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Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic

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Abstract

The natural defensive behaviors of laboratory mice have been evaluated in both seminatural and highly structured situations; and characterized in terms of eliciting stimuli, response to pharmacological agents, behavior patterns, and outcome or effect on the social and physical environment. The defense patterns of laboratory mice and rats are generally similar, but mice show risk assessment on initial exposure to highly threatening stimuli while rats do not, while rats display alarm vocalizations, missing in mice. Quantitative differences in freezing and flight for laboratory mice and rats appear to largely reflect domestication effects, with wild mice and rats more similar to each other. This nexus of detailed within-species and comparative data on defense patterns makes it possible to reliably elicit specific defenses in mice or rats in an experimental context, providing well-validated assays of the natural defensive behaviors themselves, as opposed to 'models' of defense.

The mouse–rat comparisons indicate considerable cross-species generality for these defense patterns, as does a scattered but considerable literature on other mammalian species, generally involving field studies and typically focusing on those aspects of defensive behavior that are visible at a distance, such as vigilance, or flight. Although potential homologies between normal mouse and human defense systems should ideally involve all four pattern components (stimulus, organismic factors, response characteristics, outcome), predictive validity in terms of response to drugs active against specific defensive psychopathology is the most extensively investigated of these. Flight, as measured in the Mouse Defense Test Battery shows a consistently appropriate response to panicolytic, panicogenic, and panic-neutral drugs, while some other predictive 'panic models' (dPAG-stimulation; DMH-inhibition; possibly conditioned suppression of drinking paradigms) also elicit and (indirectly) measure behaviors potentially related to flight. Models unrelated to flight (e.g. ultrasonic vocalization to conditioned stimuli); or for which flight elements may a relatively minor contributor to the behavior measured (Elevated T-maze) are less predictive of panicolytic or panicogenic action. These findings indicate that natural defensive behaviors provide a well-characterized pattern for analysis of effects of genetic or other physiological manipulations in the mouse, and may also serve as a model for analysis of defense-related human psychopathology.

Keywords: Defense; Defensive behavior; Flight; panic; Mouse models; Panicolytics; Panicogenic; Behavioral assays

The recent explosion of genetic techniques and genetically modified animals has greatly exacerbated the need for comprehensive, natural, models or assays of behavior that are relevant to mice, the mammalian species of choice for genetic research [25]; and has additionally focused attention on issues of construction and validation of these tests.

Minimally, a behavioral model/assay should enable the reliable elicitation and measurement of a qualitatively consistent behavior pattern for animals of a particular species, taking into account variables such as age, sex, strain, and the like. However, for maximal usefulness of data from these, it is helpful to recognize that the broader goal of such studies is to understand and potentially control the actions and interactions of genetic, physiological or other factors with reference to behavioral and physiological outcomes, not just in mice but in other species as well. This suggests that mouse behavior tests should be evaluated both in terms of their reliability and validity as indices of mouse behavior per se, and also with a view to their potential as analogues or homologues of human behavior. Over 20 years ago, Frank Beach [26] suggested that there are '*…two cardinal rules that should govern not only the construction of animal models for human behaviour, but for all interspecific comparisons regardless of the behaviour and the species involved. The first rule is that meaningful comparisons are based not upon the formal characteristics of behaviour,*

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but upon its causal mechanisms and functional outcomes…. The second rule is that the validity of interspecific generalization cannot exceed the reliability of intraspecific analysis. Significant comparison of a particular type of behaviour in two different species is impossible unless and until the behaviour has been adequately analyzed in each species by itself'.

This statement has a number of implications. First, it suggests that, particularly when non-human behavior patterns are to be compared with those of humans, the factor most likely to be different is the behavior itself. Analysis of behavior patterns has progressed substantially since the Beach statement was made, and methods of analyzing commonalities and differences within cross-species diversity in behavior are emerging [78,106]. Nonetheless it remains true that behavior alone, as particular types and patterning of movements, provides a much less precise analytic foundation than does a complete behavior paradigm (NIMH meeting 'It's Not Just Context' January, 1998), including eliciting stimuli, and, typical outcome; along with consideration of modulating organismic factors, of which genetic aspects are an important component. In fact when a complete behavior paradigm has been sufficiently characterized, the behaviors produced by manipulation of the stimuli and situations important in the evolution of the behavior pattern should not be considered to represent a 'model' nor even a 'simulation' [107], p. 7: What is elicited is the behavior itself, and attempts to measure it involve behavioral assays, not models.

The second of Beach's cardinal rules states that such paradigmatic analysis—for both species that are to be compared—is a necessary precursor to any truly meaningful comparison between these species. In the present context, of behavior patterns that may be useful for genetic analysis in mice, as well as for potential comparison of both behaviors and underlying mechanisms to other species, the necessity for a comprehensive inspection of the validity of these behaviors for mice is exacerbated. While this inspection, for a particular behavior pattern, is the major focus of the present treatment, an additional implication of the second Beach rule is that a corresponding inspection/analysis for other species to which generalizations may be made (e.g. rat; human) is also necessary. In this comparative context, the term 'model' is more appropriate, in that a mouse or rat may serve as a model species for which cross-species generalizations are intended.

Here, we attempt to demonstrate how these considerations may be applied in the case of defense, a behavior pattern for which there is considerable data from both mice and rats, providing the possibility of detailed comparisons to establish both intraspecies and cross species validity. In addition, defense has also been analyzed in wild as well as laboratory mice and rats, enabling some understanding of domestication effects in these species, and providing a broader basis for use of these behaviors in analyses of genetic and physiological manipulations. Finally, some of these defensive behaviors constitute a potential analogue or perhaps even homologue to human behaviors that may be important in specific defense-related psychopathologies.

1. Eliciting stimuli for defense

The eliciting stimuli for defense represent the array of dangers encountered in every natural environment. First, there are many inanimate, immobile, and relatively easily avoided threat sources such as poisons, sharp things, and high or tight places; along with a few inanimate but highly mobile dangers such as fire and floods. In terms of evolutionary mechanisms, none of these is likely to have a very profound effect on defensive behavior since defense against the first group is very simple, and against the second, virtually impossible. Neither situation provides any substantial environmental pressure for development of the rapid and finely-tuned defenses that have evolved in response to conspecific and predator threats.

The dangers that have been most influential in shaping the mammalian defense pattern are animate. They consist of attacking conspecifics, predators, and, for some species, non-conspecific competitors. In each case these dangerous animals are themselves the product of evolutionary forces that have promoted the development of size, strength, weapon systems and other behavioral and structural specializations that enhance their ability to attack, defeat, and kill conspecifics, competitors, and prey. Each of these adaptations requires a corresponding adaptation in defensiveness, if an opponent is to survive and propagate. The very close relationship between improvements in attack capabilities, and corresponding enhancement of defense systems, is particularly marked in the case of predator-prey relationships, because an inadequate antipredator defense typically results in death. Conversely, a predator whose prey escapes too often will starve. This parallel evolutionary development is an example of what has been characterized as a 'red queen' relationship, reflecting that each of the participants has to run faster and faster over time, in order to stay in the same place, vis a vis the other [84].

Although both defense against conspecific attack and antipredator defense represent evolved, adaptive, behavior patterns, predators have two clear advantages in terms of serving as stimuli for laboratory models of defensive behaviors. First, they are less likely than attacking conspecifics to change their attack behaviors because of subtle alterations in the defensive behaviors of the animal they are attacking [7]. In addition, the species of the animal selected to serve as a predator is more flexible than that of a conspecific; an attacking conspecific of a mouse, is a mouse, whereas the predator of a mouse may be a rat, a cat, or a human (e.g. graduate student). The last of these is much more amenable to experimental control than are any of the other threat sources; the speed, direction, and type of

its movements can be orchestrated in order to determine how alterations of specific predator features produce corresponding alterations in particular features of defense. Thus, although the range of antipredator defenses, and anticonspecific defenses are similar (albeit not identical) [7], predator stimuli have considerable analytic advantages. This is particularly true in pharmacological studies in which the defensive animal receives a drug, as drugged animals often elicit somewhat different patterns of attack from conspecifics [80].

An additional feature of the proximate determination of defense is the specific situation in which the threat stimulus is encountered, and the 'defensive distance' between the threat stimulus and the subject. In general, the intensity of defense increases as the defensive distance decreases, but this change is not just quantitative: very distant threat stimuli tend to elicit cessation of ongoing activity, orientation to the predator, and sensory scanning (vigilance) while closer threat stimuli may elicit flight or freezing, depending on the situation (i.e. is an escape route available or not) and the prey species involved; and very close (near contact) threat stimuli may elicit defensive threat and, as contact becomes imminent, defensive attack; again depending on the species involved. In contrast, when the nature and location of the threat source are uncertain, mammals may show not only the orientation to the potential threat, and cessation of ongoing activity that is seen also to distant predators, but may actually approach and investigate possible dangers, using a posture and type of movement that, at least in rodents, is highly specific to this 'risk assessment' situation [9].

2. Defensive behaviors in the laboratory mouse

2.1. Mice in a seminatural habitat: The visible burrow system

The Visible Burrow System (VBS) is a seminatural habitat with a large open or surface area (maintained under a 12:12 h light–dark cycle) and with tunnels and chambers (under constant red light, to which the animals are insensitive) opening from this surface area. Groups consisting of one male and several female Swiss-Webster mice (with more than one male, excessive fighting erupts), maintained in this area, utilize the surface area freely during the dark period of the light–dark cycle, sleeping in the burrows during the light period. These VBS provide an environment with a number of features (larger size, surface area, tunnels and chambers) that enable the resident groups to freely express their own defense patterns, with minimal contrivance by the experimenter.

When a live domestic cat-, or, a toy-cat (control), is presented to (Swiss-Webster) mouse groups in the VBS, both stimuli elicit flight into the burrows from all mice in the surface area of the VBS [15]. However, in contrast to mice shown the toy-cat, which re-enter the surface almost immediately and show no diminution in surface time, mice exposed to the cat return to where the tunnels open onto the surface area, peeping through the opening and apparently scanning the cat (side to side head movements can be seen in the overhead videotape records). In order to determine that the mice were deliberately re-establishing visual contact with the cat, we inserted a short Z-shaped section of tunnel pipe adjacent to the surface opening, blocking the mouse's view unless it maneuvered awkwardly through this section in order to look out at the surface area. Each and every mouse did so, repeating this risk assessment activity and scanning the open area several times during the first 5–10 min, of cat presentation; after which the mice retreated to the depths of the burrow system and remained there for some hours [15].

During the period when the stimulus was present, there was no difference in frequency of head out (of the tunnel openings onto the surface) for the toy-cat- and cat-exposed mice. However, these had different outcomes for the two groups, as the average 3.5 head outs for the toy-exposed group were associated with over six surface entries during this time period, whereas cat-exposed mice showed a similar number of head outs but no surface entries, supporting an interpretation that risk assessment can produce two, opposite, effects, depending on the situation: If a threat is confirmed (cat group), the animal switches to a more specific defense (here, retreat and freezing); if not (toy-cat group), risk assessment results in a return to normal, non-defensive, behavior. The risk assessment difference for these two groups comes much later, with a dramatic surge in head outs for cat-exposed mice during the same time period (16 h) as when they begin to systematically re-enter the surface area. These re-entries are rather protracted events, with the mouse approaching the surface opening, and scanning, then retreating, to return and repeat the scanning a few minutes later, making many more head outs than actual surface entries.

2.2. Rats in the VBS

There were two major differences between mice, and cat-exposed rats in the VBS [7]. First, the risk assessment activity just after cat presentation was marked in mice, but not seen in rats. Upon cat presentation, the rats retreated straightforwardly to the depths of the tunnels, to freeze there. Like those of mice, their freezing behaviors broke up over time and they approached the tunnel openings again, to show intermittent head-out and scanning of the open area and emergence onto the surface area, on average 7–10 h after the cat was presented and removed. The peak risk assessment period again occurred in conjunction with first re-entry onto the surface area.

However, there was an additional difference for rats and mice: Rats show alarm vocalizations to the cat [10,11]. We

have attempted repeatedly to detect mouse ultrasonic vocalizations to the presence of a cat, without success (unpublished observations). This difference may be related to the more colonial life style of rats compared to mice, since the mammalian tendency is that group-living species are more inclined to show alarm calls than solitary species. This interpretation is also consonant with findings [10] that rats fail to emit alarm cries to a cat when they are alone with the cat, and not part of a conspecific group. The relationship between alarm cries and risk assessment in the VBS may be that whereas rats reinforce and maintain (and perhaps even elicit) avoidance by their alarm cries, mice, lacking these, find it necessary to check out the threat stimulus individually. If this is true, then mice forced to stay in visual contact with the cat long enough to acquire clear knowledge of the threat stimulus, should show less subsequent risk assessment. In fact, pilot studies in our laboratory indicate that when mice are briefly kept on the surface in a cage with the cat, they run deep into the tunnels when released and stay there.

2.3. A rodent defense model

The defense of mice and rats to a cat in a semi-natural situation consists of the following sequence, unfolding over a number of hrs: Behaviors are listed in terms of time of peak incidence. (no. 2) (in italics) is different for mice as compared to rats.

1. Flight to the burrows (immediate).
2. Risk Assessment without reentering surface (mice) (first 5–10 min), alarm cries (rats) (30–60 min).
3. Freezing or immobility in the burrows (several hours).
4. Protracted pattern of approach to surface; peeping through tunnel opening; scanning surface (risk assessment).
5. Long-latency re-entry on surface.
6. Inhibition of non-defensive behaviors throughout, with gradual resumption of normal activities over many hours.

These findings suggest some specific defense differences of mice and rats, potentially related to the more colonial habitat of the latter, but set against an overwhelmingly similar pattern for the two when the two species are evaluated in a familiar situation affording and supporting a number of possible behaviors.

2.4. Defensive behaviors in a structured threat situation, the mouse defense test battery

Unfortunately, the VBS does not provide for 'on-demand' appearance of specific defensive responses. However, by using a predator (a hand-held, deeply anesthetized, rat) that systematically approaches the mouse in either an endless oval runway or (by closing off the runway) a straight alley, the Mouse Defense Test Battery (MDTB) enables well-controlled elicitation of flight, freezing, risk assessment, and also defensive threat and attack (not seen in the VBS because subjects can always escape from close contact with the cat). We have evaluated mice in the MDTB with and without the hand-held rat; in the latter case, the experimenter who holds and manipulates the rat placed his hand, bunched into a rat-grasping position, inside the alleyway and brought it up to the mouse following precisely the same protocol as with the rat stimulus. Under this condition, mice showed orientation to the hand and occasionally moved away as it approached, but the certainty and celerity of movement, as well as the high degree of precision between movements of the stimulus and movement of the subject was lost. It might be noted that these mice were reacting to a hand and part of an arm, not to the whole experimenter. The outside walls of the MDTB are high enough that the experimenter is invisible to the rat, while he or she can see the rat as a reflection in the inside wall of the MDTB.

The use of a rat as a mouse 'predator' rests on findings that rats are indeed predators of mice with hunger enhancing this tendency [71,86]. Moreover, mice appear to recognize rats as dangerous, without contact or prior experience: Rat exposure causes disruptions of pregnancy in mice [30].

The control (i.e. no drug) data for about dozen MDTB studies run in this laboratory have provided a great deal of information on the defensive behaviors of mice to the rat. Flight is the dominant response in the endless runway (escape) situation. The mouse turns and runs from the rat when a relatively consistent rat–mouse distance (about 1.1 m) is reached. Typically, it runs around the oval runway until the rat is left behind (i.e. out of sight) only to recommence moving away when the rat is brought around the curve into visual contact. When the rat approaches at a high rate of speed, the mouse flees swiftly, making several (4 + m) circuits of the runway in a short period of time. However, in keeping with VBS findings that Swiss-Webster mice show high levels of risk assessment even in situations of imminent danger, risk assessment can occur even during flight; the fleeing mouse stops abruptly, and may look back (orient) toward the oncoming rat. It sometimes reverses direction to approach and run past the predator.

When the runway is converted to a straight (inescapable) alley, and the rat is brought up to the mouse, pausing at set distances, the mouse orients to the rat, but shows little freezing. Instead it tends to approach and then withdraw from the rat, another risk assessment behavior. As the rat approaches closely, the mouse shows a defensive threat/attack pattern of upright (boxing) behavior, sonic vocalizations, jump attacks, and bites. With the exception of the two sets of risk assessment behaviors, this entire behavior pattern is very similar indeed to that of wild rats in a parallel situation.

Table 1
Behaviors of wild mice and six strains of laboratory mice in the mouse defense test battery

Sub-test	Measure	Wild mice	Lab mice
Flight/avoidance in oval runway	Avoidance distance (cm)	125.50	64.00
	Avoidance frequency (five trials)	4.90	2.65
	Flight speed (m/s)	0.69	0.57
Risk assessment (RA) in oval runway	Stops	0.30	4.25
	Reversal	1.00	1.45
	Orientation	1.50	2.35
Straight alley	Approach–withdraw	0.20	1.82
	Immobility(s)	34.50	10.40
Response to forced contact	Vocalization	0.00	2.80
	Upright posture	0.20	2.46
	Biting	0.40	1.50
	Jump attack	4.70	0.92

2.5. Wild vs laboratory rodents: Domestication effects on defense

The mice typically used in the MDTB are laboratory Swiss-Websters, while the rats used in the parallel situation were, depending on the study, first or second generation wild-trapped wild rats. The latter show a very consistent pattern [7], of flight (when an escape route is available); freezing (if it is not); and defensive threat and attack as the defensive distance between the threat stimulus (the experimenter) and the subject decreases toward zero. In contrast, laboratory rats show reduced flight, and essentially no jump attacks or biting to human approach and contact in this task. Instead, they freeze, and this single defensive behavior accounts for about 80% of behaviors seen as the experimenter approaches within a range of about 5–2 m, in an enclosed alleyway. These changes suggest that the process of domestication has had a major impact on defense.

What then of wild mice? Table 1 presents a number of measures from the MDTB, for wild mice (fourth laboratory generation, from stock originally trapped near Capalbio; Grosseto, Tuscany, Italy: [13]), compared to mean scores for laboratory mice of six different strains (combined from Refs. [13,53]).

The wild mice showed an immediate and high intensity flight response to the rat, fleeing out of visual contact (i.e. around the curve of the oval runway) on 98% of trials. When chased, they fled at an average speed of 0.69 m (about six body lengths) per second. In the laboratory mice flight frequency was reduced, although flight speed when the mice did flee was little changed: both wild and lab mice run, fast, away from the rat. When the runway was blocked, freezing to the approaching predator was high; giving way to jumps, either toward the runway wall (attempted escape) or toward (and typically around) the oncoming rat at about 50 cm prey–predator distance. These close-contact jumps toward and around the rat were so effective that sonic vocalization and biting were rare for wild mice in this test, although they were prominent when the wild mice were tested with the anesthetized rat in a more confined situation [13].

Risk assessment activities of wild mice occurred in two different subtests of the MDTB, and were qualitatively quite similar to those of Swiss-Webster mice, but less frequent than in the domesticated strains. Finally, in direct contrast to the direction of domestication effects in the rat, laboratory mice showed considerably *less* immobility than the wild mice.

These rat and mouse data from test batteries designed to elicit specific defensive behaviors strongly suggest that the basic rodent defense pattern, as exemplified in *wild rats* and *wild mice*, is quite similar except for the exception already outlined; that only the mice show active risk assessment—approach and sensory investigation—when confronted by a predator as well as to more ambiguous threat stimuli or situations (ultrasonic vocalizations have not been measured in these test batteries). Laboratory rat and laboratory mouse patterns are quantitatively somewhat less similar to each other, due to different (sometimes opposite) domestication effects in the two species; more freezing for laboratory rats, less for laboratory mice, and a reduction of biting only in rats. Nonetheless, flight, freezing, defensive threat and attack, and risk assessment all occur in both mice and rats, and show a generally similar set of relationships to eliciting stimuli and situations.

2.6. Species-specificity of defensive behaviors

This correspondence of defensive behaviors, greater between wild rats and mice than for laboratory strains of the two species, brings up a seemingly innocuous semantic point that reflects an important issue. The term 'species-specific' is often applied to defensive behaviors. Yet flight, freezing, defensive threat/attack and risk assessment (referring to behaviors often subsumed within the category of 'vigilance') occur in the defense repertory of many other

mammalian and even inframammalian species [37,100]. The commonalities in defense patterns include the types of threat stimuli that elicit these behaviors; the relationship between characteristics of the threat situation and the type of defensive behavior that is emitted; the defensive behaviors themselves, and the order in which they tend to occur, endure, and disappear in response to the appearance of a threat stimulus; and the effects these behaviors have on the environment. The degree of congruence in this pattern across mammals cannot yet be satisfactorily estimated, because the type of detailed experimental analysis of defense that has been made for rats and mice is not available for other species. However, in very brief summary of an enormous literature, chiefly involving field studies [37], there is a good deal of data suggesting that many mammal species, particularly those that are heavily predated, show defense patterns that provide an excellent fit to the 'rodent' schema. Thus it is simply incorrect to refer to defensive behaviors in general as species-specific.

That these patterns of defense are not species-specific but widely represented in mammals is crucial in terms of their potential applicability to human defensiveness and to the psychopathologies that may be related to these biobehavioral patterns. Although there is a conceptual 'missing link' in this equation—detailed and systematic information on normal human defensive behavior is badly needed—some individual defensive behaviors appear to be strikingly homologous to particular types of psychopathology, with reference to both the eliciting situations, and the responses made to them. For example, anxiety is consistently analyzed as a response to potential or ambiguous threat (with such divergent approaches as those of Freud [43] and Estes and Skinner [39] agreeing on this point). In the Diagnostic and Statistical Manual of the American Psychiatric Association III-R, generalized anxiety disorder (GAD) had as its two major behavioral manifestations 'apprehensive expectation' and 'vigilance and scanning' [2], both of which correspond well to a risk assessment pattern. First, risk assessment is elicited maximally (and enduringly) in situations in which threat is suggested by partial stimuli (odors) or learned or unlearned cues (e.g. a situation in which a predator was previously encountered; the odor of a predator). Second, risk assessment consists of 'vigilance and scanning' in response to such potential threat; a situation that permits a reasonable interpretation that, insofar as the animal is capable of such cognitions, its 'expectations' are 'apprehensive'. It is interesting that, although only 'apprehensive expectation' was maintained in DSM IV, with 'hypervigilance' becoming part of the diagnostic criteria for acute stress disorder [3], p. 432), 'Irritability' was listed as one of six symptoms, among which three or more were required for sufficient duration, to meet the criterion of GAD. This additionally suggests a link to defensive threat and attack, the other component of the anxiogenic profile based on response to clinically effective anxiolytic drugs [12].

These apparent similarities are often labeled 'face validity'; an extremely unfortunate term in this context, as it suggests that such congruence is only at a surface level, concealing underlying differences. Yet the standard method of analyzing the 'structure' of a behavior is coming to be accepted as focusing on four constituents, eliciting stimuli; organismic variables; response or behavioral characteristics; and effects or outcomes (e.g. N.I.M.H. Workshop 'It's not just Context' January, 1998), and these situational and response correspondences of risk assessment and generalized anxiety disorder provide 'face validity' for two of these four aspects. With reference to the others, the typical outcome of risk assessment is to gain information about the anticipated threat [9], as is, presumably, the goal of the 'Vigilance and Scanning' associated with GAD. That GAD sufferers find it difficult to reach this goal, and identify the threat in order to deal with it more effectively, is a primary manifestation of the status of GAD as a psychopathology, rather than a normal defensive behavior. This view suggests the specific hypothesis that similar ruminative or investigative activities that do have an appropriate informational outcome (with reduced emotionality when the situation is found to be harmless) will be found in the context of normal behavior. Moreover, at least some aspects of the organismic basis of risk assessment appear to be similar in mice and humans: Risk assessment measures respond appropriately and selectively to anxiolytic drugs, both in the MDTB (reviewed in Ref. [12]) and, when appropriate ethological measures are added to existing models such as the elevated plus maze [85]. This pharmacological 'predictive validity' is the tail that wags the dog with reference to most animal models. However, in a wider context of attempts to understand the biobehavioral systems involved in emotionality and emotional psychopathology, and the relationship of these to genetic mechanisms, similarity of response to pharmacological agents is only one of four aspects; albeit the fourth aspect to show similarity for risk assessment and generalized anxiety disorder. Insofar as an animal model shows an adequate degree of correspondence to human patterns on all four levels we suggest it may be regarded as having 'systemic validity', in essence providing an homology as opposed to an analogy to the human behavior patterns.

The ethological concept of behavioral 'homology' adds another requirement; that a behavioral system be represented in the common ancestor of the two species to be compared, and descending without interruption in intervening organisms. Given that the common ancestor of mice and men is an unknown and certainly long-extinct early eutherian that lived perhaps 50 million years ago [37], its behavior is not directly accessible. However, even the most primitive of mammals, monotremes, display immobility (associated with rolling into a ball: echidnas) and also flight and defensive threat/attack (platypus) in an antipredator context ([37]; p. 19), while many marsupials (noted for didelphidae: [37], p. 37) show flight or freezing, depending on the initial stimulus; and defensive threat and attack on being 'cornered

or captured'. We have also spent some time serving as threat stimuli for various species of kangaroos in a 'plains zoo' situation in which these animals were living under seminatural conditions in large paddocks. Based on these experiences we can attest that they show risk assessment activities to potential threat, as well. The widespread representation of these mammalian defense patterns (note the relationship of each defensive behavior to relevant eliciting stimuli, similar to that for contemporary rodents) in animals from which placental mammals had diverged by about 135 million years ago makes it very likely indeed that the common ancestor of mice and men also displayed these behaviors, in much the same circumstances and to the same effect as do present mammals.

It should be noted that claims of homology do not require that a behavior be totally unchanged [96]. Indeed, as the Beach quotation at the beginning of this article indicated, the 'formal characteristics' of behavior, involving specific movements and movement patterns, are likely to be less meaningful than are its functional outcomes, and causal mechanisms. It is obvious that species differences in locomotor systems, for example, may dramatically change what constitutes 'flight' for different species, without altering the functions of flight in escaping threat stimuli, or their 'causal mechanisms' in terms of relationships to threat stimuli and situations. Similarly, it is predictable that species evolving under conditions of heavy predation may show stronger or more specialized defenses than those that have been confronted by reduced predation pressure (see Ref. [82], for a specific example). Nonetheless, when the defensive behaviors of two species do show a clear similarity in form, as well as agreement with reference to other points of analysis, this adds to a view that they may be homologous. Certainly 'face validity' should not be taken to suggest false validity.

As this suggests, a concept of homology between some mouse and human defensive behaviors, or of systemic validity for a mouse model eliciting those behaviors, rests on more than a single criterion. Given that the bulk of work on mouse behavioral models has traditionally involved pharmacological effects, these provide a convenient means to evaluate one area of similarity, that of the organic systems underlying defense. If particular defensive behaviors provide a genuinely homologous model of some human psychopathology, then they should be capable of providing better correspondence to the known efficacy of drugs with reference to that specific disorder, than do other models. As with 'face validity' this may not be a necessary criterion: Striedter and Northcutt [96] note that physiological systems may change in evolution without destroying the basic homology of behavior between species. Nonetheless, similarity at this level represents an important analytic point, as well as a strong practical consideration for the value of the behavior as a research tool.

3. Defensive behavior and defense-related psychopathology: Panic

3.1. Panicolytic and panicogenic agents

In the context of the potential relationship between defensive behaviors and psychopathology, panic disorder (PD) is a very useful exemplar, in that a range of agents effective against PD, that provoke or exacerbate PD, or that have been found to have no effect on PD, are available. A range of tricyclic antidepressants, 5-HT reuptake inhibitors (SSRIs), inhibitors of monoamine oxidase (MAOIs) and several benzodiazepines (BZs) are effective against PD; often, however, their antipanic efficacy is seen only after chronic treatment or at quite high dose levels. Thus, although BZs have been used for many years in the treatment of panic symptoms, success was limited until the introduction of the high-potency agents alprazolam and clonazepam which offer the benefit of a rapid onset. Clobazam, clorazepate and diazepam are also effective, but the latter mostly at high doses (for reviews, see Refs. [5,17,35]). The tricyclic antidepressants clomipramine, imipramine and, to a lesser extent, desipramine have been widely used to treat panic. However, these drugs have a delayed therapeutic onset of up to 4 weeks or more, and imipramine and clomipramine induce a transient exacerbation of symptoms during the first week of treatment in approximately onethird of the patients (for reviews, see Refs. [64,70,76]). Although the irreversible MAOIs are not routinely used in the treatment of PD, phenelzine has been used to treat panic symptoms [19,28]. The reversible inhibitors of monoamine oxydase, brofaromine and moclobemide were recently introduced into therapy to minimize the risk of serious adverse reactions associated with the irreversible MAOIs. Clinical trials have shown these drugs to be effective antipanic agents [20,83,99,101]. SSRIs are active in reducing panic symptomatology and are better tolerated than BZs, tricyclic antidepressants and MAOIs. Fluvoxamine, paroxetine and sertraline have been the most extensively studied SSRIs (for reviews, see Refs. [67,104]). The problems associated with their use include a delayed onset of action (from 2 to 6 weeks) and the anxiogenic reactions many panic patients will initially experience.

In addition to antipanic drugs, there are a number of pharmacological panic-provoking agents; e.g. the 2-adrenoceptor antagonist yohimbine [21], the non-selective 5-HT-2 receptor agonist mCPP [22], and the selective CCKB receptor agonist CCK4 [18,31,63]. Moreover, a number of drugs evaluated and found to have no efficacy against PD are useful in the analysis of the specificity of drug effects found in particular panic models.

3.2. Panic disorder (PD)

PD is defined in the DSM-IV diagnostic system by the presence of at least four to 13 somatic or cognitive

symptoms, including palpitations, sweating, feeling of choking, fear of dying or paresthesias [3]. It is clear that many of the behavioral symptoms, which often rely on verbal report, can hardly be modeled in animals. However, in the DSM-IV, panic is described as often accompanied by an 'urge to escape' and PD patients 'usually report an urgent desire to flee from where ever the attack is occurring'. It has been suggested that panic may be the result when 'flight or fight' mechanisms are strongly aroused but no perceived route for escape is available [4,32]. In addition, it has been shown that electrical stimulation of the hypothalamo-periaqueductal gray fight-flight system in man elicits symptoms and autonomic changes that closely resemble panic [87]. Based on these observations, several groups have developed experimental paradigms in animals which involve measures of behaviors resembling flight or escape with relationship to an unconditioned threat stimulus. In addition to the flight-related measures of the MDTB, these include the dorsal periaqueductal gray (PAG)-induced aversion model; the elevated T-maze test; and the dorsomedial hypothalamus (DMH) inhibition model. An important caveat for all of these models aside from the MDTB is that they involve rat subjects. The propensity of laboratory rats to freeze in situations in which mice or wild rats would flee suggests that flight measures may be more complexly determined in rat studies, potentially obscuring drug effects that may be more straightforward in mice.

Table 2 presents findings of effects for a variety of psychoactive drugs in these and other panic models. In the MDTB, extensive pharmacological investigations have shown that clinically effective antipanic treatments, including two high-potency BZs (i.e. alprazolam and clonazepam) and chronic treatments with various antidepressants (i.e. imipramine, fluoxetine, moclobemide and phenelzine), reduced flight behavior [12,48,49,51]. In contrast, drug challenges known to trigger or potentiate human panic responses such as acute imipramine, fluoxetine, yohimbine or the BZ receptor antagonist flumazenil [27,54,55,90] were found to increase flight [16,48]. Also in agreement with clinical data are the findings that the traditional BZ chlordiazepoxide, the 5-HT1A receptor agonist buspirone and the 5-HT2 receptor antagonist mianserin did not modify flight behavior in this test [50,52]. Most recently, cocaine has been shown to dose-dependently increase MDTB flight measures [8,14]. Taken together these pharmacological data demonstrate that panic-modulating agents specifically and appropriately affect the flight responses of mice.

Based on clinical observations, Deakin, Graeff, and their colleagues [32,33,44] have suggested that panic may be due to the spontaneous activation of hypothalamic-PAG fight–flight mechanisms. To illustrate this idea, Graeff developed a procedure in which the activation of the rat dorsal PAG (dPAG) leads to behavioral defense manifestations (i.e. flight, jump escape) identified as panic-like [44,46]. Jenck and colleagues modified this procedure by shaping the paniclike reactions elicited by dPAG stimulation into operant self-interruption behavior [61]. Briefly, under dPAG aversive stimulation, animals show rapid acquisition and maintenance of operant self-interruption responses (e.g. lever pressing) which allow them to interrupt the stimulation and escape from it [89]. Drugs known to acutely ameliorate (alprazolam, clonazepam) or precipitate (yohimbine, caffeine, flesinoxan) panic attacks in patients were found to acutely and dose-dependently reduce or enhance, respectively, aversion induced by dPAG stimulation [59,61]. However, this model also revealed paradoxical drug effects. For example, the panic-provoking drug mCPP displayed marked antiaversive action in this test [57]. Similar effects were obtained following acute injection of the SSRIs fluoxetine [60] and fluvoxamine [62] which were both found to potentiate panic reactions in PD patients at the initiation of treatment [34,88]. Moreover, compounds that failed to alleviate panic symptoms in human (i.e. the antipsychotic haloperidol and the CCKB receptor antagonist L-365,260) produced antiaversive effects in this test [56,58]. These latter findings bring into question the validity of the dPAG-stimulation procedure to reveal a specific panic or antipanic profile of panic-modulating agents.

Tonic inhibition of GABAergic activity in the dorsomedial hypothalamus (DMH) induced by chronic infusion of the GABAA receptor antagonists bicuculline or picrotoxin has been shown to result in behavioral and physiological (e.g. increases in heart rate, blood pressure and respiratory rate) reactions in rats that were claimed to resemble a human panic attack [93,95]. Following repeated treatment with the clinically effective antipanic agents imipramine and clonazepam, effects of bicuculline infusion in the DMH were completely blocked [94]. In contrast, the panicogenic drugs sodium lactate, yohimbine and dfenfluramine were found to increase further the behavioral and physiological actions of bicuculline in the DMH [66]. It is important to note that the focus of this model is on the compromised DMH, not on the specific behavioral test (social interaction test) typically used in these studies: Nonetheless the behavioral response seen with this preparation has been described as 'escape'-oriented locomotion [93,95]. However, blocking or enhancing GABAA receptors in the DMH also produces anxiogenic and anxiolytic responses on the elevated plus maze, a test typically not selectively responsive to panic-modulating agents [85]. This suggests that DMH GABAA receptor manipulations produce a wider spectrum of defense changes, some of which but perhaps not all are responsive to panicolytic and panicogenic manipulations.

The Graeff group [45,47] have devised a T-maze apparatus derived from the elevated plus-maze [79]. This consists of three arms of equal dimensions, one enclosed by walls and perpendicular to the two open, opposed arms, all elevated above the floor. Three trials in which the animal is placed in the closed arms are followed by a single trial in which it is placed in an open arm, and latency to escape or retreat to the closed

Table 2
Effects of a variety of psychoactive drugs in animal models of panic. +, anxiolytic-like effects; 0, inactive; −, anxiogenic-like effects; (a), acute only; (c), chronic only; MDTB, mouse defense test battery; dPAG, dorsal-periaqueductal grey; DMH, dorsomedial hypothalamus; USV, ultrasonic vocalization; CSD, conditioned suppression of drinking

	Flight-based models of panic				Learning-based models of panic	
	MDTB	dPAG-stimulation	Elevated T-maze	DMH-inhibition	USV	CSD
Clinically effective antipanic treatments						
Alprazolam	+	+			+	+
Clobazam	+					
Clonazepam	+	+		+		
Clorazepate	+	+		+		
Desipramine (c)						+
Diazepam	+		0		0/+	
Fluoxetine (c)	+	+				
Imipramine (c)	+		+	+		+
Moclobemide (c)	+					+
Phenelzine (c)	+					+
Panic-provoking challenges						
Caffeine		−	0			
CCK_4		0			−	
Clomipramine (a)			0		+	
Cocaine	−					
d-Fenfluramine			+	−	+	
Flesinoxan		−			+	
Flumazenil	−				0	
Fluoxetine (a)	−	+			+	
Fluvoxamine (a)		+			+	
Imipramine (a)	−	0	+		+	0
MCPP		+	+		+	
Yohimbine	−	−	0	−	− /+	
Clinically ineffective antipanic treatments						
Amitriptyline						+
Buspirone	0		0		+	
CI-988		0				0
Chlordiazepoxide	0				0	0
Citalopram					+	
Desipramine (a)					0	0
Haloperidol		+	0		+	
Ipsapirone			0		+	
L-365,260		+			0	0
Maprotiline					0	
Mianserin	0					0
Moclobemide (a)	0		0			
Ondansetron					0	
Paroxetine (a)					+	
Phenelzine (a)	0					0
Ritanserin			0		+	

arm is measured. In validating studies, the anxiolytic agents buspirone and ipsapirone as well as the 5-HT2 receptor antagonist ritanserin did not modify this unconditioned oneway escape, a finding that fits well with clinical data showing these drugs to be inactive in patients with PD [47,73,103]. However, in these studies oneway escape was not enhanced by the panic-provoking agents yohimbine, caffeine and mCPP, or by acute administration of clomipramine, which has found to produce a transient exacerbation of panic symptoms at the beginning of treatment [64]. Instead, mCPP tended to depress one-way escape, suggesting an antipaniclike activity. Similar results were obtained with the 5-HT releaser dfenfluramine which was reported to worsen anxiety symptoms in PD patients [97]. Finally, diazepam which was found effective against panic in several clinical trials [36,75], did not reduce one-way escape over a wide dose-range. Taken together, these pharmacological data barely support the predictive validity for PD of one-way escape in the elevated T-maze.

However, recent behavioral analyses have produced a 'fine-tuned' version of the Elevated T-maze. When rats

are placed on the open arm of the Elevated T-maze, they show an average latency to move out of the open arm and into the closed arm, of about 10–12 s [45]. This, in a 50-cm arm, constitutes a locomotion rate of about .05 m/s; at best, ambling rather than fleeing. A view that these animals are not fleeing the open arm is strongly supported by findings [45] that 'escape' latencies are very similar to the initial latency to leave the enclosed arm, and, that open arm escape latencies do not change over five successive trials. However, 30 min of prior forced exposure to the open arms of the maze results in a decrease in latency to leave this arm on a later trial [98], suggesting a somewhat closer approximation to a flight behavior. With rats previously exposed to the open arms, chronic administration of imipramine increases the latency to leave the open arms. However, acute imipramine produces the same effect, albeit at the highest dose only. These findings are in agreement with the interpretation that latency to leave the open arm, in a naive rat, may reflect more than just a simple flight response, and that previous exposure may somewhat reduce behaviors, defensive or not, that potentially interfere with flight.

3.3. Animal models of panic disorder based on conditioned behavior

Several investigators have developed animal models of panic based on conditioned behaviors. Fontana and colleagues claim that the conditioned suppression of drinking (CSD), a modified version of the Geller-Seifter and Vogel conditioned conflict tests, might serve as an animal model for the screening of antipanic drugs. These authors showed (Table 2) that chronic but not acute treatments with the clinically effective antipanic compounds alprazolam, imipramine, desipramine and phenelzine produced an increase in the number of shocks accepted [24,38,40,41]. In the latter study, repeated treatments with the classic BZ chlordiazepoxide and the barbiturate pentobarbital had no effect. Similarly, the CCKB receptor antagonists CI-988 and L-365,260, inactive in clinical trials against panic [1,68,77], also did not change the behavior of rats in the CSD [29]. However, it is important to note that the tricyclic antidepressant amitriptyline which was found inactive in clinical trials against panic [6,65], produced positive effects in the CSD. Moreover, unlike what is often reported in clinical trials [35], acute imipramine did not produce anxiogenic effects. Despite these latter observations, and the absence of positive effects with panicogenic agents, the CSD appears to be a reliable animal model of PD. Its potential relationship to flight has not been investigated, although conflict situations are typically regarded as involving antagonism between approach and avoidance (flight?) tendencies.

Molewijk et al. [72] suggested that conditioned ultrasonic vocalizations (USV) elicited by reintroducing adult rats into the environment in which they previously received inescapable footshocks may serve as a screening method for panic-modulating drug effects. However, as can be seen in Table 2, major discrepancies between animal studies and clinical findings have been found with this model. For example, although 5-HT1A receptor full and partial agonists (i.e. flesinoxan, buspirone, ipsapirone) potently reduced ultrasound emission, clinical reports invariably failed to show an antipanic efficacy of these compounds [81,92,105]. Instead, panic may even be exacerbated by buspirone or flesinoxan [23,42,74,102]. Moreover, a single administration of clomipramine, imipramine, fluvoxamine, mCPP and yohimbine, which have all demonstrated panicogenic properties following acute treatment, produced clear antipaniclike activity in the USV [72,91]. Taken as a whole, these findings strongly question the predictive validity of USV as an animal model of panic.

3.4. The panic–flight connection

The high prevalence of PD in the community [69] has stimulated the search for novel treatment for this condition. A direct consequence of this research effort has been the development of a variety of different animal models of panic. One potential differentiation among these models, that some are based on learned or conditioned behaviors (USV, CSD, and dPAG-stimulation), while others reflect unconditioned behaviors (MDTB, Elevated T-maze, DMH-inhibition), does not appear to be productive, as both positive and negative outcomes have been obtained for models within each category. Among the conditioned response models the USV procedure, the most extensively used panic model for the screening of panic-modulating compounds, has shown major discrepancies between preclinical and clinical findings. The CSD and dPAG-stimulation tests, in contrast, show relatively good agreement with human studies, although with the qualification that the former has not been shown to respond bidirectionally, by enhanced suppression, to panicolytic compounds. A further division might be made on the basis that the dPAG-stimulation procedure involves a conditioned behavioral response to an unconditioned stimulus (the dPAG-stimulation itself). However, this does not seem to produce any additional insight into what does, or does not, contribute to a predictive panic model.

With respect to panic models that tap unconditioned behaviors to unconditioned stimuli, it is notable that all of these tests may involve some aspect of flight or escape: i.e. flight in the MDTB; 'one-way escape' in the elevated T-maze; and 'escape'-oriented locomotion in the DMH-inhibition paradigm. Although pharmacological data indicate that only the MDTB and the DMH-inhibition test have strong predictive validity, recent behavioral analyses suggest that the 'escape' measure of the Elevated T-maze may respond to a number of as yet unanalyzed behavior tendencies, in which flight is one component.

For the other two tests (MDTB and DMH-inhibition), flight is clearly indicated. Although the main behavioral test used with DMH-impaired rats was the social interaction

test, the salient behavior pattern displayed by these rats was 'full-blown flight' (Shekhar, personal communication). These data strongly suggest that flight may be a crucial component of the behavior seen in tests that respond to panicolytic and panicogenic drugs, even though it may not be the response that is directly measured.

3.5. Summary: Assays and models

These findings with respect to pharmacological effects on particular defensive behaviors point to the value of well-characterized behaviors, both for behavioral assays designed to determine deviations from the normal behavioral repertory of a given species, and, for evaluating the cross species validity of behavioral phenomena. These results are compatible with a view that the biobehavioral systems underlying at least some defensive behaviors—here, flight—are broadly homologous from one mammalian species to another. Although laboratory mice and rats show substantial diversity in quantitative flight measures, much of this difference appears to reflect strong (and in this case, unnatural) selection, during the process of domestication. Moreover, since strong flight occurs with GABAA inhibition in key brain sites, it seems possible that flight has been reduced in the laboratory rat because of strongly selected inhibitory mechanisms, not because the basic flight system itself has been directly impaired. Such a flight-inhibitory factor for rats may be important in findings that rat models using brain stimulation to elicit flight produce more straightforward results, both behaviorally and pharmacologically, than do models evaluating rat escape in response to complex situations such as the Elevated T-maze. Although there is little systematic information on the non-pathological elicitation of flight in humans, the existence of such a response is unquestionable. Both behavioral analyses [32] and the pharmacological findings outlined here suggest that pathophysiological changes in this system may be involved in panic.

Thus intensive analyses of defensive behaviors have produced detailed characterizations for both mice and rats, showing ethological validity with reference to seminatural habitats and demonstrating areas of similarity and difference with the wild ancestors of these laboratory species. These characterizations also outline the focal situations and stimuli that selectively produce specific defensive behaviors, and provide criteria by which these specific defenses may be evaluated and measured. These paradigms involve assays of behavior, not models or simulations, and they enable a much more direct route to analysis of particular defensive behaviors and the biobehavior systems that produce them than may be achieved through tasks designed without such information.

The mouse-rat comparisons, both for wild and laboratory strains, enable a much more detailed understanding of defense in mice, specifically, as well as an appreciation of the rapid, interactive, effects that genetic selection can produce in this pattern. This provides a firm basis for evaluation of gene-linked changes in particular mouse defensive behaviors. Finally, these analyses enable the use of mouse behaviors as models for research on the physiology and pathophysiology of emotion, potentially generalizable to human emotion and emotional disorders.

Earlier analyses of particular rodent defensive behaviors indicated that some of these provided similarities to generalized anxiety disorder both in terms of the behaviors themselves, and in response to pharmacological manipulations. Work reviewed here indicates that an additional defensive behavior, flight, has excellent predictive validity with reference to drugs active with reference to panic, both when measured directly, and, as an important component of the behaviors seen in other tasks that provide successful prediction of panic effects.

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