Chapter 6

Learned fear and innate anxiety in rodents and their relevance to human anxiety disorders

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Any effort to understand the causes of anxiety disorders must begin with an important conceptual assumption: anxiety disorders arise from a disturbance of *brain function*. Both of these words are emphasized here because both are critically important to our understanding of anxiety. Anxiety disorders are disturbances of the *brain* – whether one asserts psychological, environmental or genetic causes, the end result is a brain that creates maladaptive behavioral patterns. Anxiety disorders arise when brain *function* goes awry – that is, these disorders arise from exaggerations or maladaptive use of defensive behavioral patterns which under normal circumstances help an organism respond appropriately to threats in its environment.

The notion of anxiety disorders as disturbances of brain function raises the tantalizing possibility that we might understand what goes wrong in the brain during maladaptive anxiety states, and by extension develop treatments aimed at reversing (or overcoming) this dysfunction. Arguably, one of the best ways of going about understanding a disease state and testing potential treatments is to develop an accurate animal model. Especially with regard to diseases which affect the brain, animal models offer the best chance at careful, in depth analysis of pathophysiology (what goes wrong) and reproducible, safe tests of potential treatments. The principal issue to resolve is how to develop animal models of relevance to human disorders. This is especially problematic with symptoms of psychiatric disorders such as anxiety disorders: How do you tell if a mouse if it is anxious?

It turns out, though, that observing and quantifying anxiety-like behavior in a mouse is not as difficult as one might think. The mechanisms of normative anxiety – the behavioral and physiological characteristics of normal defensive behaviors – are actually quite well conserved across species (c.f., Chapter by Hofer). This is evident from even a cursory consideration of anxiety-related behaviors in man and mouse. In man, anxiety is typically manifest by worries about potentially threatening events, and avoidance of places or situations which make these events more likely to occur. Thus, a dark alley evokes concerns that one might be accosted by a criminal, and is avoided where possible. In mice, while it is not possible to measure worry, measuring avoidance is very simple. Given a choice between two rooms in a given apparatus, for example, a mouse will avoid a room in which it has previously received a shock; it has learned to be afraid of that room. Similarly, given a choice between a bright room and a dark room, a mouse will avoid the bright room; mice are innately afraid of bright lights, perhaps because light makes them more visible to potential predators.

Of course, the repertoire of defensive behaviors in humans and animals extends beyond simple avoidance. Consider for a moment how a person responds to a potentially threatening environment, such as the aforementioned dark alley. One can walk away from it; or approach it cautiously, alert to additional signs of danger. If there is some reason to go down that alley, say, to visit a chic night club on the other end, one ventures in slowly and quietly, alert for sudden movement or unusual objects. Students

of animal behavior might describe this as "approach/avoidance behavior." See something move, or a loud shout, and one might turn and run – an "escape" response. An unmistakably threatening stimulus – such as a gun, aimed and ready to shoot – and one freezes on the spot. Animals engage in the exact same progression of defensive behaviors. When faced with an environment suggestive of a potential threat, rodents engage in approach/avoidance behavior. An actual threat (such as the presence of a predator) evokes an escape response; an immediate threat (such as a predator about to strike) evokes freezing behaviors. The animal literature tends to classify approach/avoidance and other responses to potential threats as "anxiety," and freezing and other responses to immediate threats as "fear." How these concepts of "anxiety" and "fear" map onto human anxiety disorders is unclear.

Defensive fear and anxiety behaviors have been extensively studied in rodents, using behavioral paradigms that test such behaviors in response to both learned and innately threatening stimuli. For reasons that are not necessarily clear, "fear"-like responses to immediate threats have typically been studied in the context of learning – animals are taught that particular stimuli signal threats. These learned fear stimuli then evoke a pattern of behavior consistent with the immediate presence of danger. In contrast, "anxiety"-like responses to potential threats have typically been studied in the context of innate responses to non-learned stimuli. This chapter will review these studies of learned fear and innate anxiety, detailing the latest advances in the

understanding of neurobiological mechanisms and their implications for treatment. As this discussion proceeds, the advantages of studying fear and anxiety in rodent models will be made plain, in terms of the rich mechanistic detail these models provide. Consideration will also be given to data from human studies in order to examine the relevance of these animal models of normative defensive behaviors to the pathological anxious behaviors seen in patients with anxiety disorders.

ONCE BURNED, TWICE SHY: THE AMYGDALA AT THE CENTER OF A FEAR CIRCUIT

Once burned, twice shy, goes the adage that accurately describes learned fear behaviors. Animals and people alike tend to avoid places and situations in which they have had painful or aversive experiences. This can be adaptive – it is the rare child who will touch a hot stove twice; or maladaptive – for example, when a panic attack at work forces the agoraphobic to quit his job. A principal advantage of learned fear is that it can be easily modeled in rodents, an approach which has been exploited by numerous groups to identify the neural circuits responsible for the learning, expression and regulation of fear responses (Davis, 1997; LeDoux, 2003; Quirk & Beer, 2006). All learned fear paradigms involve the same basic elements: a standardized, neutral stimulus (for example, a particular tone); a directly threatening stimulus (such as a mild shock); and a behavioral or physiological measure of the fear response (such as freezing

behavior or increase in heart rate). For example, in the oft-studied paradigm of conditioned freezing to tone, a rat or mouse is presented simultaneously with both a neutral tone and a mild electrical shock (FIGURE 6.1A). The animal rapidly learns that the tone predicts a shock through a process known as classical conditioning. Subsequent playback of the same tone evokes a freezing response, in which the animal stops exploring its cage and remains motionless while the sound is being played.

Indeed, playback of the fear-conditioned tone induces a host of behavioral and physiological fear behaviors that can be measured in rodents, revealing a network of activated brain regions responsible for each (Davis, 1992). Increased heart and respiratory rate, dilated pupils, decreased responses to pain, facial expressions of fear, increased startle responses, defecation and urination, and stimulation of the corticosteroid release have all been documented in rodents exposed to fear-conditioned stimuli. Specific brain regions, such as the hypothalamus and various brainstem nuclei, serve as the foot soldiers responsible for specific fear responses. The activation of the lateral hypothalamus leads to increased activity in the sympathetic nervous system and tachycardia and pupilary dilation. Activation of the midbrain central gray leads to freezing behavior. The roles of each of these regions were established through a combination of lesion and electrical stimulation studies: Lesioning a specific region abolishes and stimulating that region mimics the fear response for which that region is responsible.

If these varied brain regions are the foot soldiers of the fear response, then the central nucleus of the amygdala is the general (FIGURE 6.2). Neurons in this nucleus send their axons to each and every one of the brain regions responsible for the various fear reactions. Lesions of the central nucleus prevent all of the various fear reactions to conditioned stimuli; stimulating the central nucleus mimics a variety of these responses (Davis, 1992). Thus the central nucleus serves to trigger the myriad fear-related behavioral and physiological responses to conditioned stimuli by activating specific brain areas in the hypothalamus and brainstem

But how is the central nucleus itself activated by fear stimuli? This circuit, too, has been worked out through careful study in the rodent brain. Sensory stimuli (such as the neutral tone and the mild shock) are relayed through thalamic and cortical sensory areas, to the basolateral nucleus of the amygdala, which in turn projects to and activates the central nucleus (LeDoux, 2000). It is in the basolateral nucleus that the requisite learning takes place; neurons here learn that the tone predicts the shock, and signal the central nucleus to activate a set of defensive behaviors (e.g., freezing). Basolateral neurons learn this association by virtue of the properties of a particular receptor for the neurotransmitter glutamate: the NMDA-type glutamate receptor, so named because it can also be activated by the glutamate analog N-methyl-D-aspartic acid. In response to simultaneous activation of the postsynaptic neuron and its presynaptic inputs, the NMDA receptor allows calcium to enter the postsynaptic neuron; calcium then initiates

a cascade of intracellular events that results in a change in the strength of the incoming synapses. Blocking NMDA receptors in the basolateral nucleus of the amygdala prevents the acquisition of conditioned fear (Fanselow & Kim, 1994; Miserendino, Sananes, Melia, & Davis, 1990; Walker & Davis, 2002). This finding leads to a model of fear-conditioning in which the simultaneous activation of sensory inputs carrying tone and shock information activate NMDA receptors in the basolateral nucleus, resulting in a strengthening of the synapses that signal the previously neutral tone. Following this plasticity-dependent strengthening, the now fear-conditioned tone is better able to activate basolateral neurons. These basolateral neurons in turn activate the central nucleus, triggering a fear response.

Extinction: The neural substrates of overcoming fear learning involve cortical regulation of the amygdala

Once learned, the fear association can last a long time – up to the lifetime of the animal. Yet it can also be squelched through an additional learning process, called extinction. Through re-exposure to the conditioned stimulus, this time without the reinforcing shock, the animal learns that the stimulus no longer predicts an incoming shock. The stimulus subsequently fails to elicit the fear conditioned response (FIGURE 6.1B). The extinction process has two distinct components: the initial learning or pure "extinction" phase, and a subsequent "consolidation" phase in which the extinction

memory is preserved for the long-term. Take an animal trained to associate a tone with a shock, and expose it to the same tone without any shock multiple times the following day; the animal will gradually stop responding to the tone (the fear response has been "extinguished"). Put the same animal back in the same environment 24 hours later, and again expose it to the tone; it will still fail to respond (because the extinction memory has been "consolidated")(Quirk & Mueller, 2008). Extinction and its consolidation resemble psychological treatments of anxiety disorders, many of which rely on repeated exposures to anxiety-provoking stimuli (see below).

Both extinction and consolidation are also dependent on NMDA receptors, suggesting that they utilize the same neural plasticity mechanisms as the initial fear conditioning. Nonetheless the two processes are distinguished by separable neurobiological substrates. Infuse NMDA receptor antagonists into the basolateral amygdala during extinction training and the animal will fail to learn that the stimulus no longer predicts danger (Falls, Miserendino, & Davis, 1992). Infuse NMDA receptor antagonists into the medial prefrontal cortex (mPFC) during the extinction training and the animal will initially extinguish its fear response, but it will fail to retain the extinction memory: 24 hours later the animal will again respond to the tone with a full-blown fear response (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007). The mPFC, located on the medial wall of the frontal cortex, is a brain region implicated in cognitive control of behavior and directly connected with the amygdala, hypothalamus

and other limbic structures. It is therefore perfectly situated to perform a regulatory role in the fear circuit, dampening fear expression by inhibiting amygdala output, a role supported by numerous additional studies of its role in defensive behaviors (Quirk & Beer, 2006; Quirk & Mueller, 2008).

Fear conditioning and extinction as models of anxiety disorders and their treatment

Fear conditioning has some superficial similarities to several forms of human anxiety disorders. Most obvious are the specific phobias, in which a specific stimulus (blood, spiders, snakes, airplanes, heights, etc.) is assigned an inappropriately threatening value, evoking extreme defensive behaviors. One can easily posit that in patients with specific phobias, the phobic stimulus activates the central nucleus of the amygdala, which in turn activates brain regions responsible for autonomic and behavioral signs of anxiety. While it is not clear that fear conditioning plays an important role in the development of most cases of specific phobias, heightened amygdala responses to phobic stimuli have been demonstrated in several studies (Etkin & Wager, 2007; Schienle, Schafer, Walter, Stark, & Vaitl, 2005; Straube, Mentzel, & Miltner, 2007).

Fear conditioning plays a more obvious role in anxiety disorders that involve generalization of a fear response, such as panic disorder with agoraphobia and post-traumatic stress disorder (PTSD). In panic disorder, patients often describe their illness

as beginning with an attack of extreme anxiety that comes out of the blue. Over time, however, many patients begin to associate their attacks with the places or situations in which they occur. A patient who has a panic attack at work, for example, may then experience heightened anxiety whenever she goes to work; indeed, the anxiety may be so severe that she avoids going to work altogether. It is as if this patient were a victim of a fear conditioning experiment, with the worksite as the tone, and the panic attack as the shock! Unfortunately, unlike the limited shocks delivered to the rodents discussed above, panic attacks may continue to occur in patients with the disorder, resulting in an ever-widening circle of places and situations that the patient avoids; the end result is agoraphobia, in which the severe patient cannot even leave his or her home for fear of experiencing a panic attack. Similarly, PTSD patients start out avoiding situations and places that remind them of their traumatic experience. Fear evoked in closely related situations or places result in further generalization so that severely affected patients are subject to anxiety in a great variety of situations, greatly limiting their ability to function. An analogous process can be observed in fear-conditioned rodents. Pairing a fear conditioned stimulus with an additional neutral stimulus – such as a second novel tone – can result in so-called "second order conditioning," such that the animal develops fear responses to the second tone, despite the fact that it was never directly paired with shock (Gewirtz & Davis, 1998). Studies of neural activity in patients with either panic disorder or PTSD have also shown hyperactivity in the amygdala,

underscoring the similarities between these disorders and models of fear conditioning, although in humans amygdala activation also occurs with emotional stimuli that are not fear-inducing.

Where does extinction fit in? One intriguing possibility is that the same mechanisms that govern mPFC-mediated long-term extinction of fear conditioning processes might underlie successful treatments of these related forms of anxiety. Indeed, the premise that mPFC and amygdala oppose each other in the generation of defensive behaviors is an attractive one, as the mainstay of psychotherapeutic approaches to anxiety involves enhancing cognitive control over anxiety symptoms, in the context of repeated exposure to feared stimuli (Berkowitz, Coplan, Reddy, & Gorman, 2007; Garakani, Mathew, & Charney, 2006; Quirk & Beer, 2006). Accordingly, several studies have shown that successfully treating some anxiety disorders results in increased mPFC activity and/or decreased amygdala activity, strongly suggesting that at least part of the therapeutic response involves harnessing the same sorts of mechanisms involved in extinction of learned fear (Etkin & Wager, 2007; Rauch, Shin, & Phelps, 2006; Roffman, Marci, Glick, Dougherty, & Rauch, 2005).

Using neurobiological understanding of learned fear to develop novel therapeutic approaches

The recognition that at least some forms of anxiety disorders and their treatment rely on mechanisms similar to those identified in fear conditioning models raised the seductive possibility that the neurobiological understanding might be exploited to develop new treatment strategies. One approach of increasing prominence (and promise) is to exploit the neural plasticity mechanism involved in extinction learning. As noted above, NMDA receptors in the amygdala and mPFC are required for extinction learning to result in long-term suppression of learned fear responses. This requirement was discovered by blocking NMDA receptors with an antagonist. The converse experiment also works: Augmenting NMDA receptors, using the allosteric modulator D-cycloserine, enhances extinction (Walker, Ressler, Lu, & Davis, 2002). Normally, a few unreinforced exposures to the fear-conditioned tone do not result in full-blown extinction of the fear response in rodents. Administration of D-cycloserine augments the otherwise minimally effective regimen, resulting in complete and long-lasting extinction of the fear response.

Would such an approach also work in patients with anxiety disorders? Ressler and colleagues (2004) tested this idea by utilizing a standardized, virtual-reality based exposure therapy that had been shown to be successful in acrophobia (fear of heights). This treatment paradigm is thought to work through an extinction-like mechanism – exposing patients to ever-increasing virtual heights using standard anxiety-reduction techniques, patients slowly learn that heights do not pose any real danger to them.

Patients typically require seven sessions of such therapy to experience significant reductions in discomfort and avoidance of heights. To test the effects of augmenting NMDA receptor activity with D-cycloserine, the authors combined a minimally effective regimen of two exposure therapy sessions with two single doses of the drug, given at the time of the therapy. The shorter therapy plus drug regimen was as effective as the more time-consuming therapy alone regimen, perhaps the best example to date of a successfully translational neuroscience finding applied to psychiatry. Studies of the effectiveness of D-cycloserine augmentation of extinction therapies in other anxiety disorders relevant to fear conditioning have also yielded promising positive findings (Guastella, Dadds, Lovibond, Mitchell, & Richardson, 2007; Guastella, Richardson, Lovibond, Rapee, Gaston, Mitchell, & Dadds, 2008; Hofmann, Meuret, Smits, Simon, Pollack, Eisenmenger, Shiekh, & Otto, 2006; Kushner, Kim, Donahue, Thuras, Adson, Kotlyar, McCabe, Peterson, & Foa, 2007; Norberg, Krystal, & Tolin, 2008; Storch, Merlo, Bengtson, Murphy, Lewis, Yang, Jacob, Larson, Hirsh, Fernandez, Geffken, & Goodman, 2007; Wilhelm, Buhlmann, Tolin, Meunier, Pearlson, Reese, Cannistraro, Jenike, & Rauch, 2008).

WHAT YOU ALREADY KNOW CAN HURT YOU: INNATE ANXIETY PARADIGMS REVEAL ADDITIONAL COMPONENTS OF A DEFENSIVE BEHAVIOR CIRCUIT

While learned fear paradigms have indeed taught us a lot about anxiety, not all fear responses are learned. Think of the dark alley mentioned in the introduction to this chapter: darkness increases one's level of anxiety even without prior learning. Human fear of the dark is *innate*. Innate fears tend to be species-specific. Laboratory-bred monkeys with no experience of snakes nonetheless exhibit avoidance, escape and even freezing responses in when presented with a rubber snake, much less a real one. Rodents are innately fearful of bright lights, as anyone who has had a mouse infestation and a penchant for midnight snacks has indubitably discovered while turning on the kitchen light. Turn on a light suddenly, and rodent will escape to the dark; if escape is impossible it will freeze. Given a choice of a bright or dark room, a rodent will spend most of its time exploring the dark room.

Several behavioral paradigms have been developed over the years to measure anxiety-related behaviors in rodents (FIGURE 6.3). These anxiety tests explore the conflict between approach/avoidance behaviors displayed by rodents placed in a novel environment. When exposed to a new place, rodents have a drive to explore the environment, an adaptive trait considering that, in their natural habitat, rodents depend on foraging to find food. However, a novel environment is also potentially threatening for a rodent, for it may be a site where it is more exposed to predators. Rodents therefore take a cautious approach to exploring novel settings, exhibiting approach/avoidance behaviors and physiological signs of arousal. Moreover, rodents

tend to spend more time in the safer (e.g., less exposed) areas of the new environment, an easily measured trait that has been exploited in several laboratory-based tests of innate anxiety (Whishaw, Gharbawie, Clark, & Lehmann, 2006). In the open field test, for example, a large, well-illuminated circular arena is surrounded by high walls; mice and rats tend to avoid the brightly lit center and spend most of their time near the walls (Belzung & Griebel, 2001; Crawley, 1985; Prut & Belzung, 2003). The fraction of time spent in the periphery vs. the center of the field is used as behavioral measure of anxiety. A similar preference for closed dark spaces is seen in the elevated plus maze test, in which rodents prefer either of two enclosed arms to two open ones, and the light-dark test, in which they spend most of their time in the dark half of a two-chambered environment (Belzung & Griebel, 2001; Montgomery, 1955; Pellow, Chopin, File, & Briley, 1985; Rodgers, 1997). Findings such as elevated plasma levels of the stress-related hormone corticosterone supports the notion that these tests are indeed anxiogenic (Cruz, Frei, & Graeff, 1994; Pellow et al., 1985).

Not all tests of anxiety rely on physical aspects of the environment to induce defensive behaviors. In the social interaction test, a paradigm used as a model of social anxiety, a similar approach/avoidance conflict occurs (File & Seth, 2003). In this test the dependent variable is the time two rats spend in social interaction (sniffing, grooming, etc). The conflict in this test is between the drive to interact socially and the risk of being harmed by the other animal. More anxious rodents will therefore spend less time

interacting with others. Although the nature of the stimuli in this test is different from that in the EPM and in the open field, all these tests fundamentally exploit approach/avoidance conflicts to measure anxiety-related behavior. Interestingly, there are suggestions of both similarities and differences in the neurobiology underlying these different tests of innate anxiety. In general, animals that perform on the anxious end of the scale on one test often tend to perform on the anxious end of the scale on other tests. However, rigorous mathematical analysis of these tendencies – called factor analysis – suggests that there are several independent factors contributing to anxiety in the tests (Aguilar, Gil, Flint, Gray, Dawson, Driscoll, Gimenez-Llort, Escorihuela, Fernandez-Teruel, & Tobena, 2002; Griebel, Blanchard, & Blanchard, 1996; Ramos, Berton, Mormede, & Chaouloff, 1997). Thus, any given animal might avoid the open arms of the plus maze, for example, and subsequently fail to avoid a novel animal in the social interaction test. Such findings raise the possibility that there are different kinds of innate anxiety, just as there are different kinds of anxiety disorders in humans.

The principal advantage of these tests is that they explore innate behaviors. They are thus thought to explore ethologically relevant sources of anxiety, and may reflect a different neural circuitry compared to learned behaviors. Given that many human anxiety disorders are not fully explained by learned responses to fearful stimuli, understanding the neural circuitry of innate anxiety in animals (and how it differs from the neural circuitry of learned fear paradigms) may be of some use in understanding

anxiety disorders. Moreover, these tests of anxiety have been exploited both to screen for novel pharmacological compounds, as described below, and to screen genetically altered mice for anxiety-related phenotypes (see Hen Chapter).

The effects of anxiolytic and anxiogenic drugs on innate anxiety tests in rodents

Although these innate tests appear to accurately model aspects of normal and pathological anxiety in humans, such tests would not be very useful if they lacked pharmacological validity: Drugs that reduce or increase anxiety in humans, ought to have similar effects in these laboratory-based tests of rodent behavior. As the most commonly used anxiolytic drugs in humans are benzodiazepines (such as diazepam) and serotonin reuptake inhibitors (SSRIs; such as fluoxetine), these two classes of drugs have been used extensively to validate rodent conflict anxiety tasks. Benzodiazepines reduce anxiety in virtually all tests of innate anxiety. In the open field these drugs increase time spent in the aversive center (Britton & Britton, 1981; Crawley, 1985; Pellow & File, 1986; Schmitt & Hiemke, 1998; Siemiatkowski, Sienkiewicz-Jarosz, Czlonkowska, Bidzinski, & Plaznik, 2000). In the social interaction test, they increase interaction time (de Angelis & File, 1979; File & Hyde, 1979). In the elevated plus maze, they increase time spent on the exposed arms (Cruz et al., 1994; Pellow & File, 1986), and in the light-dark test these drugs increase time spent exploring the bright compartment (Bourin & Hascoet, 2003).

The effects of SSRIs are much more complex (Gordon & Hen, 2004). It is noteworthy that SSRIs are anxiogenic in humans when given acutely (Grillon, Levenson, & Pine, 2007), and anxiolytic during chronic treatments (Gorman, Kent, & Coplan, 2002). The effects of both acute and chronic SSRI treatment have been tested in animal models of anxiety. Similarly to the data in humans, in the open field, chronic but not acute fluoxetine was found to be anxiolytic, increasing center time (Dulawa, Holick, Gundersen, & Hen, 2004), although others have failed to see such effects (Durand, Berton, Aguerre, Edno, Combourieu, Mormede, & Chaouloff, 1999). In the social interaction test, acute treatment with the SSRIs appears to be anxiogenic, decreasing interaction time (Bagdy, Graf, Anheuer, Modos, & Kantor, 2001; Dekeyne, Brocco, Adhumeau, Gobert, & Millan, 2000; To & Bagdy, 1999). The effects of chronic treatment with the SSRI fluoxetine in the social interaction test are unclear, as it has been reported to be both anxiogenic (Kantor, Graf, Anheuer, & Bagdy, 2001) or to have no effect (To & Bagdy, 1999). The effects of SSRI treatments in the elevated plus maze are similarly inconclusive. Generally acute treatment with SSRIs are anxiogenic, increasing avoidance of the open arms (Drapier, Bentue-Ferrer, Laviolle, Millet, Allain, Bourin, & Reymann, 2007; Griebel, Moreau, Jenck, Misslin, & Martin, 1994), in agreement with human studies. The effect of chronic SSRIs in elevated plus maze, however, are unclear, as some studies show increased anxiety (Griebel, Cohen, Perrault, & Sanger, 1999; Silva & Brandao, 2000) while other reports show decreased anxiety (Durand et al., 1999;

Griebel et al., 1994; Kurt, Arik, & Celik, 2000). The one consistent finding from these studies is that acute SSRI administration is anxiogenic, as it seems to be in anxiety disorder patients.

The inconsistent effects of chronic SSRIs in tests of innate anxiety are troubling, given that these drugs are typically effective in many patients with anxiety disorders. However, not all patients (and not all disorders) benefit from even chronic SSRI administration. Furthermore, the inconsistency in the animal literature may be the result of methodological differences between laboratories in what is a relatively young field of research. While the SSRI data at present to not firmly support the validity of these tests as models of human anxiety disorders, it is perhaps too early to make a final conclusion on this issue.

Towards a neural circuitry of innate anxiety

The validity of learned fear models has been confirmed in part by neuroanatomical approaches in both animals and humans, suggesting a fear circuit centered on the amygdala that is hyperactive in normal and pathological fear states (see above). Does the neuroanatomy of innate anxiety shed light on the issue of relevance to the human condition?

The neuroanatomical basis of innate anxiety tests suggest a network of sites, closely aligned with but somewhat separate from the learned fear network. Unlike the

clear effects of amygdala lesions and manipulations on fear conditioning, most lesion studies suggest that the amygdala is not required for innate anxiety (Kjelstrup, Tuvnes, Steffenach, Murison, Moser, & Moser, 2002; Kondo & Sakuma, 2005; Moller, Wiklund, Sommer, Thorsell, & Heilig, 1997). Instead, lesions of a related network of sites disrupt anxiety-like behavior in these tests, including the ventral hippocampus, the mPFC, and the bed nucleus of the stria terminalis. Lesions of these regions result in effects that are similar to those of benzodiazepines in several of these tests, reducing open-arm and bright chamber avoidance and increasing social interaction, among other effects (Bannerman, Rawlins, McHugh, Deacon, Yee, Bast, Zhang, Pothuizen, & Feldon, 2004; Gonzalez, Rujano, Tucci, Paredes, Silva, Alba, & Hernandez, 2000; Kjelstrup et al., 2002; Lacroix, Spinelli, Heidbreder, & Feldon, 2000; Shah & Treit, 2003; Treit, Aujla, & Menard, 1998). The ventral hippocampus, mPFC and bed nucleus are components of the limbic system, a circuit long known to be involved in the generation of emotional behaviors; each also is tightly connected with the amygdala. Moreover, these areas are also send efferent output to many of the same downstream regions as the central nucleus of the amygdala, such as the hypothalamus and brainstem. They are therefore well-situated to generate and/or modulate anxiety-related behaviors either independent from or in concert with the amygdala.

The distinction between amygdala-dependent and amygdala-independent anxiety responses has been perhaps most clearly demonstrated in an interesting

paradigm that can be used to test defensive responses to both learned and innate stimuli, the potentiated startle paradigm. In the learned version, usually called fear-potentiated startle, the effect of a learned fear stimulus on the strength of animal's startle response is measured. Normally, a rat will startle to a loud noise by jumping up in the air; the force the rat uses to make the jump is a reliable measure of this "startle response." The rat's baseline startle response is measured, and then the rat is trained to associate a stimulus (like a tone) with a shock, in much the same manner as discussed above. The tone is then presented immediately before the startle stimulus, and the strength of the rat's startle response is again measured. The startle response is much larger when the startle stimulus is given along with the learned tone, as opposed to when the startle stimulus is given alone. The strength of this increase is "fear-potentiated startle" (Campeau & Davis, 1995).

In the innate version of this paradigm, no training is given, but the startle response of rodents is measured in the presence or absence of bright light. Rats will naturally startle more in the light; the strength of the increase in startle response with light is called "light-enhanced startle. Intriguingly, fear-potentiated startle, a learned behavior, requires the amygdala; light-enhanced startle, which is innate, does not require the amygdala (Walker & Davis, 1997). Rather, it requires the bed nucleus, a region implicated in other innate forms of anxiety as noted above (Walker & Davis, 1997). The dependence of light-enhanced startle on other brain regions involved in

innate anxiety (such as the ventral hippocampus and mPFC) has not yet been tested. Notably, lesions of the BNST have no effect on fear-potentiated startle (Hitchcock & Davis, 1991), completing the double-dissociation and supporting the separability of the two anxiety-related circuits.

SEPARABLE BUT NOT SEPARATE: A UNIFIED LIMBIC CIRCUIT OF ANXIETY

While the aforementioned lesion studies have shown that the amygdala is not required for normal anxiety-like behavior in innate anxiety tests, there is considerable evidence that it nonetheless plays an accessory role. Infusing drugs (typically inhibitory agents such as GABA receptor agonists and benzodiazepines) into an otherwise intact amygdala has profound effects in several of these tasks, including the elevated plus maze, open field and social anxiety tests (Green & Vale, 1992; McNamara & Skeleton, 1993; Pesold & Treit, 1995; Sanders & Shekhar, 1995; Zangrossi Junior & Graeff, 1994).

Neuroanatomical data supports the notion that the various structures implicated in innate forms of anxiety are part of the same circuit as the amygdala. For example, both the ventral hippocampus and the mPFC project to many of the same brainstem structures involved in producing defensive behaviors, such as the periaqueductal grey (Burwell, Witter, & Amaral, 1995; Vertes, 2004), as does the central nucleus of the amygdala (Veening, Swanson, & Sawchenko, 1984). Moreover, the BNST, mPFC and ventral hippocampus each project directly to the amygdala itself (Alheid, de Olmos, &

Beltramino, 1995; Burwell & Witter, 2002; Vertes, 2004), suggesting that the separable innate anxiety and learned fear pathways nonetheless are capable of interacting. Importantly, both the mPFC and the ventral hippocampus receive highly processed contextual information from association cortices and rhinal cortices (Hoover & Vertes, 2007). This suggests that the mPFC and the ventral hippocampus are in an ideal position to evaluate threats in the environment and activate downstream structures (such as the brainstem) to induce defensive responses.

Studies of neural activity also confirm that notion that the hippocampus, mPFC and amygdala work together in both innate anxiety and learned fear. For example, although the hippocampus is not required for normal freezing responses to fear-conditioned tones, neural activity in the hippocampus nonetheless synchronizes with activity in the amygdala during presentation of the tone (Seidenbecher, Laxmi, Stork, & Pape, 2003). We have recently found similar synchronization between the hippocampus and the mPFC in mice exposed to the elevated plus maze and a novel open field (A.A. & J.A.G., unpublished observations).

These findings suggest that the mPFC, hippocampus, amygdala and BNST are indeed part of a functional circuit involved in the generation and modulation of defensive behaviors (FIGURE 6.4). While certain elements of the circuit play particularly important roles in certain forms of anxiety (BNST for light-enhanced startle, an innate response; amygdala for fear conditioning to tone, a learned response), it is

likely that under normal circumstances the circuit operates together to compare and evaluate threats in the environment and generate the appropriate specific defensive responses.

FUTURE DIRECTIONS: RELEVANCE OF THE EXTENDED ANXIETY CIRCUIT TO ANXIETY DISORDERS IN HUMANS

Considerable questions remain with regard to the relevance of this combined circuit to human anxiety disorders. As noted above, the key elements of the learned fear pathway – chiefly the amygdala and mPFC – have been clearly implicated in anxiety disorders through neuroimaging studies. Moreover, knowledge about the mechanisms underlying learned fear – particularly the requirement for NMDA receptor-mediated plasticity in extinction of learned fear – has led directly to novel treatment approaches. Can our neurobiological understanding of innate fear contribute further to our knowledge of and ability to treat pathological anxiety? Are the brain regions required for innate anxiety involved in human anxiety disorders? Will further investigation of the patterns of neural activity responsible for innate anxiety lead to novel therapeutic targets and strategies?

Anxiety disorders clearly have learned and unlearned components, suggesting that both types of animal models are of potential relevance. The generalization seen in panic disorder and PTSD – where patients "learn" to avoid situations and places which

trigger anxiety symptoms – seems to be a phenomenon akin to learned fear. Simple phobias may arise from learning, such as the child who becomes afraid of dogs after getting bitten. Yet there are also examples of phobias without any evidence of prior threatening exposure, suggesting direct relevance of models of innate anxiety. Finally, generalized anxiety disorder offers perhaps the most compelling case for the relevance of innate anxiety, in that patients worry about numerous aspects of their lives without any logical rationale or previous experience. To the extent that such patients are overly anxious in response to typical threats, they are akin to the genetically altered mouse which responds to elevated plus maze with increased open-arm avoidance: They are biased towards a stronger defensive reaction.

Moving beyond such phenomenological comparisons to hard data demonstrating the relevance of innate anxiety to human disorders will require further study. Neuroimagers might focus on neglected components of the extended anxiety circuit. For example, the anterior hippocampus (the human analog of the rodent ventral hippocampus) is located quite close to the amygdala, suggesting the possibility that the abnormal amygdala activity occurs in concert with abnormal hippocampal activity. Functional connectivity between the mPFC and the amygdala has been a focus of neuroimaging studies; might the increased functional activity we have reported between the mPFC and the hippocampus also be seen in anxiety disorder patients? Focusing on patients with disorders less readily explained through learning, such as

specific phobics without prior exposure, or patients with generalized anxiety disorder, might be particularly helpful to further explore the relevance of animal models of innate anxiety. Studies, such as these, that take into account multiple animal models of anxiety, have the potential to identify additional critical elements of abnormal brain function that lead to pathological anxiety. If such efforts succeed, they promise to enhance our understanding and treatment of anxiety disorders in all their various forms.

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FIGURE LEGENDS

FIGURE 6.1. Conditioning and extinction of learned fear. *A,* Conditioning of learned fear. Pairing a neutral tone and a shock (*left*) results in the animal learning that the tone predicts the shock. Subsequent exposures to the tone evokes defensive behaviors such

as freezing (*right*). *B,* Extinction. Repeated exposure to the fear-conditioned tone teaches the animal that the tone no longer predicts shock (*left*). Subsequent re-exposure to the tone fails to elicit defensive behaviors (*right*).

FIGURE 6.2. Fear conditioning circuit. Shock and tone information is integrated in the basolateral amygdala, which, through NMDA receptor activation, learns the association. The basolateral amygdala then activates the central nucleus, which in turn activates downstream regions in the hypothalamus and brainstem that are responsible for different elements of the fear response.

FIGURE 6.3. Two innate tests of anxiety. A, the elevated plus maze. B, the open field.

FIGURE 6.4. A putative unitary anxiety circuit. Information processing by the medial prefrontal cortex (*mPFC*) and ventral hippocampus (*vHPC*) guide the amygdala and bed nucleus of the stria terminalis (*BNST*). The former is primarily responsible for learned fear behaviors, the latter for innate anxiety.

FIGURE 6.1

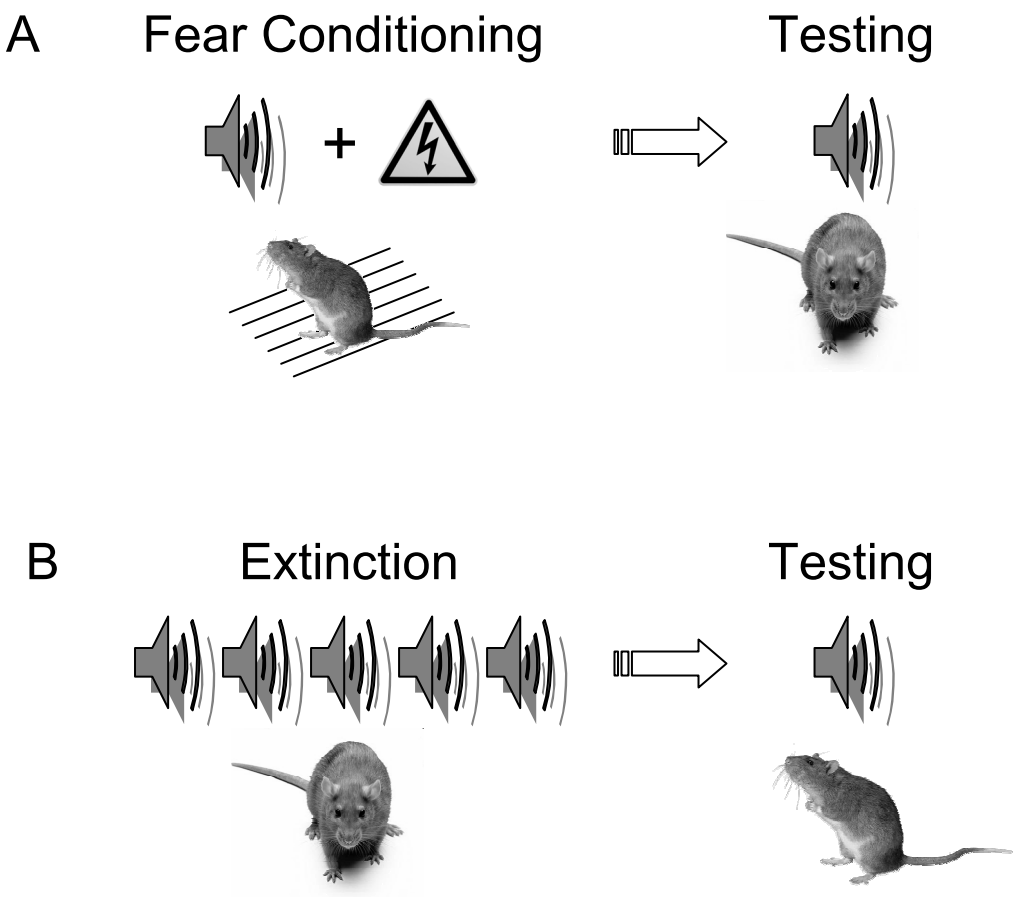
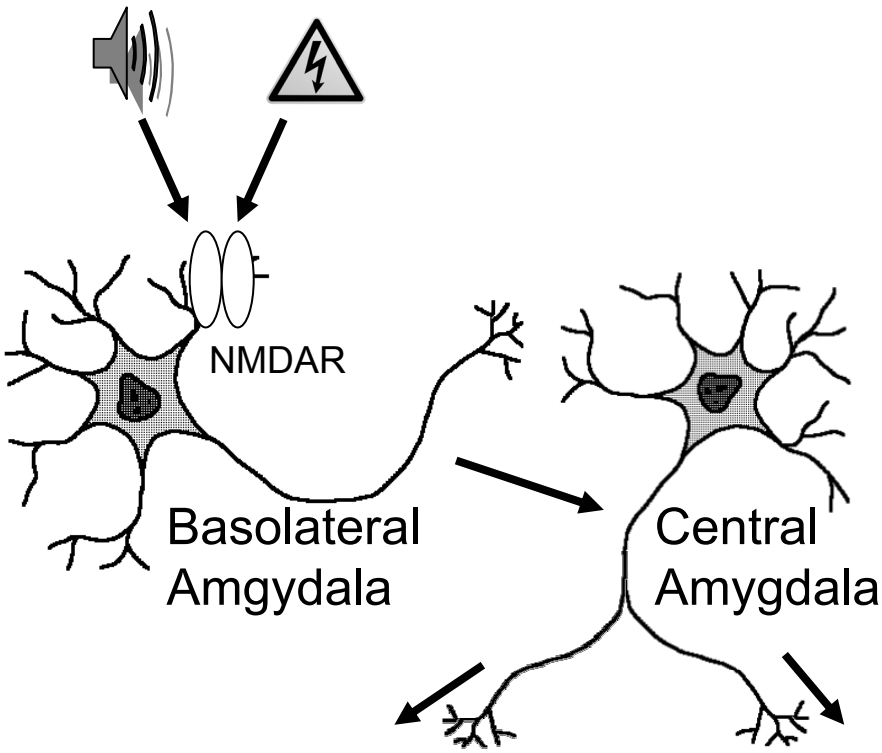


FIGURE 6.2



Hypothalamus

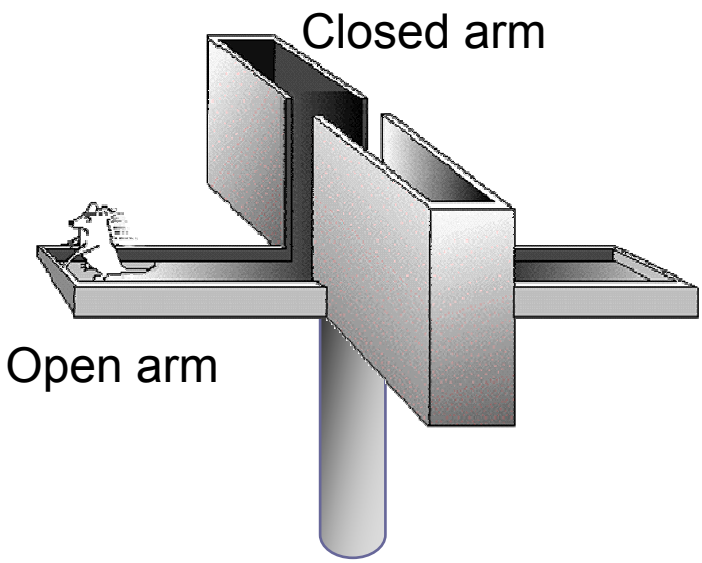
- Lateral: autonomic responses
- Paraventricular: corticosterone release

Brainstem

- Periacqueductal Gray: freezing, suppression of pain
- Locus ceruleus: arousal, attention
- Parabrachial nucleus: tachypnea
- Trigeminal/facial motor nuclei: facial expressions of fear

FIGURE 6.3

A. Elevated plus maze



B. Open Field

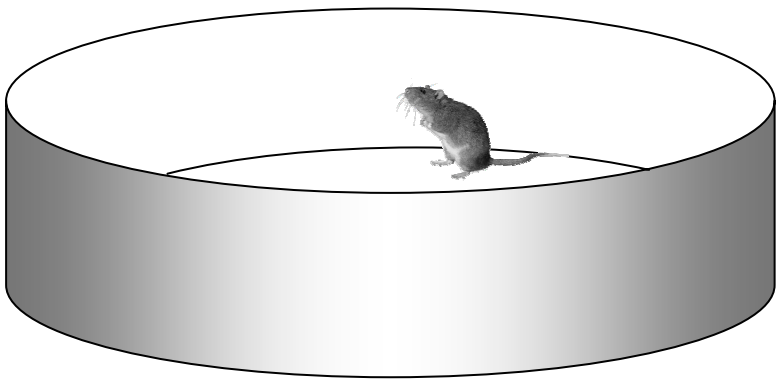


FIGURE 6.4