship and aggression (Clowney et al., 2015; Hoopfer et al., 2015). Although P1 neurons are not persistently active during this state, a group of downstream neurons, named pCD neurons, exhibit long-lasting activation during this internal state (Figure 7A; Jung et al., 2020). Activity in these neurons is required for stable behavioral changes during the P1-induced state and transient inactivation of pCD neurons attenuates their persistent neural response to P1 activation, providing evidence that continued pCD activity supports its own persistence. Transient inactivation of pCD neurons also suppresses persistent aggressive behavior elicited by recent exposure to a female fly. This study highlights how neural circuits with recurrent excitation can maintain a persistent internal state.

Studies in mammals have also implicated recurrent connectivity in the control of internal states. Activation of VMHdmSF1 neurons in the VMH can elicit a state of fear or anxiety (Kunwar et al., 2015). As a group, the VMHdmSF1 neurons show persistent activation in response to social sensory cues that can evoke an anxiety state (Kennedy et al., 2020). However, the dynamics of the neurons within this population vary, with some neurons displaying immediate onset activation and others ramping slowly. Moreover, neurons in the population respond differently to different social cues. Several computational models were constructed to determine whether they could recapitulate features of the population activity. Interestingly, only the models that included recurrent connectivity and neuromodulation were able to do so, suggesting that recurrent connectivity and neuromodulation may co-occur in this circuit to support stable population dynamics (Figure 7B). It is worth noting that there is an additional similarity between P1 interneurons and VMH neurons, which is that they can both induce different behavioral states in different sensory contexts. This specific topic has been reviewed previously in Anderson (2016).

Although we note examples here of state persistence driven by recurrent circuits, persistence can also be achieved by neuromodulatory control of cellular excitability (as discussed above). It is not well understood whether these mechanisms are

interdependent or used in different cases to achieve similar outcomes depending on the contexts, circuits, or timescales involved.

CONCLUSIONS

In this review, we have discussed our current understanding of internal states: how they are defined, measured, generated by neurons, as well as how they affect the brain and behavior. Building upon the insights from many other authoritative reviews about internal states (Anderson, 2016; Bargmann, 2012; Bargmann and Marder, 2013; Getting, 1989, etc.; Lee and Dan, 2012; Marder, 2012; McCormick et al., 2020; McGinley et al., 2015a; Taghert and Nitabach, 2012; Tye, 2018, etc.), here, we have emphasized advances in the classification of internal states, the insights from studying brain-wide populations, and some of the many biological mechanisms through which neuromodulators can influence states. Importantly, we have emphasized common principles found across model species.

Although the field has made enormous progress, many fundamental questions about internal states and their neural basis remain unanswered or completely unexplored. How do sensorimotor circuits integrate state-relevant information to drive adaptive behavioral responses? To what extent do neuromodulators have unique versus redundant effects? Are brain-wide dynamics required for the expression of states or just a consequence of a massively interconnected brain? Why are some states controlled by a handful of neurons, whereas others are controlled by neurons distributed across multiple brain regions?

As the field resolves these mechanistic questions, it may be important to reflect on the challenges of defining internal states. How do different co-occurring states interact with each other, and would it be more useful in certain instances to simply refer to the animal's overall state? Can states always be inferred from behavior and/or physiology? When do measurements of the brain, behavior, and physiology reflect the same underlying state and when do they reveal unexpected distinctions? Is there a true distinction between motor actions, sequences of motor actions, and states, or does behavior simply unfold along a continuum of timescales? Can behavior in natural environments be adaptive in the absence of long timescale state organization?

One key issue regarding the definition of internal states is their degree of independence. How do we know that fear represents a unique internal state, distinct from others such as anxiety? Is the ability to distinguish such states dependent on the tools we use for measuring their observable output? Would we be able to further splinter internal states into smaller substates if we had better tools? How does selection of model organism affect our ability to isolate and define an internal state? Given the wide variability in model organisms as well as experimental approaches, would we benefit from a definition of internal states as they pertain to biological relevance and their importance to survival?

These questions and more can be addressed using the emerging methodological approaches discussed herein, including more rigorous quantification of states using integrated datasets and ML approaches, precise observation and control of electrical and biochemical activity across entire nervous systems, and better theoretical frameworks understanding the utility of internal states.

As with any search for common principles in biology, this field of neuroscience will benefit greatly from studying an expanded set of animal species, challenging animals with more natural and varied behavioral conditions, and welcoming scientists to approach these questions with diverse views, expertise, and experiences.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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