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Review
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A Framework for Studying Emotions across Species

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Since the 19th century, there has been disagreement over the fundamental question of whether "emotions" are cause or consequence of their associated behaviors. This question of causation is most directly addressable in genetically tractable model organisms, including invertebrates such as *Drosophila*. Yet there is ongoing debate about whether such species even *have* "emotions," as emotions are typically defined with reference to human behavior and neuroanatomy. Here, we argue that emotional behaviors are a class of behaviors that express internal emotion states. These emotion states exhibit certain general functional and adaptive properties that apply across any specific human emotions like fear or anger, as well as across phylogeny. These general properties, which can be thought of as "emotion primitives," can be modeled and studied in evolutionarily distant model organisms, allowing functional dissection of their mechanistic bases and tests of their causal relationships to behavior. More generally, our approach not only aims at better integration of such studies in model organisms with studies of emotion in humans, but also suggests a revision of how emotion should be operationalized within psychology and psychiatry.

> "Even insects express anger, terror, jealousy and love, by their stridulation."—Charles Darwin, The Expression of the Emotions in Man and Animals

Introduction

The ongoing revolution in the development of genetically based tools for studying the activity, anatomy, and function of neural circuits in diverse model organisms has opened up new vistas into the mechanistic study of fundamental brain processes historically rooted in psychology, such as perception, cognition, learning, and memory. One of the most intriguing yet elusive of these processes is emotion. The paradox of emotions is that, on the one hand, they seem self-evident and obvious when examined introspectively; on the other hand, they have been extremely difficult to define in objective scientific terms. Attempts to achieve a consensus definition that is accepted across fields from neuroscience to psychology to philosophy have repeatedly failed, to the extent that at least one prominent emotion researcher has suggested that we excise the word "emotion" altogether from our scientific vocabulary (LeDoux, 2012). Yet this would deprive the study of fundamental aspects of animal and human behavior of a unifying topic, preventing comparisons. But how can we study a topic so important if we cannot even agree on operational criteria for what it is?

Most researchers would probably agree that emotions include (but are not limited to) certain expressive behaviors that are associated with internal brain states that we, as humans, subjectively experience as "feelings" (Dolan, 2002). Such behaviors in humans include facial expressions such as frowning, vocalizations such as screaming or sobbing, and physiological expressions such as tearing or blushing. Identifying instances of emotional expression is intuitively obvious to a lay person. Darwin, in his 1872 monograph The Expression of the Emotions in Man and Animals, was the first to consider the unique nature of emotional expression from the functional and evolutionary standpoint. He assumed that instances of emotional expression are easily recognizable not only in humans (Figure 1A), but also in closely related mammalian species such as chimpanzees, as well as in domestic pets such as cats and dogs (Figures 1B–1D). In fact, Darwin went further and asserted that, even in insects, certain behaviors such as stridulation reflect the expression of emotions homologous to our own (such as "anger" and "terror"). However, in so doing, he provided no consistent, operational criteria for identifying instances of emotional expression in such evolutionarily distant species, other than his own intuition—much of which was based on unabashed anthropomorphizing. But arriving at such objective criteria would seem essential if we are to apply the powerful genetic tools available in invertebrate model organisms, such as *C. elegans* or *Drosophila*, to understand the evolutionary origins and neurobiological underpinnings of emotion. The principles that are learned from the use of such model organisms could generalize across phylogeny, including

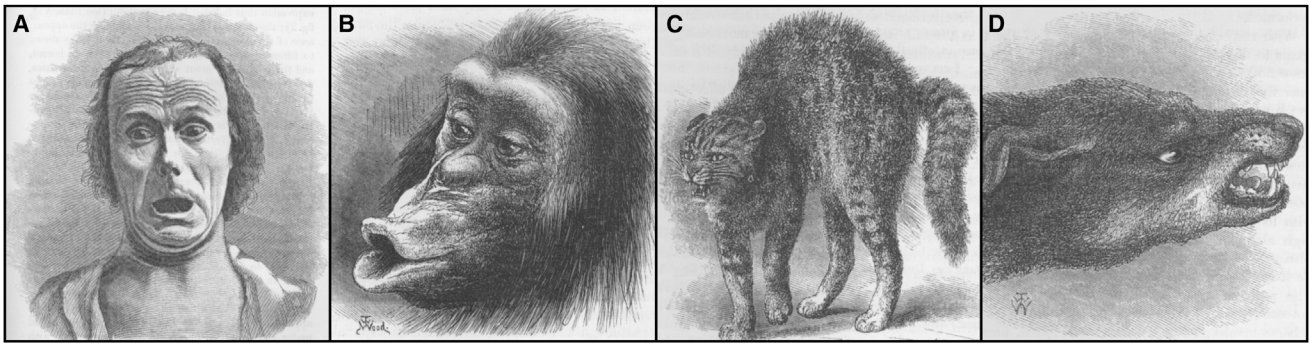

Figure 1. Charles Darwin's Examples of Emotional Expressions
(A) Expression of terror in a human. (B) Chimpanzee "disappointed and sulky." (C and D) hostility in a cat (C) and a dog (D). From Darwin (1872).

humans, and may even shed light on psychiatric illnesses such as mood and anxiety disorders.

Emotions are Central, Causative States

Here, we will argue that an "emotion" constitutes an internal, central (as in central nervous system) state, which is triggered by specific stimuli (extrinsic or intrinsic to the organism). This state is encoded by the activity of particular neural circuits that give rise, in a causal sense, to externally observable behaviors, as well as to associated cognitive, somatic, and physiological responses (Figure 2B). This view differs from the majority of psychological accounts of emotion (e.g., Russell, 2003; Scherer, 2009; Barrett and Russell, 1999; Barrett et al., 2007), as well as some neurobiological accounts (Salzman and Fusi, 2010), which typically conceive of an emotion as encompassing all of these effects, notably including the subjective experience (Figure 2A). Indeed, according to many views, emotional experiences are a consequence, not a cause, of the various responses that are evoked by particular stimuli (Box 1).

We agree with Darwin that phylogenetically distant, invertebrate model organisms have primitive emotion states that are expressed by externally observable behaviors. However, in contrast to Darwin, we argue that, in such organisms, these primitive emotion states are not necessarily homologous to the specific psychological categories that define human emotions (fear, anger, happiness, and so forth). Rather, these states have certain fundamental properties, which we term "emotion primitives," or evolutionary building blocks of emotion, which are shared across emotions and across phylogeny, even if the species-typical behaviors that express them are not. According to our view, therefore, the question is not whether flies have "fear" or some other emotion present in humans that one should try to "model" in *Drosophila* (Iliadi, 2009) but, rather, whether they have central states that have features that are characteristic of emotion states in general. If so, then one can begin to apply the tools available in invertebrate models to mechanistically dissect the neural circuit basis of these central states and to test directly their causal relationship to observable behavior. This approach allows us to investigate general features of emotion using model organisms without having to link them to anthropocentric labels like "fear," "anger," or "sadness."

To develop this view, we will address several issues that are central to arriving at operational criteria for emotion that are applicable across phylogeny. These include: (1) the causal relationship between emotions and observable behavior; (2) the relationship between emotion states and subjective "feelings" in humans; (3) the characteristic features of emotion states that generalize across specific emotions; (4) whether there are uniquely human features of emotion.

Our hope is to suggest a way of thinking about emotion, and its evolution, which will facilitate its study at the neural circuit level in model organisms. This would allow rapid progress because of the new methods available for imaging and manipulating neural circuit analysis in such systems (e.g., Venken et al., 2011), as well as quantitative and objective, machine vision-based methods for measuring the behavior of such model organisms (Dankert et al., 2009; Branson et al., 2009; Kabra et al., 2013). Most importantly, we seek to provide a unified view of emotion that would afford more cohesion with the study of this topic in mammalian systems, including humans.

The Relationship between Emotion States and Observable Behavior

> "Certain states of the mind lead…to certain habitual movements."—Darwin

Much of the literature on emotion is confusing for two reasons. One reason is that there is disagreement about the causal direction in which behavior is related to emotion. A second is that there is equivocation regarding the difference between emotions and feelings. In the next sections, we briefly clarify our view of the relationship of central emotion states to emotional behaviors and to subjective feelings.

As mentioned earlier, emotional behaviors can be thought of as a class of behaviors that are associated with internal states. A central issue in the debate over emotions has been the question of the direction of causality between these behaviors and states. A common lay intuition is that the state causes the expression: I cry *because* I am sad. As reflected in the quotation above, Darwin shared this intuition, but it is not the predominant psychological view of emotions (Figure 2A), which typically makes the behavior a part—and even a cause—of the emotion. Most famously, the American psychologist William James

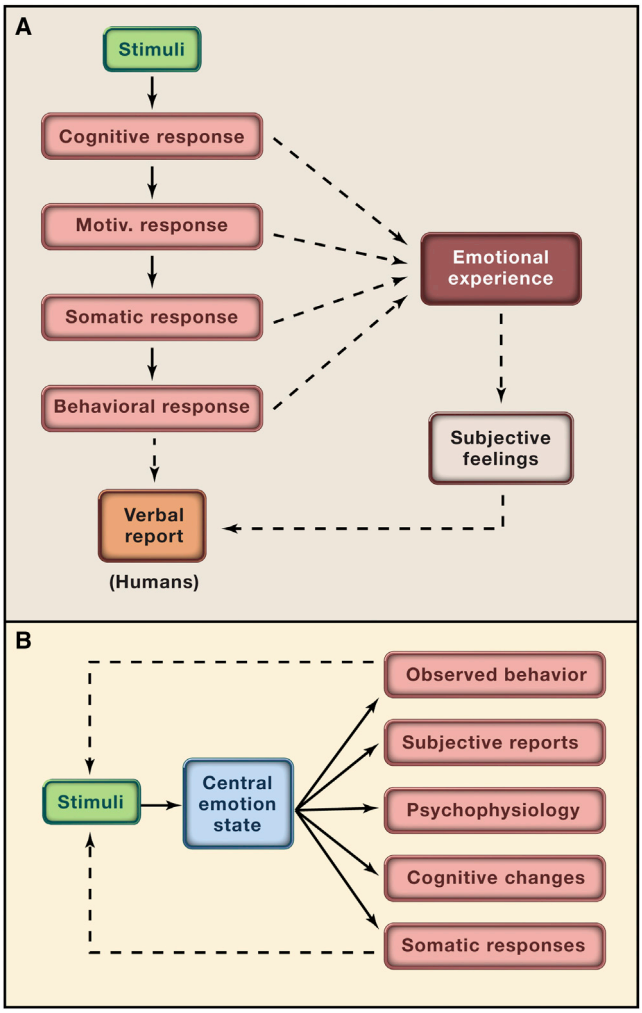

Figure 2. Emotions as Central, Causative States

(A and B) Proposed (B) and alternative (A) views of the causal relationship between emotions and behavior.

(A) In more conventional views, emotions are distinguished by multiple components that need to be coordinated and often synchronized (Barrett et al., 2007; Russell, 2003; Scherer, 2009; Salzman and Fusi, 2010). Although we agree that emotions involve all these components, our view differs in not including these components as part of the emotion state itself but, rather, as consequences of it. Reproduced with modification from Moors (2009).

(B) In our model, a central emotion state causes multiple parallel responses. "Stimuli" include both exteroceptive and interoceptive (feedback) components. Reproduced with permission from Calder et al. (2001).

(1884) argued that the direction of causality is, in fact, the reverse of what one might think: "I feel 'afraid' because I run from the bear; I do not run because I feel afraid," goes the famous (albeit oversimplified) paraphrase of his theory. In other words, to the extent that subjective feelings are equated with emotions in humans (but see below), these feelings are a consequence, not a cause, of observable expressive behaviors (Figure 3B). Although this view of the relationship between emotion and behavior may seem counter-intuitive and others have argued against it (Cannon, 1927; Panksepp, 1998), it remains a defended view.

Box 1. Psychological Theories of Emotion

Psychological theories of human emotion have emphasized the multicomponent nature of emotions, typically including subjective experience and neurophysiological processes, as well as somatic and endocrine ones (Barrett et al., 2007; cf. Figure 2A). For instance, "appraisal theories" have proposed architectures for how these diverse components might be related, often in a specific adaptive sequence of so-called stimulus evaluation checks (Lazarus, 1991; Scherer, 2009). In brief, the idea is that an organism continuously evaluates a stimulus within a context; this process is not analogous to a quick snapshot categorization that results in a single, final emotion state. Instead, it is more akin to the continuing layers of experience that a wine connoisseur might experience upon savoring a good wine. There is some evidence for such a sequential evaluation from studies of the dynamics of human facial expressions, but simpler examples are abundant in animal behavior (such as the example of the octopus fleeing that we mention in the text).

Two points are important to make in relating appraisal theory to our view. First, in agreement with what we write here, appraisal theory stresses that emotions involve highly coordinated (often synchronized) effects in behavior, body, and brain. The flexibility of emotions seen in pleiotropy, stimulus degeneracy, and trans-situationality emphasizes this aspect. Second, in disagreement with our view, appraisal theory takes all of these varied effects to be literally part of the emotion state (cf. Figure 2A), whereas we view them as consequences that are caused by a central emotion state (Figure 2B). Appraisal theory bears considerable resemblance to the kind of decision tree envisioned by the ethologist Niko Tinbergen (Tinbergen, 1950). A fruitful direction for research would be to determine the extent to which the emotion states found in different species are indeed hierarchically organized.

This is not to say that behavior cannot also influence emotion states: of course, our behaviors, once expressed, become stimuli in their own right, and there is a causal loop from emotion states to behaviors and back to emotion states (dashed lines in Figure 2B). Indeed, some theories argue from this fact that emotion states are so dynamic that it becomes impossible to say whether the behavior is cause or consequence (Salzman and Fusi, 2010). This disagreement over causality is, in part, a result of the purely observational approaches that have been used to study the link between emotions and behavior in the field of psychology. In contrast, the virtue of studying the neural basis of primitive emotion states in model organisms is that one can directly and rigorously test the causal relationship between such states and behavior through functional manipulations of the neural components of such states. We also believe that, insofar as these primitive emotion states ultimately led to human emotions through evolution, a similar analysis may be possible in phylogenetically diverse organisms, provided that such functional manipulations are possible. New technologies for genome modification, such as CRISPR/Cas9 (Gaj et al., 2013), may make genetic manipulations of neuronal activity more feasible in a variety of animal species.

The Relationship between Central Emotion States and Subjective Feelings

A frequent point of confusion in arguments about emotion, for semantic as well as conceptual reasons, is the relationship between subjective feelings and emotion states. The colloquial usage of the word "emotion" refers to "feelings," our subjective

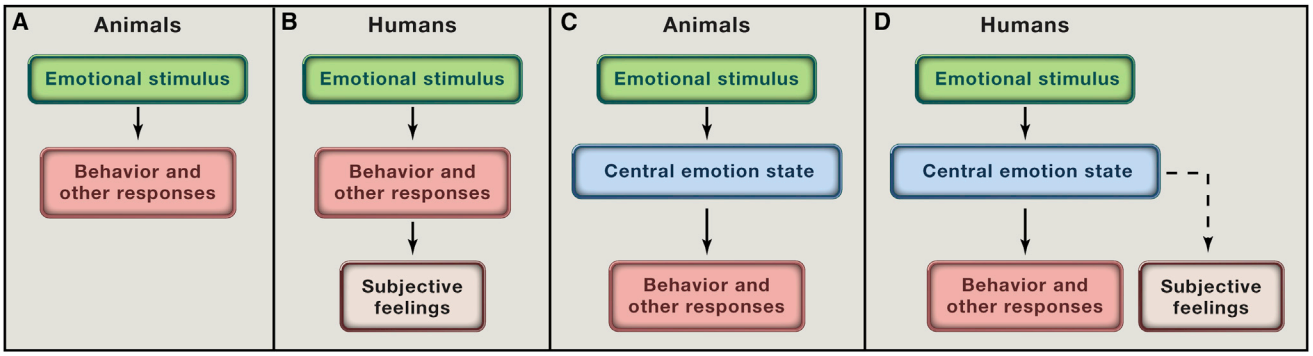

Figure 3. The Relationship between Central Emotion States and Subjective Feelings

(A and B) Behaviorist version of view in which emotional stimuli evoke behavior and other responses in animals (A) without the involvement of any causative central state. In humans (B), the subjective feeling of emotion is assumed to arise from our conscious awareness of the behavioral and somatic responses to the stimuli (James, 1884).

(C and D) In our view, responses to emotional stimuli are mediated by central emotion states, which are evoked by those stimuli in both animals (C) and humans (D). Those central states produce subjective feelings in parallel with behavioral and somatic responses in humans (D). We argue that central states also play an important role in emotional expression in animals (C), irrespective of whether they have a subjective perception of those states or not.

perception of emotion states and their accompanying somatic responses (although recent theories have been careful to make a clear distinction between emotion and feelings [Damasio, 2003]). The existence of "feelings" can at present only be assessed by verbal report and therefore is currently uniquely accessible to study in humans (Figure 3B). However, if one were to accept the colloquial definition of "emotions" as subjective feelings, then because we cannot know whether animals incapable of verbal report have such feelings (Figure 3A), it would follow that we cannot study "emotions" in any organism other than *Homo sapiens* (LeDoux, 2012).

Our view is that animals, like humans, have central emotion states even if they are not consciously aware of them (Figure 3C). We, like others before us (Dolan, 2002; Damasio, 2003; Panksepp, 1998; Rolls, 1999; Salzman and Fusi, 2010), argue that there is no reason a priori to exclude this possibility and that the evolutionary similarities between emotional expressions in humans and animals, as observed by Darwin, suggest that animals—invertebrates as well as vertebrates—have central emotion states as well. Consistent with this view, there is some evidence that even humans may have emotions of which they are not consciously aware (Winkielman and Berridge, 2004); and conversely, there are views that animals have emotion states that provide basic building blocks for feelings, only much less elaborated in nature (Damasio, 2003; Berridge and Kringelbach, 2013; Panksepp 1998).

The idea that animals have central emotion states with certain general and fundamental properties (which we will discuss below) and that these states play a causal role in transforming certain kinds of stimuli into characteristic, species-typical behaviors should prompt the search for such states and the neural circuit-level mechanisms that encode them in model organisms. By analogy, in the same way that we have learned a great deal about the neurobiology of vision by studying animal models without worrying about trying to solve the problem of how we have conscious visual experiences, we can learn much about the neural encoding of central emotion states in animals without concerning ourselves with the subjective, conscious perception of such states.

Emotion "Primitives" and Their Behavioral Expression

Below, we attempt to delineate some of the evolutionary "building blocks," or "emotion primitives," that describe central emotion states. These features are common to different emotions in different animal species. It is possible and even likely that these features independently evolved to subserve multiple behavioral and cognitive functions and are combined by the brain in a specific manner to produce emotion states (Salzman and Fusi, 2010). Even if that is the case, however, we argue that understanding such features in terms of neural circuit activity and brain chemistry will move us a step closer to understanding the brain mechanisms underlying emotions.

Scalability

> "He who will attend to the starting of his horse…will perceive how perfect is the gradation from a mere glance at some unexpected object…to a jump so rapid and violent that the animal probably could not voluntarily whirl round in so rapid a manner."—Darwin

Emotion states have often been classified according to their valence (positive or negative) and their intensity (Figure 4A) (Russell, 1980). One can be annoyed, angry, furious, or enraged or sad, despondent, or grief-stricken. Some of this gradation may reflect differences in the level of arousal that is associated with a particular emotion. Whether such arousal is generic (Pfaff et al., 2005) or specific to a particular behavioral system (Devidze et al., 2006) is not yet clear. Arousal in *Drosophila* has been studied using assays that test for increases in locomotor activity or sensitivity to noxious sensory stimuli (van Swinderen and Andretic, 2003; Greenspan et al., 2001) or using electrophysiological recordings (Nitz et al., 2002; van Swinderen et al., 2004). Some evidence has been provided for at least two forms of arousal in the fly, which are regulated in opposite directions by dopamine (DA) acting through the fly homolog of the D1 DA receptor (Lebestky et al., 2009).

Gradations in emotional intensity are also associated with qualitative shifts in the behaviors associated with those states. "Predator imminence" theory, for example, posits that, as the

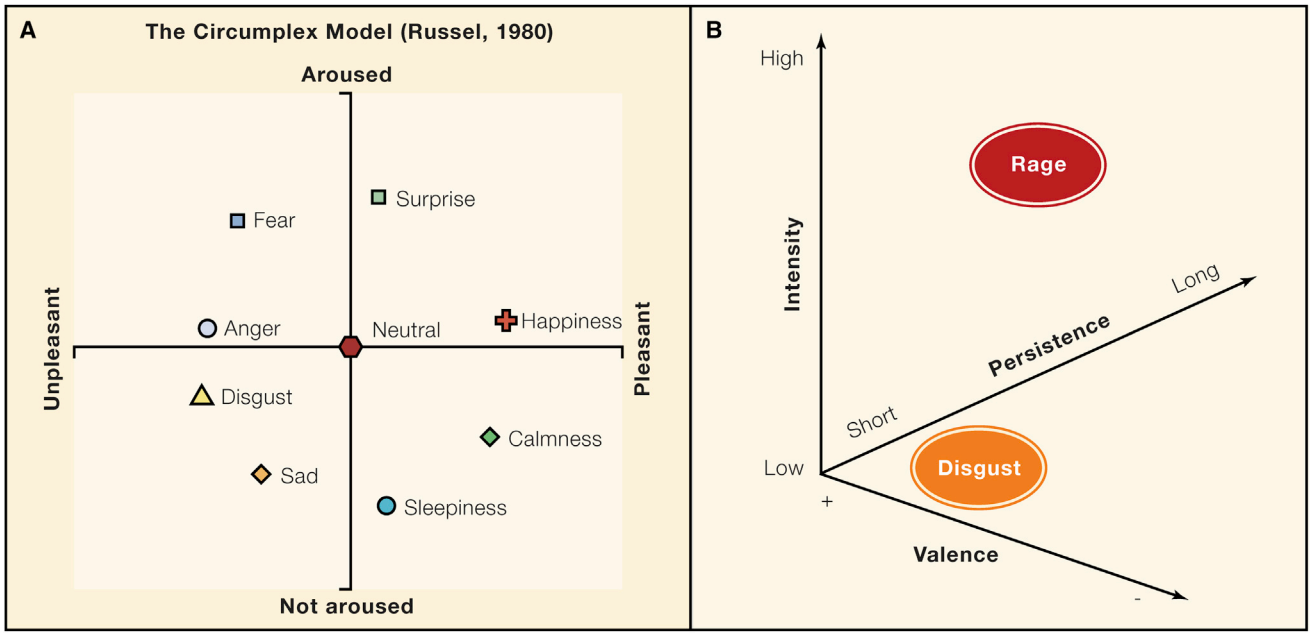

Figure 4. Dimensional Models of Emotion

(A) A two-dimensional space representing what is often called "core affect," the most popular construct in psychological theories of emotional experience (Barrett and Russell, 1999; Russell, 2003) but also applied more broadly to other animals (Mendl et al., 2010; Rolls, 1999).

(B) Example of a multidimensional model for separating different emotions into different domains of a state space. According to some views, the space in which emotion states can be located is extremely high dimensional, consisting of all of the different parameters that one can measure (e.g., Salzman and Fusi, 2010) and essentially formalizing a multivariate version of emotion as depicted in Figure 4B.

encounter between a prey animal and a predator becomes more imminent, the defensive behavior of the former switches from freezing (which avoids detection) to flight (which avoids entrapment) (Bolles and Fanselow, 1980; Blanchard et al., 1998). In octopi, there is a switch from crypsis (camouflage) behavior to ink jetting and propulsion as a potential threat becomes more proximate (http://www.youtube.com/watch?v=eS-USrwuUfA). There are relatively few such examples in *Drosophila*. "Low-intensity" versus "high-intensity" aggressive behaviors have been identified through ethograms (Chen et al., 2002); however, the transitions are not as stereotyped as in the case of the cricket, *G. bimaculatus* (Stevenson et al., 2005). The development of tractable model systems that display this behavioral phenomenon would allow one to approach the question of the underlying neural mechanisms that link graded states of arousal/drive/motivation to action selection, a process that is currently poorly understood. Furthermore, the ability to quantitatively manipulate the level of excitability in genetically defined neuronal subpopulations using techniques such as optogenetics may make it possible to investigate whether scalability implemented as graded differences in spiking activity can lead to graded or qualitative differences in emotional expression (Lee et al., 2014).

Psychological models of emotion in humans all feature scalability as well (Harris et al., 2012). Often this is simply incorporated as an arousal dimension (Lang et al., 1993; Russell, 1980, 2003; Russell et al., 1989) (Figure 4A and Box 2), but many theories also acknowledge phase transitions with parametric increases of some variable, such as transitioning from mild concern to anxiety to fear to panic (thus leading to these three emotions seen as distinct in many views; McNaughton and Corr, 2004). Multidimensional models, however, may capture more fully the range of different emotion states (Figure 4B).

Valence

> "When actions of one kind have become firmly associated with any sensation or emotion, it appears natural that actions of a directly opposite kind…should be unconsciously performed…under the influence of a directly opposite sensation or emotion."—Darwin

In our daily life, we infer the existence of a particular emotion in others through its behavioral expression. In his monograph, Darwin articulated three principles to explain why certain emotions are expressed by particular behaviors. The second of these he called the "Principle of Antithesis." According to this principle, emotions come in pairs of opposites (e.g., joy versus anger; happiness versus sadness), which are expressed by physically opposite and complementary behaviors (Figures 5A and 5B). Thus, one operational criterion for recognizing instances of emotional expression in animals is to look for behaviors that appear to be related as such "antithetical pairs."

In model organisms, the simplest example of such an antithetical pairing is directed locomotor activity: this activity may result either in approach toward or withdrawal from a particular object or stimulus. In *C. elegans*, the neural circuitry underlying

Box 2. Evolution of Emotion Primitives versus Specific Emotions

Valence and intensity can be thought of as evolutionary building blocks of emotion, or emotion "primitives." Indeed, they are often considered the two defining aspects of emotion that distinguish emotions from all other kinds of mental states (Russell, 2003; Salzman and Fusi, 2010). In considering the evolution of emotion, it is important to distinguish between the appearance of emotion per se versus the appearance of *specific* emotions: an insect may exhibit a behavior whose properties reflect emotion primitives, even if it does not correspond to a specific human emotion. One reason that it is difficult to bridge basic biological principles of emotion with psychological studies of emotion can probably be traced to the fact that the psychological studies invariably emphasize details about specific human emotions (of note, aspects based on emotional experience, social cognition, and language, e.g., Barrett et al., 2007). In humans, many studies have argued for a small set of so-called "basic" emotions, including happiness, fear, anger, disgust, and sadness, which are thought to be culturally universal, especially in their facial expressions (Ekman, 1992). Interestingly, the axes of valence and intensity have often been used to categorize these different emotions, according to their degree of similarity (Russell, 2003): some emotions may be high arousal but differ in their valence (joy versus rage), whereas others may be of similar valence but differ in their intensity (annoyance versus fury) (Figure 4). Valence and intensity (or two dimensions much like them [Rolls, 1999]) thus typically capture much of the variance in emotional behaviors in human psychological studies (Russell et al., 1989; Watson and Tellegen, 1985). Interestingly, functional MRI studies in humans have provided evidence for dissociated representations of valence and intensity with respect to gustatory or olfactory stimuli (Small et al., 2003; Kringelbach, 2005). The appearance of valence and intensity as early emotion primitives may have provided a framework for diversifying different types of emotions, thereby linking these two aspects of emotional evolution.

approach versus avoidance to olfactory stimuli is relatively well understood (reviewed in de Bono and Maricq, 2005; Sengupta, 2007). In *Drosophila*, male wing posture is orthogonal during courtship versus aggressive behavior: in courtship, males extend their wings horizontally and vibrate them to generate a "song" that attracts females (Figure 5C2**)** (Dickson, 2008); the neural circuitry underlying this behavior has been dissected in detail (von Philipsborn et al., 2011). During agonistic interactions with conspecific males, male flies raise their wings vertically into a "wing-threat" (Figure 5C1) (Chen et al., 2002). From Darwin's perspective, these appendicular postures might constitute an example of "antithesis." If so, then the fly's wings may express an internal emotion state in a manner analogous to the expressive tail of a cat or a dog. In this context, it is important to note that emotional expression often (but not always) has a social communication function either to conspecifics or heterospecifics; courtship song in *Drosophila* is a clear example of this feature.

The neurobiological mechanisms underlying the Principle of Antithesis remain to be understood. At the limit, the simplest "antithetical" actions are those controlled by antagonistic pairs of flexor and extensor muscles (as noted by Darwin) and their corresponding motor inputs. It makes intuitive sense that "opposite" emotion states might exert complementary biases on particular flexor-extensor pairs, but the underlying neural mechanisms remain to be elucidated. One clue comes from the famous experiments of Kravitz and colleagues demonstrating that injection of octopamine versus serotonin in lobsters can trigger subordinate versus dominant postures, respectively (Livingstone et al., 1980), although the neurobiological mechanisms underlying this effect remain unclear (Kravitz and Huber, 2003). Some neuroimaging studies in humans have also suggested that responses to oppositely valenced stimuli are represented in distinct regions of the brain (Small et al., 2003; Kringelbach, 2005).

In psychological theories of emotion, valence (antithesis) and arousal (intensity) are taken to be essential features of all emotions and ones that define what in the psychological literature is referred to as "core affect" (Russell, 2003; Barrett et al., 2007) (Figure 4A). In this respect, these two features of emotion states are thought also to distinguish emotions from other mental states that we might attribute to an organism. Recent work has emphasized that these two attributes need not correspond to aspects of the conscious experience of emotion but can be thought of as parameters that define a similarity space in which all emotion states can be related to one another (Salzman and Fusi, 2010). It is also worth noting that there may be instances of antithesis that do not seem to fall on opposite ends of a positive versus negative valence dimension. For instance, Susskind et al. (2008) have shown that fear and disgust expressions in humans have opposite effects on increasing versus decreasing the intake of sensory information, respectively (fear widens the eyes and nostrils to acquire cues about potential danger; disgust squints the eyes and nostrils to shut out aversive taste and odors). The precise psychological dimension corresponding to Darwin's original concept of "antithesis" (which was entirely behaviorally defined) thus remains to be fully understood.

Persistence

> "A man may have his heart filled with the blackest hatred or suspicion, or be corroded with envy or jealousy…these feelings…commonly last for some time."—Darwin

A key feature that distinguishes emotional behaviors from simple stimulus response (SR) reflexes is that these behaviors, or associated state variables, often outlast the stimuli that elicit them. For example, heart rate, blood pressure, and levels of stress hormones can remain elevated for many minutes following exposure to a threat or other stressor. In humans, anxiety or depression can continue for very prolonged periods of time, with a sustained and pervasive effect on experience, cognition, and behaviors. This feature of persistence makes emotions powerfully flexible in how they can control cognition and behavior and therefore is worth searching for in model systems. In *Drosophila*, repeated presentations of a noxious mechanical stimulus (air puffs) promote a persistent state of elevated locomotor activity (Figure 6A), the duration of which is controlled by dopamine (Lebestky et al., 2009). Studies in *C. elegans* have identified neuropeptides, biogenic amines, and the underlying circuitry that controls opposing, persistent behavioral states such as roaming in remarkable detail (Chalasani et al., 2007; Flavell et al., 2013) (Figures 6B and 6C). Recent studies in *Drosophila* have shown that brief optogenetic activation of a specific population of brain interneurons controlling courtship

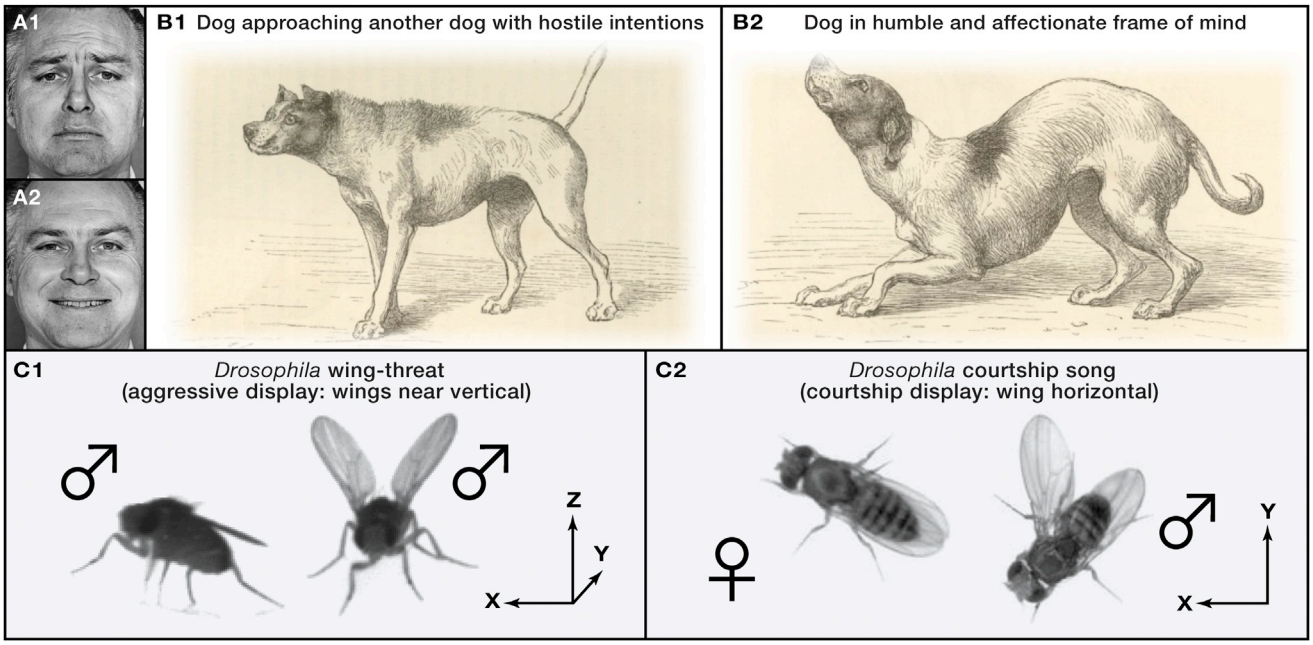

Figure 5. Examples of Darwin's Second Principle of Antithesis

According to this principle, opposite emotions produce behaviorally opposite expressions.

(A) In humans, sadness (A1) and happiness (A2) are expressed by opposite configurations of the mouth.

(B) Antithetical postures in dogs, from Darwin (1872).

(C) A potential example of antithesis in *Drosophila*. Male flies elevate both wings close to the vertical in a "threat display" during agonistic interactions with conspecific males (C1), whereas they extend one wing horizontally to vibrate it in order to produce a courtship "song" during mating (C2). Axes indicate the different angles of view (C1, frontal; C2, overhead). This example also illustrates the social communication function of some types of emotional expression.

song (von Philipsborn et al., 2011) can lead to persistent singing lasting for minutes (Inagaki et al., 2014) (Figure 6D). The neural mechanism underlying persistence in this case remains to be elucidated.

Interestingly, some forms of persistence may be intimately related to scalability at the level of neural circuits (Major and Tank, 2004). Increases in the scalar value of state parameters (e.g., spiking rate of some neurons or levels of a neuromodulator) during an encounter with a predator or during a social interaction with a conspecific may reflect the integration or accumulation over time of sensory inputs. This integration may be used in at least two ways, not mutually exclusive: to provide cumulative information leading to behavioral decisions and action selection and to increase the state of arousal/drive/motivation of the animal. Many instantiations of neural integrators require persistent activity of some sort either at the level of individual neurons or at the circuit level (Major and Tank, 2004; Ratcliff and McKoon, 2008). Persistent activity underlying neural integrators may continue even after the sensory inputs being integrated are no longer present. In this way, persistence could be a natural consequence of the neural coding mechanisms that underlie scalability.

Generalization

> "When any sensation, desire, dislike, etc. has led during a long series of generations to some voluntary movement, then a tendency to the performance of a similar movement will almost certainly be excited, whenever the same, or any analogous or associated sensation…is experienced."—Darwin

One consequence of persistence is that an emotion state induced by one stimulus can generalize to a different context and thereby influence subsequent responses to different stimuli. In this way, emotions bias cognition and behavior. This criterion amounts to context generalization, or "trans-situationality." This property well illustrates the pervasive effects of emotions on behavior and offers another respect in which they differ from SR reflexes. Applying this criterion would, for example, allow one to distinguish whether the response of an insect to an aversive stimulus, such as a shadow (Card and Dickinson, 2008), is simply a reflex or involves a persistent internal state that can generalize to other contexts or affect subsequent behavioral decisions. In honeybees, traumatic stress (vigorous mechanical shaking) caused a persistent "pessimistic cognitive bias" in terms of the behavior the bees showed in an ambiguous odor choice assay (Bateson et al., 2011), implying the induction of an internal state caused by the shaking that could operate across contexts (e.g., during the odor choice) (Mendl et al., 2011).

Another aspect of generalizability of emotion states comes from two features that could be called "stimulus generalizability" (or stimulus "degeneracy") and "pleiotropy": the sensory causes of a given emotion state can "fan in" from a multitude of stimuli; in turn, the consequences of an emotion state "fan out" to a multitude of effects. The feature of stimulus generalizability is described by Darwin in the first of his three principles of

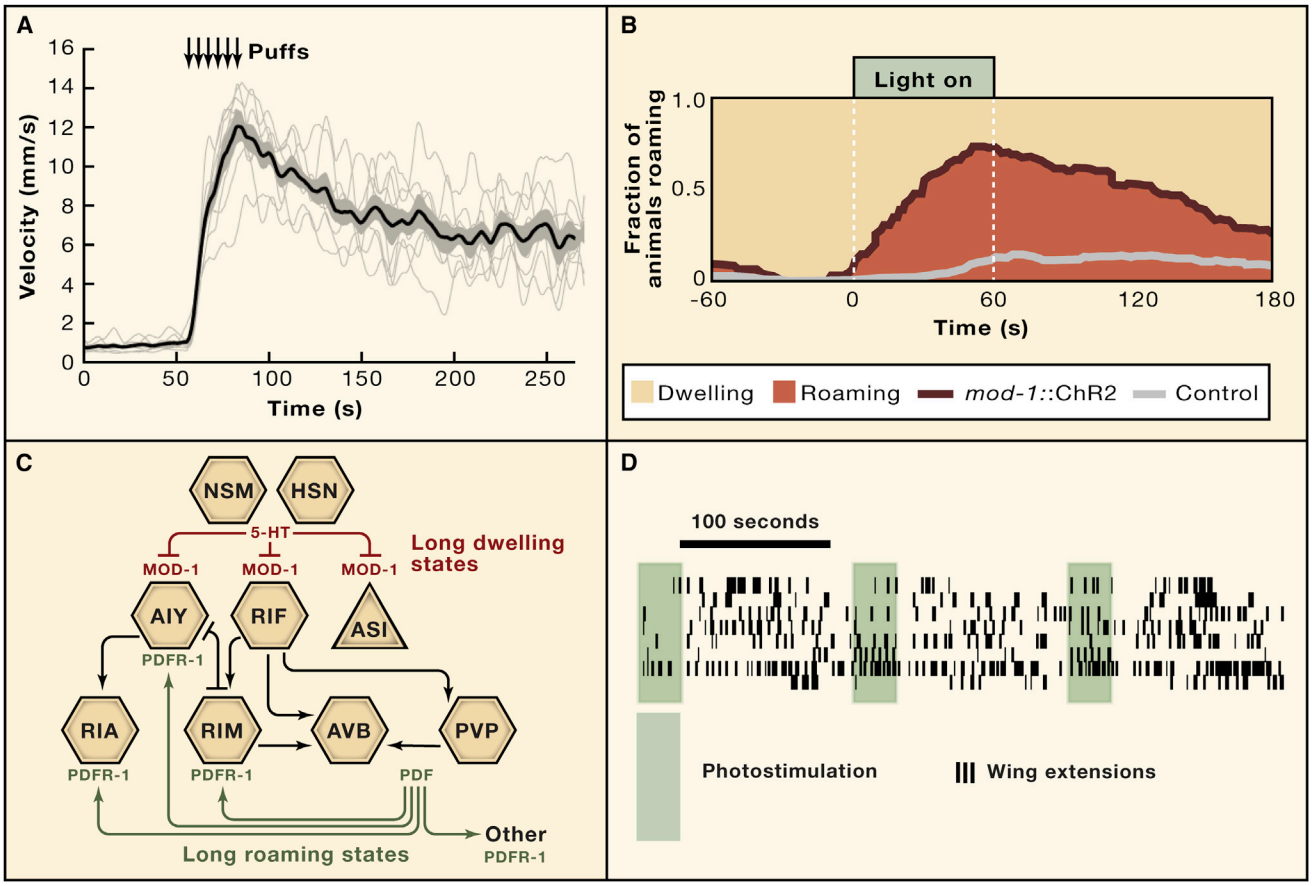

Figure 6. Experimental Examples of Persistent Activity in Flies and Worms

(A) Persistent elevation of locomotor activity evoked by repeated mechanical startle (using brief air puffs) in *Drosophila*. Modified from Lebestky et al. (2009).
(B and C) Persistent roaming behavior in *C. elegans* evoked by optogenetic stimulation of a specific subset of interneurons under the control of the mod-1 promoter. (C) Circuit model summarizing control of persistent and opponent dwelling and roaming states (B and C). Modified from Flavell et al. (2013).
(D) Transient optogenetic activation of P1 neurons in *Drosophila* using a red-shifted version of channelrhodopsin-2 (green bars) evokes persistent wing extension behavior (black rasters). Modified from Inagaki et al. (2014).

emotional expression, called the principle of Serviceable [Useful] Associated Habits (Darwin, 1872). According to this principle, the same behavioral expression can be triggered by many different stimuli and different contexts, including those for which the behavior appears to serve no useful ("serviceable") purpose, if those stimuli evoke the same internal emotion state. Darwin's classic example of this phenomenon is that of a cat that kneads its paws on a soft blanket. This behavior is "serviceable" (useful) in kittens to stimulate the flow of milk from a nursing mother but has no clear utility in relation to the blanket in an adult cat. Darwin argues that, in such cases, the behavior becomes associated either through learning ("habit") or inheritance with the central state (in this example, presumably "pleasure") to the degree that any stimulus that elicits that same state will elicit the same behavior. As Darwin and later Ekman noted, a source of positive selection for some apparently "useless" behavioral expressions is that they may indeed have utility in the context of communicating the animal's internal emotion state (Darwin, 1872).

Darwin noted that the strong link between stimuli and the emotion states that they elicit can be either inherited or associated by habit—in other words, through learning and memory. A familiar example of such "emotional learning" is Pavlovian fear conditioning, in which a neutral "conditioned" stimulus (CS), such as a tone, is able to evoke an emotional behavior, such as freezing, following repeated pairing of that CS with an "unconditioned" stimulus (US) that innately evokes emotional behavior, such as a footshock. The amygdala, a structure whose role in emotion we already mentioned above, is known to be necessary for Pavlovian fear conditioning in rodents (Davis, 1992) as well as in humans (Bechara et al., 1995). There is now a large literature from reinforcement learning, applied widely across species, that provides important intersection with the study of emotion (Clark et al., 2012; see Box 3).

Emotion states are also pleiotropic, meaning that they have multiple, parallel effects: they influence many different aspects of behavior and also have internal somatic effects, as well as effects on cognition (Figure 2B). For example, responses caused by a fear-like central state not only include defensive behaviors, such as freezing or flight, but also endocrine changes such as increases in stress hormone levels; changes in autonomic

Box 3. Emotions and Learning

There is substantial intersection between the study of emotions and the burgeoning field of appetitive and aversive classical conditioning. The prototypic example of Pavlovian fear conditioning illustrates the basic phenomenon: while an initially restricted class of stimuli that are innately aversive (e.g., electric shock) elicit behaviors that look emotional (e.g., jumping), there is considerable flexibility in the system. A much larger set of stimuli (e.g., a tone reliably paired with the shock) can elicit fear behaviors after learning (and moreover, those fear behaviors are also more diverse and flexible than simply duplicating the unconditioned response: whereas a mouse may jump when shocked, it may freeze when it hears the sound). Much of the plasticity for such emotional learning occurs at the interface between sensory processing and the central emotion state, thus allowing multiple stimuli, through learning, to access or evoke an emotion state that they could not causally influence before.

Conditioned olfactory avoidance and its molecular and neural circuit basis have been extensively studied in *Drosophila* (Keene and Waddell, 2007). However, this assay is not entirely analogous to mammalian fear conditioning in that the CS odor is typically not neutral but, rather, innately aversive. Perhaps closer approximations to emotional learning are provided by the conditioned place preference/aversion (CPP/CPA) tests in which an animal learns to prefer or avoid a specific but neutral environment (chamber) that is paired with a pleasant or unpleasant stimulus, respectively. For example, crayfish have been shown to exhibit CPP to cocaine (Huber et al., 2011). A modification of this test, involving associations with neutral odors, has been used to demonstrate that alcohol has rewarding properties in *Drosophila* (Kaun et al., 2011). More recently, Heberlein and colleagues have shown that male *Drosophila* can be positively conditioned to an odor-laced location by presenting that odor during mating with a virgin female (Shohat-Ophir et al., 2012). This observation and the finding that the response to virgin females involves neuropeptide Y (NPY), which also controls ethanol-based reward learning, implies that mating does not simply involve a series of concatenated and serially dependent SR reflexes (sometimes referred to as "stigmergy" [Giuggioli et al., 2013]) but, rather, involves the induction of a rewarding internal state.

Box 4. The Somatic Component of Emotions

"*Joy quickens the circulation, and this stimulates the brain, which again reacts on the whole body*."—Darwin

Ever since William James, the somatic component of emotional reactions has received particular attention in emotion theories (e.g., Craig, 2008; Damasio, 2003). These somatic components involve autonomic reactions such as changes in heart rate, blood pressure, breathing, and sweaty palms, as well as changes in the state of internal organs such as the gut. Importantly, as recognized by Darwin (see above), somatic components of an emotional response are not only caused by the central emotion states, but also react back on the brain to further modify those states (Figure 2B), a process termed "interoception," the brain's detection of the body's internal state. It has been argued that a central feature of "feeling states" in humans reflects our conscious experience of interoception (Craig, 2008; Damasio, 2003). But that does not mean that animals that (may) lack such a subjective perception, or conscious awareness, of interoceptive states necessarily lack somatic responses to emotional stimuli, or interoception, per se: the ability to detect such somatic responses with their brains. There is much to be learned about the neurobiology of interoception, not only in mammalian systems but especially in model organisms such as *Drosophila*, without trying to fathom the basis of its subjective perception in humans.

function such as increased heart rate, blood pressure, and sweaty palms; and changes in attention and memory encoding (in humans). Psychological theories of human emotion have also emphasized the multicomponent nature of emotions, typically including subjective experience and neurophysiological processes, as well as somatic and endocrine ones. Simple reflex responses are not typically associated with such multidimensional features.

Internal emotion states also alter sensorimotor information processing. For example, in *Drosophila* and in other insects, the state of hunger (sometimes called a "homeostatic emotion" [Craig, 2003]) increases behavioral sensitivity to sucrose (Inagaki et al., 2014; Dethier, 1976). This increase is mediated by an increase in the tonic activity of certain dopaminergic neurons (Marella et al., 2012), which release dopamine onto the terminals of sugar-sensing gustatory neurons, increasing calcium influx in response to sucrose (Inagaki et al., 2014). Such studies illustrate the utility of *Drosophila* for identifying neural mechanisms of state encoding and demonstrating their causality in state-dependent behavioral changes.

Finally, it is important to note that the features of context/stimulus generalization and pleiotropy also mean that the causal architecture within which an emotion state operates can become quite complex. This complexity, together with the persistence feature that we noted earlier, means that, as an emotion state unfolds over time, the very behavior that it causes can in turn feed back onto the state (Figure 2B). This feedback aspect, which was already noted by William James, has been given much attention specifically in terms of the somatic effects of an emotion (Box 4).

Recognizing Emotional Expression in Mammals, Model Organisms, and Martians

In any nonhuman model organism, to study experimentally the neural underpinnings of emotion states, it is necessary to identify expressive behaviors that can serve as a phenotypic "readout" of experimental manipulations of brain circuitry and chemistry. Emotional behaviors in mammals are typically recognized by homology to human behaviors and more recently by the involvement of homologous neuroanatomical structures involved in specific human emotions. For example, fear behaviors such as freezing and the experience of fear require the amygdala in humans (Feinstein et al., 2011). Rodents and other mammals exhibit many similar fear behaviors such as freezing, and this requires the amygdala as well (Vazdarjanova et al., 2001; Choi and Kim, 2010). Thus, it is reasonable to conclude that behaviors like freezing can be "emotional" behaviors in mammals, whether or not the animal has a conscious or subjective experience of "fear" as we recognize it in ourselves (LeDoux, 2012).

However, these criteria are difficult to apply to phylogenetically distant organisms that do not freeze and that lack an amygdala. We have argued that model organisms, such as *Drosophila* or *C. elegans*, may exhibit primitive emotional behaviors even if those behaviors are not homologous to our own. But how can one identify such behaviors and distinguish them from simple (SR) reflexes? Put another way, if we landed on Mars and little

green men approached our spaceship, how would we know if they had emotions or not? As mentioned earlier, Darwin provided little general guidance on this issue, other than anthropocentric homology. However, given his assumption that central emotion states are expressed by observable behaviors, we suggest that, as a starting point, one may look for certain behaviors that exhibit some or all of the core properties that we attribute to internal emotion states themselves, as described above. One can then begin to investigate whether the properties of such behaviors are indeed causally controlled by internal brain states through experimental identification and perturbation of the mechanisms that underlie such properties.

An alternative to the view that invertebrate model organisms have emotions is that their behavior instead reflects a series of dependent, concatenated SR reflexes, a process sometimes referred to as "stigmergy" (Giuggioli et al., 2013). According to this view, for example, courtship behavior in *Drosophila* would not express a central emotion state but, rather, would comprise a series of reflexive responses triggered by specific sensory cues. (These responses may, in turn, feed back as additional stimuli to elicit further reflex actions.) One implication of this view is that, in contrast to humans and other mammalian species, flies do not mate because sex is associated with a state of reward but, rather, because they are genetically programmed to do so if they encounter a potential mate emitting appropriate "releasing signals" (Tinbergen, 1950). Arguing against this view, however, are experiments showing that *Drosophila* males can be conditioned to be attracted to an odor that has previously been paired with an episode of mating (Shohat-Ophir et al., 2012), suggesting that mating is rewarding (Panksepp, 1998; see also Box 2). Furthermore, as mentioned earlier, transient activation of certain courtship neurons can evoke a persistent state of courtship behavior (Inagaki et al., 2014). Therefore, although stigmergic processes may explain some complex insect behaviors, this does not exclude the possibility that other behaviors are caused by central emotion states.

Uniquely Human Features?

There may well be emotion states that are unique to humans, or primates, or mammals—with likely candidates being some of the "social" or "moral" emotions (Tangney et al., 2007) (it seems unlikely that flies have pride or embarrassment). The emotion of awe has sometimes been proposed as being truly unique to humans (Keltner and Haidt, 2003). However, it would seem that all of the features that apply to emotions more generally also apply to these emotion states that may be species specific.

A different question is whether there are any *features* of emotions as such that may be unique to humans (or primates or mammals). Three leading candidates are volitional control, subjective report, and stimulus-decoupled elicitation, aspects that we briefly discuss next.

Volitional Control

Control over one's emotions is a feature of adult human emotions that is not typically observed in nonhuman animals (although to some extent this can be trained in certain species), nor in human infants or children (where again extensive training is required throughout development to reach the adult level of control). There are good neurological reasons for the developmental emergence: regulation of emotion in humans is known to rely substantially on signals from the prefrontal cortex, a brain region whose connectivity is still immature in childhood. Prefrontal cortex is one of the latest regions in development to become myelinated, and its protracted developmental timeline accounts for the difficulties that young children have in metacognition, aspects of attention, and volitional control over behavior, thought, and emotion (Thompson et al., 2000).

A major mechanism for psychopathology in humans is thought to be an impaired ability to regulate one's emotions. Psychiatric disorders, in particular, arise in large part from dysfunction in the regulation of emotion, with examples ranging from posttraumatic stress disorder to phobias. Cognitive-behavioral routes to therapy capitalize on this insight and utilize various strategies to re-establish cognitive control over one's emotions (exposure therapy perhaps being the clearest case). There is relatively little work yet at the neurological level, though neuroimaging studies support the general idea of a role for prefrontal cortex in such regulation (Ochsner and Gross, 2005) (the issue is somewhat complicated by the fact that humans can regulate their emotions in at least two different ways, cognitive re-interpretation of a situation or active suppression of emotional reactions [Gross, 2002]).

Volitional control over emotional expressions has consequences for their social communicative role (an important function of many emotional expressions), opening the door to deception and manipulation of conspecifics—something that humans engage in all the time. Indeed, skilled humans who are capable of convincingly counterfeiting emotions on cue can command salaries of tens of millions of dollars—we call them "actors." There is, however, scant evidence of emotional deceit in other animals. As with other features possibly unique to humans, volitional control over emotions—to the degree that adult humans have it—does not violate any of the above features that we noted but expands upon them to permit an even more flexible interface between central emotion states and the rest of cognition and behavior.

Subjective Report

Psychological investigations of emotion in humans are not generally based on observations of behavior but on verbal report (Figures 2A and 2B). Indeed, in our own case, we typically identify emotion states within ourselves without resort to behavioral observation, unlike what we do for other people or animals. Some work using functional imaging in humans, as well as studies in rodents, has pointed to particular brain structures, such as parts of orbitofrontal cortex and the nucleus accumbens, that may be particularly important for the subjective experience of emotions. Moreover, there is some evidence for topographic segregation of emotional experiences, albeit only at the coarse level of "pleasure" versus "aversion" (corresponding to the dimension of valence we noted above) (for review, see Berridge and Kringelbach, 2013).

It is important, however, to note that the above facts do not impinge on the features of emotion that we enumerated: they hold for an emotion state regardless of how it is identified (whether through behavioral observation, verbal report, or other means). Given our view that emotions should be construed as central states, we would suggest that both behavior and

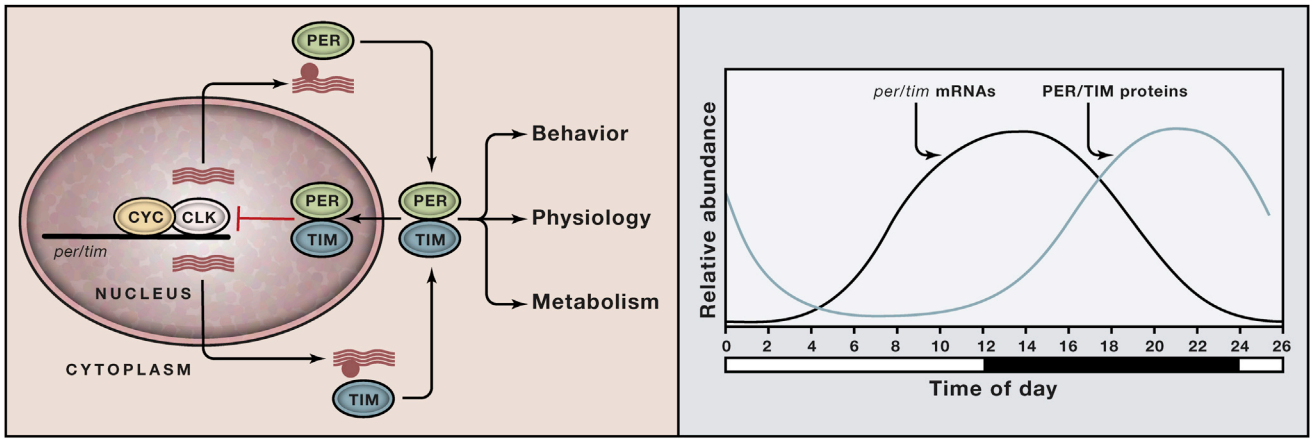

Figure 7. Schematic Illustrating Components of the Central Circadian Oscillator in *Drosophila*
PER, TIM, CYC, and CLK are transcription factors that participate in a negative-feedback autoregulatory loop. The output of this oscillator coordinates multiple organismal processes that display circadian periodicity. Modified from Nitabach and Taghert (2008).

subjective report are caused by a common central emotion state (Figure 2B). As we noted earlier (and in contrast to predominant psychological theories Figure 2A), neither behavior nor feeling are themselves part of the emotion state but instead should be viewed as consequences of it (and can be pieces of evidence for it). As with behavior, this reformulation frees us of the need to identify human-like feelings (or indeed any feelings) in other animals (Figures 3C and 3D). Emotion states cause certain behaviors in *Drosophila*, somewhat different sets of behaviors in rodents, and yet different behaviors in humans. Analogously, emotion states cause subjective reports of experiences that we call emotional feelings in humans, may cause different kinds of experiences in other animals if only we knew how to measure them, and may cause none at all in yet other species. If subjective report is no longer considered a defining feature of central emotion states, then to the extent that we can measure such states and their associated behaviors in model organisms, we can study their mechanistic underpinnings and their causal roles in emotional behavior.

Stimulus Decoupling

Another feature that is prominent in humans could be considered an elaboration on stimulus generalizability that we mentioned above. Not only may a given emotion state be caused by a larger set of eliciting stimuli in humans than in other animals—it can be caused by no direct stimulus at all. In humans, many and perhaps most emotion states are caused not by direct confrontations with specific stimuli (e.g., a predator) but, rather, by the anticipation or recollection of such stimuli. The increased metacognitive abilities of humans also make possible the elicitation of emotion states through thoughts, or imaginings, about all kinds of situations that one has not in fact experienced: one's own mortality, for instance—a large topic of research in psychology (e.g., studied in terror management theory) that is unlikely to find a parallel in other animals. Once again, none of this is in conflict with the criteria that we list for an emotion: it simply notes that the sets of eliciting conditions and the kinds of behavioral patterns that are linked to an emotion state are vastly more complex in humans than in other animals.

Experimental Investigation of Central Emotion States

We have argued that "emotions" are a type of central neural state that are caused by sensory stimuli or memories and that, in turn, control a panoply of behavioral, cognitive, and somatic changes (Figure 2B). These central states have certain properties that generalize across different emotions in the same species and across different species whether they have the same particular emotions or not.

So, how should we look for examples of such central states? And how would we know if we found one? We do not even know the level of biological organization or function at which such states are instantiated—they could be a neuromodulatory system, a neuroanatomical structure, a distributed neural network, a type of firing pattern (e.g., oscillation at a certain frequency), or all of the above. As mentioned earlier, we do not even know whether such states are instantiated in a unitary mechanism or, rather, are cobbled together from multiple interacting mechanisms. Indeed, one of the major challenges facing modern neuroscience is to understand how functional states, whether emotional or not, are instantiated in the brain.

One example illustrating the way that one may distinguish between a "central state" and its outputs is provided by the discovery of the mechanisms underlying circadian rhythms in *Drosophila*. Like emotions, circadian oscillators control a "central state"—in this case, cyclical changes in system-wide biological processes (including behavior, physiology, and metabolism) that are entrained to the 24 hr day-night cycle. Genetic (Konopka and Benzer, 1971) and molecular studies (reviewed in Nitabach and Taghert, 2008) have revealed that the central circadian oscillator is instantiated in a collection of transcription factors that function in an autoregulatory negative-feedback network. Importantly, loss- or gain-of-function genetic manipulations in components of this central oscillator machinery changed the pattern (period, amplitude) of oscillations in multiple biological outputs of the clock, in a parallel and synchronous manner (Figure 7). In contrast, analogous genetic manipulations of a single output of the clock changed only the oscillations of that output without affecting other outputs in a parallel manner.

Only through such genetic tests of causality, therefore, was it ultimately possible to distinguish the ''coding'' of the central state itself from the outputs of the state.

The point of this example is to show that a defining feature of a central state is that experimental perturbations of that state should affect multiple outputs of that state in a parallel, coordinated manner. That acid test requires the ability to manipulate components of the state, whether they are genes or cells, and such manipulations are most readily performed in genetically tractable model organisms.

So how should one search for the components of such a central state? We favor the idea that central emotion states are most likely instantiated at the neural circuit level. One potential example of such an instantiation in mammals is circuits that involve the amygdala, a structure which has long been associated with negative emotions such as fear but which has increasingly been associated with positive emotions as well (Gallagher and Chiba, 1996; Paton et al., 2006; Tye et al., 2011; Jennings et al., 2013). Importantly, the amygdala is not a unitary structure but, rather, consists of multiple substructures (''subnuclei'') (Pitkänen et al., 1997), whose collective functional properties control several of the different dimensions that constitute outputs of an emotion state (Figure 2B). For example, loss- and gain-of-function manipulations of the central nucleus, medial subdivision (CeM), by electrical stimulation, lesions, or more recently optogenetic perturbations (Johansen et al., 2012) affect behavioral, autonomic, and endocrine correlates of the ''fear'' state in a parallel manner. These parallel ''pleiotropic'' outputs are mediated by projections from CeM to distinct downstream structures (Davis, 1992; LeDoux, 1995). The basolateral amygdala, in contrast, projects to the medial prefrontal cortex (Senn et al., 2014), and this projection may underlie cognitive or subjective aspects of the ''fear'' state in higher organisms, including humans (for a more complex scheme whereby amygdala-prefrontal circuits implement an emotion state in a dynamic fashion, see Salzman and Fusi [2010]). Finally, the lateral amygdala is well known for its role in fear conditioning (Maren and Quirk, 2004), though circuits in the lateral subdivision of CeA may contribute as well (Ehrlich et al., 2009).

A drawback of mammalian systems, however, is that it is currently difficult if not impossible to search for such emotional circuit nodes in an unbiased and systematic manner. Although candidates for such nodes can be sought in humans by brain-wide functional MRI, the ability to test the causal relationship between the activity of such nodes and emotion states is extremely limited and is dependent on serendipitous, rare patients with lesions in brain structures of interest. In *Drosophila*, by contrast, it is now possible to carry out systematic, unbiased screens for neurons whose functional perturbation results in measurable behavioral alterations (Simpson, 2009; von Philipsborn et al., 2011). Using such an approach, it was recently possible to identify a small cluster of neurons whose experimental activation or inhibition altered the levels of multiple, distinct aggressive behaviors in parallel and in the same direction (Asahina et al., 2013). These cells also appear to control an internal state that may correspond to ''aggressiveness'' or aggressive arousal via release of the neuropeptide tachykinin.

Importantly, it is not necessarily the case that all of the functional properties of a given central emotion state are instantiated in a single brain structure or circuit. Instead, they may have been assembled during evolution by combining pre-existing, behaviorally relevant functional ''neural modules'' in a manner that allowed the generation of the more complex central states that we call ''emotion.'' In that case, the properties of the central state that we have delineated here would be distributed among distinct but coupled systems, some of which may individually be used for non-emotional processes. Even in that case, however, a mechanistic understanding of such ''emotion primitives'' in model organisms should provide important insights into the control of emotional behavior and would allow tests to ascertain the way in which such modules are coordinated.

Future Directions

There is no shortage of challenges to a scientific understanding of emotions. Simply put, much of the work remains to be done. What we hope to have done here is to break the ground, so to speak, by specifying core features that can form the basis for studies of emotion across phylogeny, from worms to flies to rodents to primates, including humans. Four experimental directions are: (1) to apply our list of features to identify and study specific central emotion states and their associated behaviors in a specific species; (2) to understand how those states (particularly scalable, persistent states) are encoded in the brain; (3) to understand the causal relationship of those states to behavior; and (4) to explore the features, or a subset of them, in parallel studies across a range of species. Ultimately, this should allow us to move beyond the question of when specific emotions like fear evolved to the more fundamental question of when and how emotion states per se first appeared in evolution. Most importantly, the focus on emotions as central states with common, general properties should create a common language that will facilitate interactions between scientists studying this elusive property in humans and those working on less complex but more experimentally tractable model systems.

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REFERENCES

Asahina, K., Watanabe, K., Duistermars, B.J., Hoopfer, E., Gonzalez, C.R., Eyjolfsdottir, E.A., Perona, P., and Anderson, D.J. (2013). Male-specific tachykinin-expressing neurons control sex differences in levels of aggressiveness in *Drosophila*. Cell *156*, 221–235.

Barrett, L.F., and Russell, J.A. (1999). The structure of current affect: controversies and emerging consensus. Curr. Dir. Psychol. Sci. *8*, 10–15.

Barrett, L.F., Mesquita, B., Ochsner, K.N., and Gross, J.J. (2007). The experience of emotion. Annu. Rev. Psychol. *58*, 373–403.

Bateson, M., Desire, S., Gartside, S.E., and Wright, G.A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. Curr. Biol. *21*, 1070–1073.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., and Damasio, A.R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science *269*, 1115–1118.

Berridge, K.C., and Kringelbach, M.L. (2013). Neuroscience of affect: brain mechanisms of pleasure and displeasure. Curr. Opin. Neurobiol. *23*, 294–303.

Blanchard, R.J., Hebert, M.A., Ferrari, P.F., Palanza, P., Figueira, R., Blanchard, D.C., and Parmigiani, S. (1998). Defensive behaviors in wild and laboratory (Swiss) mice: the mouse defense test battery. Physiol. Behav. *65*, 201–209.

Bolles, R.C., and Fanselow, M.S. (1980). A perceptual-defensive-recuperative model of fear and pain. Behav. Brain Sci. *3*, 291–301.

Branson, K., Robie, A.A., Bender, J., Perona, P., and Dickinson, M.H. (2009). High-throughput ethomics in large groups of Drosophila. Nat. Methods *6*, 451–457.

Calder, A.J., Lawrence, A.D., and Young, A.W. (2001). Neuropsychology of fear and loathing. Nat. Rev. Neurosci. *2*, 352–363.

Cannon, W.B. (1927). The James-Lange theory of emotions: a critical examination and an alternative theory. Am. J. Psychol. *39*, 106–124.

Card, G., and Dickinson, M.H. (2008). Visually mediated motor planning in the escape response of Drosophila. Curr. Biol. *18*, 1300–1307.

Chalasani, S.H., Chronis, N., Tsunozaki, M., Gray, J.M., Ramot, D., Goodman, M.B., and Bargmann, C.I. (2007). Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature *450*, 63–70.

Chen, S., Lee, A.Y., Bowens, N.M., Huber, R., and Kravitz, E.A. (2002). Fighting fruit flies: a model system for the study of aggression. Proc. Natl. Acad. Sci. USA *99*, 5664–5668.

Choi, J.S., and Kim, J.J. (2010). Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. Proc. Natl. Acad. Sci. USA *107*, 21773–21777.

Clark, J.J., Hollon, N.G., and Phillips, P.E.M. (2012). Pavlovian valuation systems in learning and decision making. Curr. Opin. Neurobiol. *22*, 1054–1061.

Craig, A.D. (2003). A new view of pain as a homeostatic emotion. Trends Neurosci. *26*, 303–307.

Craig, A.D. (2008). Interoception and Emotion: A Neuroanatomical Perspective. In Handbook of Emotions, Third Edition, M. Lewis, J.M. Haviland-Jones, and L. Feldman Barrett, eds. (New York: Guilford Press), pp. 272–288.

Damasio, A. (2003). Looking for Spinoza: Joy, Sorrow, and the Feeling Brain (Orlando, Florida: Harcourt, Inc.).

Dankert, H., Wang, L., Hoopfer, E.D., Anderson, D.J., and Perona, P. (2009). Automated monitoring and analysis of social behavior in Drosophila. Nat. Methods *6*, 297–303.

Darwin, C. (1872). The Expression of the Emotions in Man and Animals (London: Murray).

Davis, M. (1992). The role of the amygdala in fear and anxiety. Annu. Rev. Neurosci. *15*, 353–375.

de Bono, M., and Maricq, A.V. (2005). Neuronal substrates of complex behaviors in C. elegans. Annu. Rev. Neurosci. *28*, 451–501.

Dethier, V.G. (1976). The hungry fly: a physiological study of the behavior associated with feeding (Cambridge, Mass.: Harvard University Press).

Devidze, N., Lee, A.W., Zhou, J., and Pfaff, D.W. (2006). CNS arousal mechanisms bearing on sex and other biologically regulated behaviors. Physiol. Behav. *88*, 283–293.

Dickson, B.J. (2008). Wired for sex: the neurobiology of Drosophila mating decisions. Science *322*, 904–909.

Dolan, R.J. (2002). Emotion, cognition, and behavior. Science *298*, 1191–1194.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., and Lüthi, A. (2009). Amygdala inhibitory circuits and the control of fear memory. Neuron *62*, 757–771.

Ekman, P. (1992). An argument for basic emotions. Cogn. Emotion *6*, 169–200.

Feinstein, J.S., Adolphs, R., Damasio, A.R., and Tranel, D. (2011). The human amygdala and the induction and experience of fear. Curr. Biol. *21*, 34–38.

Flavell, S.W., Pokala, N., Macosko, E.Z., Albrecht, D.R., Larsch, J., and Bargmann, C.I. (2013). Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans. Cell *154*, 1023–1035.

Gaj, T., Gersbach, C.A., and Barbas, C.F., 3rd. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. *31*, 397–405.

Gallagher, M., and Chiba, A.A. (1996). The amygdala and emotion. Curr. Opin. Neurobiol. *6*, 221–227.

Giuggioli, L., Potts, J.R., Rubenstein, D.I., and Levin, S.A. (2013). Stigmergy, collective actions, and animal social spacing. Proc. Natl. Acad. Sci. USA *110*, 16904–16909.

Greenspan, R.J., Tononi, G., Cirelli, C., and Shaw, P.J. (2001). Sleep and the fruit fly. Trends Neurosci. *24*, 142–145.

Gross, J.J. (2002). Emotion regulation: affective, cognitive, and social consequences. Psychophysiology *39*, 281–291.

Harris, R.J., Young, A.W., and Andrews, T.J. (2012). Morphing between expressions dissociates continuous from categorical representations of facial expression in the human brain. Proc. Natl. Acad. Sci. USA *109*, 21164–21169.

Huber, R., Panksepp, J.B., Nathaniel, T., Alcaro, A., and Panksepp, J. (2011). Drug-sensitive reward in crayfish: an invertebrate model system for the study of SEEKING, reward, addiction, and withdrawal. Neurosci. Biobehav. Rev. *35*, 1847–1853.

Iliadi, K.G. (2009). The genetic basis of emotional behavior: has the time come for a Drosophila model? J. Neurogenet. *23*, 136–146.

Inagaki, H.K., Jung, Y., Hoopfer, E.D., Wong, A.M., Mishra, N., Lin, J.Y., Tsien, R.Y., and Anderson, D.J. (2014). Optogenetic control of Drosophila using a red-shifted channelrhodopsin reveals experince-dependent influences on courtship. Nat. Methods *11*, 325–332.

James, W. (1884). What is an emotion? Mind *9*, 188–205.

Jennings, J.H., Sparta, D.R., Stamatakis, A.M., Ung, R.L., Pleil, K.E., Kash, T.L., and Stuber, G.D. (2013). Distinct extended amygdala circuits for divergent motivational states. Nature *496*, 224–228.

Johansen, J.P., Wolff, S.B.E., Lüthi, A., and LeDoux, J.E. (2012). Controlling the elements: an optogenetic approach to understanding the neural circuits of fear. Biol. Psychiatry *71*, 1053–1060.

Kabra, M., Robie, A.A., Rivera-Alba, M., Branson, S., and Branson, K. (2013). JAABA: interactive machine learning for automatic annotation of animal behavior. Nat. Methods *10*, 64–67.

Kaun, K.R., Azanchi, R., Maung, Z., Hirsh, J., and Heberlein, U. (2011). A Drosophila model for alcohol reward. Nat. Neurosci. *14*, 612–619.

Keene, A.C., and Waddell, S. (2007). Drosophila olfactory memory: single genes to complex neural circuits. Nat. Rev. Neurosci. *8*, 341–354.

Keltner, D., and Haidt, J. (2003). Approaching awe, a moral, spiritual, and aesthetic emotion. Cogn. Emotion *17*, 297–314.

Konopka, R.J., and Benzer, S. (1971). Clock mutants of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *68*, 2112–2116.

Kravitz, E.A., and Huber, R. (2003). Aggression in invertebrates. Curr. Opin. Neurobiol. *13*, 736–743.

Kringelbach, M.L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. Nat. Rev. Neurosci. *6*, 691–702.

Lang, P.J., Greenwald, M.K., Bradley, M.M., and Hamm, A.O. (1993). Looking at pictures: affective, facial, visceral, and behavioral reactions. Psychophysiology *30*, 261–273.

Lazarus, R.S. (1991). Emotion and Adaptation (New York: Oxford University Press).

Lebestky, T., Chang, J.S., Dankert, H., Zelnik, L., Kim, Y.C., Han, K.A., Wolf, F.W., Perona, P., and Anderson, D.J. (2009). Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron *64*, 522–536.

Lee, H., Kim, D.-W, Remedios, R., Anthony, T.E., Chang, A., Madisen, L., Zeng, H., and Anderson, D.J. (2014). Scalable control of mounting and attack by ESR1+ neurons in the ventromedial hypothalamus. Nature. http://dx.doi.org/10.1038/nature13169.

LeDoux, J.E. (1995). Emotion: clues from the brain. Annu. Rev. Psychol. *46*, 209–235.

LeDoux, J. (2012). Rethinking the emotional brain. Neuron *73*, 653–676.

Livingstone, M.S., Harris-Warrick, R.M., and Kravitz, E.A. (1980). Serotonin and octopamine produce opposite postures in lobsters. Science *208*, 76–79.

Major, G., and Tank, D. (2004). Persistent neural activity: prevalence and mechanisms. Curr. Opin. Neurobiol. *14*, 675–684.

Marella, S., Mann, K., and Scott, K. (2012). Dopaminergic modulation of sucrose acceptance behavior in Drosophila. Neuron *73*, 941–950.

Maren, S., and Quirk, G.J. (2004). Neuronal signalling of fear memory. Nat. Rev. Neurosci. *5*, 844–852.

McNaughton, N., and Corr, P.J. (2004). A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. Neurosci. Biobehav. Rev. *28*, 285–305.

Mendl, M., Burman, O.H.P., and Paul, E.S. (2010). An integrative and functional framework for the study of animal emotion and mood. Proc. Biol. Sci. *277*, 2895–2904.

Mendl, M., Paul, E.S., and Chittka, L. (2011). Animal behaviour: emotion in invertebrates? Curr. Biol. *21*, R463–R465.

Moors, A. (2009). Theories of emotion causation: A review. Cogn. Emotion *23*, 625–662.

Nitabach, M.N., and Taghert, P.H. (2008). Organization of the Drosophila circadian control circuit. Curr. Biol. *18*, R84–R93.

Nitz, D.A., van Swinderen, B., Tononi, G., and Greenspan, R.J. (2002). Electrophysiological correlates of rest and activity in Drosophila melanogaster. Curr. Biol. *12*, 1934–1940.

Ochsner, K.N., and Gross, J.J. (2005). The cognitive control of emotions. Trends Cogn. Sci. *9*, 242–249.

Panksepp, J. (1998). Affective Neuroscience (New York: Oxford University Press).

Paton, J.J., Belova, M.A., Morrison, S.E., and Salzman, C.D. (2006). The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature *439*, 865–870.

Pfaff, D., Westberg, L., and Kow, L.M. (2005). Generalized arousal of mammalian central nervous system. J. Comp. Neurol. *493*, 86–91.

Pitkänen, A., Savander, V., and LeDoux, J.E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci. *20*, 517–523.

Ratcliff, R., and McKoon, G. (2008). The diffusion decision model: theory and data for two-choice decision tasks. Neural Comput. *20*, 873–922.

Rolls, E.T. (1999). The Brain and Emotion (New York: Oxford University Press).

Russell, J.A. (1980). A circumplex model of affect. J. Pers. Soc. Psychol. *39*, 1161–1178.

Russell, J.A. (2003). Core affect and the psychological construction of emotion. Psychol. Rev. *110*, 145–172.

Russell, J.A., Weiss, A., and Mendelsohn, G.A. (1989). Affect grid: a single-item scale of pleasure and arousal. J. Pers. Soc. Psychol. *57*, 493–502.

Salzman, C.D., and Fusi, S. (2010). Emotion, cognition, and mental state representation in amygdala and prefrontal cortex. Annu. Rev. Neurosci. *33*, 173–202.

Scherer, K.R. (2009). Emotions are emergent processes: they require a dynamic computational architecture. Philos. Trans. R. Soc. Lond. B Biol. Sci. *364*, 3459–3474.

Sengupta, P. (2007). Generation and modulation of chemosensory behaviors in C. elegans. Pflügers Archiv. Eur. J. Phycol. *454*, 721–734.

Senn, V., Wolff, S.B., Herry, C., Grenier, F., Ehrlich, I., Gründemann, J., Fadok, J.P., Müller, C., Letzkus, J.J., and Lüthi, A. (2014). Long-range connectivity defines behavioral specificity of amygdala neurons. Neuron *81*, 428–437.

Shohat-Ophir, G., Kaun, K.R., Azanchi, R., Mohammed, H., and Heberlein, U. (2012). Sexual deprivation increases ethanol intake in Drosophila. Science *335*, 1351–1355.

Simpson, J.H. (2009). Mapping and manipulating neural circuits in the fly brain. Adv. Genet. *65*, 79–143.

Small, D.M., Gregory, M.D., Mak, Y.E., Gitelman, D., Mesulam, M.M., and Parrish, T. (2003). Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron *39*, 701–711.

Stevenson, P.A., Dyakonova, V., Rillich, J., and Schildberger, K. (2005). Octopamine and experience-dependent modulation of aggression in crickets. J. Neurosci. *25*, 1431–1441.

Susskind, J.M., Lee, D.H., Cusi, A., Feiman, R., Grabski, W., and Anderson, A.K. (2008). Expressing fear enhances sensory acquisition. Nat. Neurosci. *11*, 843–850.

Tangney, J.P., Stuewig, J., and Mashek, D.J. (2007). Moral emotions and moral behavior. Annu. Rev. Psychol. *58*, 345–372.

Thompson, P.M., Giedd, J.N., Woods, R.P., MacDonald, D., Evans, A.C., and Toga, A.W. (2000). Growth patterns in the developing brain detected by using continuum mechanical tensor maps. Nature *404*, 190–193.

Tinbergen, N. (1950). The hierarchical organization of nervous mechanisms underlying instinctive behaviour. In Physiological Mechanisms in Animal Behaviour (New York, NY: Academic Press, Inc.), pp. 305–312.

Tye, K.M., Prakash, R., Kim, S.Y., Fenno, L.E., Grosenick, L., Zarabi, H., Thompson, K.R., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature *471*, 358–362.

van Swinderen, B., and Andretic, R. (2003). Arousal in Drosophila. Behav. Processes *64*, 133–144.

van Swinderen, B., Nitz, D.A., and Greenspan, R.J. (2004). Uncoupling of brain activity from movement defines arousal States in Drosophila. Curr. Biol. *14*, 81–87.

Vazdarjanova, A., Cahill, L., and McGaugh, J.L. (2001). Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. Eur. J. Neurosci. *14*, 709–718.

Venken, K.J., Simpson, J.H., and Bellen, H.J. (2011). Genetic manipulation of genes and cells in the nervous system of the fruit fly. Neuron *72*, 202–230.

von Philipsborn, A.C., Liu, T., Yu, J.Y., Masser, C., Bidaye, S.S., and Dickson, B.J. (2011). Neuronal control of Drosophila courtship song. Neuron *69*, 509–522.

Watson, D., and Tellegen, A. (1985). Toward a consensual structure of mood. Psychol. Bull. *98*, 219–235.

Winkielman, P., and Berridge, K.C. (2004). Unconscious emotions. Curr. Dir. Psychol. Sci. *13*, 120–123.