SECTION 2

Animal Models of Anxiety, Fear and Defense
CHAPTER 2.1

Theoretical approaches to the modeling of anxiety in animals

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Abstract: Theory influences what we mean by the word “anxiety”, what we require of any animal model, and what specific theoretical constructs are embedded in any specific animal model of anxiety. We argue that, in the ideal case, the animal models we use should be embedded in a large-scale theory that integrates all of the theoretical levels of each animal model. We argue that face validity of a model should be ignored and that true predictive validity reduces ultimately to construct validity. So all models should aim to have construct validity based on strong theory. Theoretical analysis shows that anxiety should be distinguished from fear; that, different anxiety disorders should be distinguished from each other; and that the components of any single apparent type of anxiety can have distinct neural control. Theory can show how a model is unsatisfactory, but it can also show that it is not the model but rather our translation from the clinical situation that is faulty. To model the many flavors of clinical disorder and variations in drug effectiveness, we must use theory to link multiple animal models, neural analysis and pharmacological analysis. The goal is to provide us with truly predictive tests that can be used for drug discovery as well as drug development. Most importantly, theory is required if we are to correctly match a particular measure from a particular model with the clinical entity we desire to model.

Keywords: anxiety; fear; defense; pharmacology; theory; model; anxiolytic; panicolytic

I. Introduction

Theory can impact on the assessment of animal models of anxiety at several levels. What we mean by the word “anxiety” is itself a theoretical issue that needs to be settled, at least in a preliminary fashion, before we can define what is to be modeled. What is required of an animal model depends on one’s theoretical perspective on such models. Finally, specific theoretical constructs are embedded in any specific animal model of anxiety – either during its construction or, post hoc, when a new theory has to account for the properties of an older model. We will discuss these three levels in turn and argue that, in the ideal case, the animal models we use should be embedded in a large-scale theory that integrates all of the theoretical levels of each animal model.

II. The nature of anxiety

It is tempting to take the meaning of the word “anxiety” as approximately given and then proceed to uncover the neural or behavioral basis of this entity. But not only are we then assuming rather than demonstrating an answer to the question “What is anxiety?”, we are assuming an answer to the fundamental question posed by William James over 120 years ago, “What is an
emotion?” (James, 1884). The problem is that, despite our everyday use of “an emotion” to talk about some entity we think is inside us, there may be no strictly matching scientific concept. “An emotion” may not be a single entity within an organism. Where this is true, we may need multiple animal models, each one assessing its own independent aspect of “the emotion”. Or, we may need to choose a model in which multiple different measures capture each of the different aspects.

II.A. What is emotion?

We have argued elsewhere (McNaughton, 1989), that the key feature of whatever are variously called emotions (by lay, academic or clinical persons) is that they are products of evolution (Darwin, 1965). Certainly, phylogenetic continuity is an implicit assumption made by all who use “animal models”. The superficial form of an emotion is shaped by development and includes species-specific details. But its underlying structure is conserved not only between individuals but, in terms of things like the action patterns of muscles, across species (Ekman et al., 1980; Ekman, 1982; Redican, 1982; Ekman and Friesen, 1986). In some respects this is not contentious. But we would go further and suggest that it is the recurrence of a particular class of evolutionary requirement – of a specific adaptive pressure – that not only shapes the parts of an emotion (as labeled by us) but also allows the parts to been seen as combined into a nominal whole.

The problem is that evolution does not work in the logical, tidy, way that a human engineer would like. It is the result of a continuous interaction between available mutations and local adaptive advantages. Critically, there are many cases where a number of specific neural mechanisms have each evolved to provide a particular “rule of thumb” (Krebs et al., 1983) that provides a local simple answer to part of a more complex global problem. It is the agglomeration of a sufficient number of such independent reactions that then provides the illusory appearance of a single, higher-order, class of response to that adaptive requirement across many situations.

An example of this is provided by “separation anxiety”. This is easily and regularly identified both by the means of producing it (removal of the primary caregiver, usually the mother) and by the characteristic pattern of responses that then ensues in children and in the young of other mammals such as rats, dogs and primates. In rats, as in humans, separation anxiety is manifest in both behavioral and autonomic responses. These appear together when the mother is removed and disappear together when she is returned.

The behavioral and autonomic components of this “emotion” give the appearance of joint outputs of a single, unified central state. Certainly, one could argue that, if either output were missing, the result would not be separation anxiety. However, it has been shown that, in rats, the behavioral reactions can be eliminated by the presence of a non-lactating foster mother, whereas the autonomic reactions can be eliminated by regular feeding with milk-but not, in either case, vice versa (Hofer, 1972). Thus, the two effector aspects of the “one emotion” can be doubly dissociated in the laboratory.

“Separation anxiety” remains a nameable set of entities that are coherent under normal ecological circumstances and our analysis does not require any change in the everyday use of the term. The combined occurrence of the behavioral and autonomic components of the emotion is guaranteed by the fact that, under normal ecological circumstances, removing the mother necessarily removes, simultaneously, the separate stimuli (milk and mothering) that drive the separate autonomic and behavioral reactions. But, for scientific purposes, we must view the term as grounded in a particular class of evolutionarily recurring situation (loss of parents) which gives rise to a consistent set of adaptive requirements and so a consistent effector pattern (behavioral and autonomic) that constitutes a fairly consistent central state. However, “separation anxiety” does not refer to, or in any way imply, a single internal control mechanism governing the two sorts of pattern and guaranteeing their co-occurrence (Fig. 1).
II.B. What is anxiety?

Having concluded that we may need to invoke more than one measure or model to encompass “anxiety”, we now need to distinguish models of anxiety from models of other emotions. In the past, anxiety has often been conflated with fear and panic:

- anxiety may be focused on an object, situation, or activity, which is avoided (phobia), or may be unfocused (free-floating anxiety). It may be experienced in discrete periods of sudden onset and be accompanied by physical symptoms (panic attacks). When anxiety is focused on physical signs or symptoms and causes preoccupation with the fear or belief of having a disease, it is termed hypochondriasis. (DSM-III-R, 1987, p. 392)

This conflation continues in current clinical classifications – DSM, for example, continues to include phobias, panic and generalized anxiety within a single class of “anxiety disorders” (American Psychiatric Association, 1994). However, while extreme anxiety can result in panic as a symptom (Marks, 1988) panic in its purest sense can occur in the absence of anxiety (Holt, 1990; Carter et al., 1994; Shear and Maser, 1994) and elimination of anxiety can reduce arousal-related panic while leaving primary panic attacks intact (Franklin, 1990). Critically, some drugs, at doses that alleviate generalized anxiety and social anxiety, do not alleviate panic or specific phobia, whereas other drugs do (Table 1). This suggests a neural and functional separation between phobia and panic on the one hand and anxiety on the other. So, how are we to distinguish such entities?

If, as we have argued, a historically recurring adaptive requirement in phylogeny is what links the parts of an emotion, it follows that we can define an emotion by analysis of its possible functional significance. … Important and pervasive human action tendencies, particularly those which occur across a wide range of cultures and specific learning situations, are very likely to have their origin in the functionally significant behavior patterns of non-human animals. … This approach, working through the characteristic behavior patterns seen in response to important ecological demands (e.g. feeding, reproduction, defense) when animals are given the rather wide range of behavioral choices typical of most natural habitats, is called ethoexperimental analysis. It involves a view that the functional significance of behavior attributed to anxiety (or other emotions) needs to be taken into account.

1 This can be loosely translated as “purpose” but without any implication of a purposer. Strictly, it should be referred to as teleonomy (Pittendrigh, 1958).
account; and that this functional significance reflects the dynamics of that behavior in interaction with the ecological systems in which the species has evolved, implying that these dynamics can be determined far more efficiently when the behavior is studied under conditions typical of life for the particular species. (Blanchard and Blanchard, 1990a,b, p. 125)

Such detailed ethoexperimental analysis suggests a categorical separation of fear from anxiety in the defensive responses elicited by a predator (Blanchard and Blanchard, 1988, 1989, 1990a,b). The immediate presence of a predator elicits a distinctive set of behaviors that are identifiable with a state of fear. These behaviors, defined purely ethologically, turn out to be sensitive to anti-panic, but not anti-anxiety drugs (Blanchard et al., 1997). The potential presence of a predator (i.e., its recent disappearance from view, or the presence only of its odor) produces a quite different set of behaviors (especially “risk assessment”) that are identifiable with a state of anxiety. These behaviors, again defined purely ethologically, turn out to be sensitive to anxiolytic drugs. This analysis of fear predicts, for example, the well-demonstrated insensitivity to anxiolytic drugs of active avoidance in a wide variety of species and of phobia in humans (Sartory et al., 1990) and the sensitivity of passive avoidance to anxiolytic drugs (Gray, 1977). The critical factor distinguishing fear and anxiety (Gray and McNaughton, 2000) appears to be what can be called “defensive direction” (McNaughton and Corr, 2004). Fear operates to allow an animal to leave a dangerous situation (active avoidance); anxiety operates to allow an animal to enter a dangerous situation (e.g., cautious “risk assessment”, approach behavior) or withhold entrance (passive avoidance).

In constructing “animal models of anxiety”, then, we need to distinguish anxiety from fear. We should also note that different drugs (Table 1) could have different relative potencies in relation to, for example, generalization anxiety as compared to social anxiety. So, even within the category of anxiety (or fear) we may need different models to best approximate different sub-types. It should be noted, also, that the theoretical approach taken to the concept of anxiety in this section has been highly general. Our choice of animal models will also be shaped by specific theories of anxiety and fear but we will deal with this issue later when discussing specific models.

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<th>Unipolar depression</th>
<th>Atypical depression</th>
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BDZ₁, early benzodiazepines, e.g., chlordiazepoxide and diazepam administered at typical antianxiety doses. Other sedative antianxiety drugs (barbiturates, meprobamate) have similar effects. BDZ₂, later high-potency benzodiazepines, e.g., alprazolam. The antipanic effect is achieved at higher doses and this has also been reported with equivalent high doses for BDZ₁ (Noyes et al., 1996). BUS, buspirone and related 5HT₁A agonists; CMI, clomipramine; IMI, imipramine and related tricyclic antidepressants, but excluding clomipramine; MAOI, monoamine oxidase inhibitors, e.g., phenelzine; SSRI, selective serotonin reuptake inhibitors, e.g., fluoxetine, citalopram.

Different patterns of response in the table can be attributed to the variation in receptor occupancy or interaction by particular drugs in different parts of the brain. No drug or drug class produces a specific limited effect (despite the omission of side effects from the table) but the variation in relative effectiveness across the different aspects of disorders of fear and anxiety argues for distinct neural control of each effect.


Symbols: 0, no effect; –, reduction; —, extensive reduction; +, increase; (–), small or discrepant effects.
II.C. What is pathological anxiety?

There is a final issue that we need to consider when choosing an animal model of anxiety: what we understand to be the nature of an anxiety disorder. Anxiety can present as a symptom of some physical, non-neural pathology. In such cases we would seek an animal model of the physical pathology (expecting anxiety to be a symptom) but we would not seek an animal model of anxiety in any general sense. Anxiety disorders, in general, as classified by systems such as DSM, involve unwanted reactions that are either extreme or occur to inappropriate eliciting stimuli but which are otherwise indistinguishable from normal behavior. Thus, when the “reaction is beyond that expected for the child’s developmental level”, separation anxiety becomes separation anxiety disorder (American Psychiatric Association, 1994).

In practice, the more recently developed animal models of anxiety generate anxiety with ecologically relevant stimuli; and the resultant changes in behavior are (as noted above) sensitive to drugs that treat the symptoms of human clinical anxiety. This suggests that “anxiety disorder” will often simply represent an extreme tail of the normal distribution of population sensitivity to anxiety-provoking stimuli. However, as we develop deeper, neurally based, theories of defensive disorders it is likely that in some cases there may be genuine neural pathology underlying the symptoms. This seems likely with some cases of panic and obsession (notably those that lack any concurrent symptoms of anxiety); and, where this is true, we may want to develop pathology-specific models.

We have treated fear and anxiety, here, as specific distinct emotional states that can occur in both an adaptive and a pathological form. This matches the loose forms of common usage (where neither word entails pathology) and to some extent what is said within DSM. However, it should be noted that there are those who use the term anxiety to mean “pathological fear” (with “fear” being by definition adaptive) and, contrariwise, those who use the term fear to mean “extreme anxiety” (with the implication that this is often pathological). We think that a major advantage of the usage we have adopted, based on both animal experimental data and ethoexperimental analysis, is that it provides a clear basis for a consistent terminology that roughly encompasses the various different prior usages – and resolves the inconsistencies between them. Critically, clinical categories (such as social anxiety) that involve approach-avoidance conflict are sensitive to anxiolytic drugs such as diazepam and buspirone; but those (such as simple phobia) that involve pure avoidance are not sensitive to these drugs.

III. The nature of an animal model

The nature of an animal model, and the division of models into subtypes, has been approached from a wide range of perspectives (Joel, 2006; van der Staay, 2006). We think it preferable to use the word “model” rather loosely and to not attempt to characterize types and subtypes of model. Pragmatically, an animal model is something you use instead of a direct test on humans. The animal model may be a restricted, simplified, measure or set of measures and so “model” aspects of the test animal’s neural or behavioral repertoire as well as aspects of the human condition. But the model could also involve a complex situation that can simultaneously produce many aspects of a particular human condition. In this case we are likely to have chosen to use an animal for economical, ethical or pragmatic reasons rather than for simplicity.

III.A. Validating animal models

The key issue in relation to an animal model is whether it delivers measures that can stand in for ones that we would have taken in a direct human test. This is an issue usually referred to as “validity” and we will consider types of validity and their relation to each other below. It is important to bear in mind, given our discussion of the parts of an emotion and of distinctions between types of defensive emotion that a model may be a valid measure of some aspect of anxiety but be an invalid measure of some other aspect.
So, in determining the fitness for our current purpose of a particular model, it is as important to be as clear about the aspect of anxiety that we wish to assess as it is to know the validity of the model in relation to the aspect that it nominally measures.

While different authors may disagree on terminology and classification, there seems to be a wide agreement that it is impossible to develop an animal model that mimics a psychiatric syndrome in its entirety, and that therefore the criteria that an animal model must satisfy to establish its validity depend on the purpose of the model (Matthysse, 1986; McKinney, 1988; Willner, 1991; Geyer and Markou, 1995). In the context of neurobiological research, in which the aim of animal models is to promote our understanding of the modeled condition by elucidating its neurobiological mechanisms (Geyer and Markou, 1995), it is widely agreed that a common physiological basis of the model and the modeled condition contributes greatly to the model's validity, although authors disagree on whether this contributes to the model's face, predictive, and/or construct validity (Yadin et al., 1991; Rapoport et al., 1992; Altemus et al., 1996; Nurnberg et al., 1997; Sagvolden, 2000; Bourin et al., 2001; Geyer et al., 2001; Szechtman et al., 2001). It should be noted that a critical component in the demonstration of a common physiological basis is the demonstration of a similar response to treatment, because the latter suggests similarity in the neurotransmitter systems involved. This makes pharmacological isomorphism an important factor in assessing the validity of an animal model, and indeed, the validation process of most animal models of psychopathology involves testing the effects of relevant pharmacological treatments. Joel (2006)

As indicated by Joel, the type of validation required for animal models of disorder is not clear; (Willner, 1984, 1985, 1991; Joel, 2006; van der Staay, 2006). We will argue that a higher-order theoretical validity (linked to the neurobiological isomorphism focused on by Joel) is to be preferred. To see why, we will consider the most common forms of validity invoked in relation to animal models: face validity, predictive validity and construct validity. We think these are all best evaluated in the context of the evolutionary perspective on anxiety described above.

**III.B. Face, predictive and construct validity**

“Face validity” normally refers to a superficial resemblance of the measures taken to the equivalent human measures. In practice, such similarity provides no guarantee that changes in a measure will predict changes in the equivalent human measure and so “face validity”, although still used as a descriptive term, is not generally seen as conferring validity on an animal model in any real sense (Joel, 2006; van der Staay, 2006). This issue is neatly exemplified by the facial expressions of monkeys. The monkey expression most similar, in a photograph, to a wide human smile uses quite different muscles from a smile and is a display of aggression. By contrast, the “play face” uses the same muscles as a human smile, and is displayed in similar emotional contexts, and despite its superficial difference is a true homolog (Redican, 1982). We will argue below that, with anxiety and fear, homology is an important ingredient for validity. That is, as argued in the quotation from the Blanchards above, it is the matching of functional significance of behaviors across species that is important not the matching of the detailed behaviors themselves.

“Predictive validity” is, at first blush, the only validity required of a model: the proven capacity to predict, from the model, future findings in the modeled case. Certainly, where prediction truly fails, a model must be invalid. However, a model that, for example, detects classical anti-anxiety drugs such as benzodiazepines and predicts their clinical effects may simply involve a GABA<sub>A</sub> receptor, have no physiological relationship to the generation of anxiety, and so fail to detect the effects of novel anti-anxiety drugs that act via the 5HT<sub>1A</sub> system. The latter do not interact with GABA<sub>A</sub>, and so have quite different effects on euphoria, muscle relaxation and addiction, while having the same capacity to alleviate anxiety. True predictive validity, then, should have a guarantee that the model should detect quite novel classes of
drug in the future and not simply have been shown to detect many members of older classes of drug in the past. In this stronger form, predictive validity is, essentially, construct validity.

“Construct validity” is conferred on animal models “if their procedures are theoretically sound. The construct validity is not established by determining the relation between a test and an accepted criterion but is instead based on the establishment of relationships, which are in turn based on the definition of a trait. Implicitly, a construct is defined by a network of associations” (van der Staay, 2006). The key point here is that the model embodies a construct that, in turn, is part of a theory. A properly developed theory summarizes, integrates and encapsulates a vast database and it is the capturing of the essence of this database that validates the construct and, subject to the validity and generality of the theory, provides it with the strong form of predictive validity that we desire.

When anxiety is viewed from an evolutionary point of view, it is important to note that, whatever the surface behaviors that fulfill a function, the neural and hormonal systems controlling behavior will contain components that are conserved. Indeed, since substantial alteration in an existing defensive system is likely to be catastrophic in evolutionary terms, we can expect the core aspects of defensive systems to be particularly well conserved. The properties of the homologous systems in other animals are likely, therefore, to be highly similar to those in humans. Comparative, and particularly neural, theories of anxiety and fear should thus provide a particularly strong theoretical base for the derivation of animal models of specific aspects of human anxiety.

There are two unique aspects of construct validity that flow on from its theoretical derivation and do not follow from face or simple predictive validity. First, is that novel models can be generated directly from a theory. A model that has only current predictive validity, with no obvious construct validity, can only have been discovered by accident. By contrast, a theory provides a range of constructs from which a suitable model can be extracted by design. Second, is that apparent failure of predictive validity will not automatically invalidate a model but can lead instead to a deeper understanding of the human condition being modeled.

III.C. Deriving a model from a theory

To derive a model from a theory, one first needs a substantial theory. Probably one of the most substantial and well-developed theories of the neural basis of anxiety is that of Jeffrey Gray. This arose, over 30 years ago, in the hypothesis that anti-anxiety drugs change hippocampal function (Gray, 1970) and that this structure is an important part of a “behavioral inhibition system” (Gray, 1976). Further development of the theory, based only on analysis of classical (GABA-acting) anxiolytic drugs resulted in a full theory of the “Neuropsychology of Anxiety” (Gray, 1982). This has recently been modified and elaborated (Gray and McNaughton, 2000; McNaughton and Corr, 2004).

Given Gray’s theory, the demonstration that an anxiolytic drug can reduce the frequency of hippocampal theta rhythm (McNaughton and Sedgwick, 1978) provided a potential model of anti-anxiety drug action with high construct validity; although there was no obvious way to assess face validity and, initially, no data on predictive validity. To date this model has shown predictive validity with ethanol (Coop et al., 1990); benzodiazepines (McNaughton et al., 1986); the 5HT1A agonist, buspirone (Coop and McNaughton, 1991); the tricyclic antidepressant, imipramine (Zhu and McNaughton, 1991) and the specific serotonin reuptake inhibitor, fluoxetine (Munn and McNaughton, submitted). Of particular interest, it shows a similarly linear dose–response curve with 5HT1A anxiolitics as with GABA_A anxiolytics. By contrast, many behavioral models show only weak effects, and inverted-U dose–response curves, with 5HT1A drugs.

We would argue that the demonstrated robustness of this model derives from its tight construct validity – it assesses a core construct of the theory. Indeed, from the theoretical point of view, its apparent robustness is greater than expected as there are aspects of anxiolytic action, within the most recent versions of the theory (Gray and
McNaughton, 2000) that should be mediated by other systems and so not be detectable in this model (see section on neuropsychology, below, and the following chapter by Engin and Treit).

III.D. Does failure of prediction automatically invalidate a model?

Genuine failure of prediction will, of course, invalidate any model. However, where construct validity is high, we may want to ask how far an apparent failure reflects a failure of the model, as such, or a failure of our understanding of the situation being modeled.

As noted above, a number of behavioral models that detect classical (GABA A) anxiolytic drugs show weak responses and inverted U-shaped dose–response curves with novel (5HT) anxiolytics. Fixed-interval responding is an interesting example of this (Panickar and McNaughton, 1991). It is a prime test of behavioral inhibition and so reflects a key construct in Gray’s theory of the behavioral inhibition system, mentioned above. Further, anxiolytic drugs have been shown to affect fixed interval responding through the control of theta rhythm (Woodnorth and McNaughton, 2002), changes in which (as we saw above) appear to have strong predictive validity.

The failure of buspirone to affect fixed interval responding, except at very low doses, turns out not to be entirely general. First, if buspirone and a benzodiazepine are each given repeatedly for some time before training starts, rather than being tested with acute injections, they turn out to have the same effect on fixed-interval responding, with the chronic benzodiazepine having a relatively smaller effect than usual and buspirone having a larger one (Zhu and McNaughton, 1995). This matches the clinical situation where the two types of drug only have equivalent effects after longer-term administration. Second, buspirone releases, and benzodiazepines block the release of, stress hormones and, if these effects are blocked, again the acute effects of the two on fixed-interval responding become similar as a result of a decrease in the benzodiazepine’s and an increase in buspirone’s effects (McNaughton et al., 1996). Thus, close inspection of the fixed-interval model’s apparent failure shows that: (1) where the human treatment matches the treatment applied to the model then the model does not fail; and, (2) the differences in effects of the major classes of anxiolytic, both in the model and in the clinic, can be attributed to their additional, opposite, effects on the pituitary–adrenal axis interacting with the fundamental anxiolytic effect.

III.E. Conclusions

In discussing the nature of emotion in general, and anxiety in particular, we concluded that we must use multiple models (or at least measures) to capture the different aspects of “a single emotion” and to be able to assess different defensive emotions (e.g., fear and anxiety) and different subtypes of those emotions (e.g., generalized anxiety and social anxiety). This imposes one type of requirement on the validity of a model: the chosen model must map to the specific aspect of the specific subtype of anxiety (or fear) in which we are interested.

In discussing types of validity, we rejected face validity as being appropriate and concluded that in its strongest form predictive validity reduces to construct validity. We also argued that construct validity needs to be based on a strong, general theory and that, where this is the case, carefully chosen models can detect quite novel classes of compound and on occasion inform us about unexpected aspects of the modeled condition.

Both the nature of anxiety and the nature of animal models, then, require us to base our models (and our choice of a particular model for a particular purpose) on well-developed neuropsychological theory. In the remainder of this chapter we will look at particular models and their relation to particular theories to gain a more detailed picture of the interactions between the two.

IV. The nature of a specific test: the elevated plus-maze

We have provided a council of perfection: models, if they are to correctly perform their modeling
function, should be derived from neuropsychological theory. However, this first requires an adequate neuropsychological theory. Such theories must be derived from behavioral, neural and pharmacological experiments. These experiments often use paradigms that are either intended to be, or in practice are used as, models. In such cases, even with models that are only partially successful, we need to analyze them, post hoc, in terms of available (especially competing) theories.

As an example of such analysis we will look at the theoretical concepts behind, and alternative interpretations of results in the most popular animal model of anxiety of the last two decades: the elevated plus-maze. Briefly, this test, originally developed by Handley and Mithani (1984), consists of two opposed arms enclosed by walls and united, in perpendicular, to two open arms of equal dimensions, which are devoid of lateral walls. The whole apparatus is elevated above the floor. It has been shown that rats and mice, when freely exploring the maze, have a clear preference for the enclosed arms when compared to the open arms.

There are a number of reasons for the popularity of the elevated plus-maze. It is fast, requires no food deprivation or delivery of shock, and uses very simple apparatus. The matching disadvantage is that, without extensive analysis, it is not clear what the reinforcers are – whether, for example, any particular behavior is driven by positive (approach arm A) or negative (avoid arm B) drives. With operant tests these things are explicit and controlled – but at considerable expense. So, we will provide a theoretical analysis of the elevated plus-maze both in terms of experiments testing variations on its basic parameters and by linking these findings to the results of other operant and ethologically oriented tests.

IV.A. Theoretical analysis of the elevated plus-maze

The elevated plus-maze test was based on previous work by Montgomery (1955) that intended to analyze the factors underlying animals’ exploratory behavior in novel situations. Using Y-shaped mazes comprising different numbers of enclosed and open alleys, he showed that rats prefer to explore the closed arms. He attributed the exploration of both types of arm to the positive value of novelty. However, novelty also has negative aspects that can lead to avoidance behavior. Both the open and the closed arms evoke both positive and negative aspects of novelty. Montgomery stated that open arms evoke a greater strength of fear drive than enclosed arms. Hence, the animals tended to avoid the open arms and to explore the closed arms. However, he did not make clear the source of this greater fear and his context implies that it could have been negative aspects of novelty.

When the elevated plus-maze was developed as a model of anxiety, it was based on the fundamental conclusions of Montgomery’s studies. It presented rats with a simple choice of exploring open or protected closed alleys. The opposition of two arms of the same type meant that a genuine choice could be made. If there were only one open and one closed arm, the animal might stay in a closed arm simply through inertia – that is, a lack of exploratory drive. But, in the plus-maze, leaving a closed arm (as a result of a reduction in its novelty) presents the rat with a choice (initially) of two open and one closed arm, all of equal novelty. Choice of the other closed arm then indicates either a preference for closed arms or an avoidance of open arms.

There are two issues that need to be settled, here, in assessing the nature of the plus-maze in terms of possible influences of anxiety. The first is whether the behavior is approach to the closed arm (which would result from a positive exploratory drive and so be unlikely to result from anxiety which is, especially in the clinic, perceived as negative). The second issue is, assuming that the measured behavior is the result of avoidance, whether the test can be seen as one driven simply by avoidance, or by a conflict between approach and avoidance.

Treit and co-workers (1993) examined the extent to which stimuli in the open arms of the elevated plus-maze motivate avoidance behavior. They showed that open arm avoidance did not habituate across trials. It was also not reduced in animals
that received three previous confinement sessions to the open arms (flooding exposures). Neither negative nor positive aspects of novelty could, then, account for the pattern of behavior. The level of open arm avoidance was, also, similar in animals tested in plus-mazes elevated to different heights, leading to the conclusion that height is not an anxiogenic stimulus in this test. In contrast, they reported that rats explored an open arm with a raised Plexiglas edge more than an open arm with a standard flat edge. This suggests that fear of open spaces is the main aversive stimulus in the elevated plus-maze. This has been related to the fact that rats in open spaces are more exposed to predation. They show thigmotaxis—a preference, in a range of apparatus and situations, to stay near vertical surfaces and avoid the center of open spaces.

We have, then, good reason to see the plus-maze test (however elevated) as pitting two different motivations against each other. The positive (albeit mixed) motivation driving exploration of all the arms is novelty. The negative motivation specifically associated with the open as opposed to closed arms, is the avoidance of open spaces. We will deal later with the issue of whether the conflict between the motivations is critical or whether the positive one simply provides a baseline against which the effects of the negative can be assessed.

**IV.B. Pharmacological analysis of the elevated plus-maze**

Handley and Mithani (1984) used the ratio of the number of entries into open arms relative to closed as a potential measure of anxiety and challenged this with putative or clinically effective anxiolytic and anxiogenic compounds. They observed that non-sedative doses of anxiolytic drugs such as diazepam and amylobarbitone increased exploration of the open arms, without significantly changing exploration of the enclosed arms. Anxiogenic drugs such as picrotoxin and ACTH caused the opposite effect. Since these first observations, the anxiolytic-like effect of benzodiazepines and barbiturates in the elevate plus-maze has been extensively replicated. This highly reliable pharmacological isomorphism coupled with its simplicity, economy and lack of either lengthy training procedures or the use of food/water deprivation or electric shock made the elevated plus-maze enormously popular.

However, confidence in the elevated plus-maze test was undermined when buspirone was introduced in the clinic for the treatment of generalized anxiety disorder. Unlike GABA-related anxiolytics such as the benzodiazepines and barbiturates, buspirone is a 5HT1A receptor agonist. It was found that the elevated plus-maze did not consistently demonstrate an anxiolytic effect of this new class of anxiolytics. This was also true regarding the anxiolytic effects of other 5HT-modulating drugs such as imipramine and fluoxetine (Griebel, 1995).

To understand this failure of the test’s predictive validity, we need to return to the issue of whether the key element of the plus-maze as a model is simple avoidance or approach-avoidance conflict. This is theoretically important as conflict is a pervasive concept associated with anxiety. It emerged from the psychoanalytic idea that human anxiety results from inner conflict. While there is considerable scientific doubt about many psychoanalytic premises, the association between approach-avoidance conflicts and anxiety is much more a superficial description of situations and behavior than specific to psychoanalytic theory. The idea of conflict in one form or another, then, remains a cornerstone for many current theories (Blanchard and Blanchard, 1990a,b; Blanchard et al., 1991; Gray and McNaughton, 2000; Graeff, 2002).

One could simply argue that, since anxiolytic drugs affect passive but not active avoidance in many learning-based tests that the effect of the drugs in the plus-maze must also be on conflict and not on simple avoidance. However, we do not have to rely on such inference. A variant of the elevated plus-maze, the elevated T-maze, was developed to separate out the different elements embedded in the plus-maze (Viana et al., 1994; Zangrossi and Graeff, 1997; Graeff et al., 1998; Jardim et al., 1999; Sanson and Carobrez, 1999).
This model was designed to separate different motivational components by shutting the entrance of one of the enclosed arms of the plus-maze and presenting the animal with specific trials rather than allowing free behavior. To assess inhibitory avoidance, the rat is placed at the end of the remaining enclosed arm and the latency to exit this arm with the four paws is recorded in three successive trials made at 30-s intervals. Learning can be indicated by an increase in withdrawal latency across trials. To assess active escape, the rat is placed, 30 s after the completion of avoidance training, at the end of one of the open arms and the withdrawal latency from this arm is similarly recorded.

As would be expected from previous data in operant experiments, benzodiazepine anxiolytics such as diazepam and midazolam, but also 5HT-related drugs such as buspirone, ipsapirone and ritanserin impair inhibitory avoidance in the T maze while leaving one-way escape unchanged (Graeff and Zangrossi, 2002). This selective effect in favour of defensive approach correlates with the clinical effectiveness of these drugs on generalized anxiety, as opposed to their lack of efficacy (at least at anxiolytic doses) on panic disorder. Similarly, one-way escape is impaired by chronic (but not acute) administration of the tricyclic antidepressants such as imipramine (Teixeira et al., 2000) and clomipramine as well as by the selective 5HT reuptake blocker fluoxetine (Poltronieri et al., 2003) – all drugs that are used to treat panic disorder.

We have shown that the plus-maze is a mixed test, in the sense that the rat can display multiple different strategies of defense while exploring any specific part of the maze. This has been recognized for some time and can explain the inconsistent effects of 5HT-acting drugs (Handley et al., 1993). As indicated by results in the elevated T-maze, at least two of them can be clearly named: avoidance of open arms when the rat is in an enclosed arm, and escape from an open arm toward an enclosed arm. Since it has been shown that 5HT pathways may influence these defense strategies in different, even opposite ways (for a review see Graeff, 2002, and Chapter 4.3 in this book) the effect of 5HT-acting drugs in the plus-maze would vary as a function of the predominance of one or the other of these defense reactions.

**IV.C. Ethological analysis of the elevated plus-maze**

In parallel to the introduction of buspirone in the clinic, there was a worldwide acceptance of the separation of “anxiety” disorders into distinct diagnostic categories, a trend that was initiated by the DSM III classification of psychiatric disorders (American Psychiatric Association, 1980). (Note that DSM conflates what we would see as distinct anxiety and fear disorders.) Later studies showed that buspirone was effective in treating generalized anxiety disorder and depression but not panic, post-traumatic stress disorder or obsessive-compulsive disorder.

This led to a different way of dealing with the failure of predictive validity of the elevated plus-maze. The idea was that the successful detection of a drug effect could depend on the types of defensive behaviors actually measured in this test. On this view, as we have already argued on the basis of both the earlier work and Treit’s results, the plus-maze does in fact contain the critical elements required of an animal model of anxiety. But, in contrast to Montgomery’s focus on choice behavior (and so, as we have seen, a contaminated measure of behavioral inhibition), we can focus on the kind of elicited behaviors, such as risk assessment, that are fundamental to the ethological approach advocated by the Blanchards.

Application of this ethological approach showed that the incorporation of defensive acts and postures toward risk assessment in the scoring of the plus-maze notably improved the test’s capability for detecting the effects on anxiety of 5HT-modulating drugs (Griebel et al., 1997). Again we see that careful theoretical analysis of the content of a test can account for, and lead to ways to deal with, apparent failures of the test as a model. In this case by using measures of elicited behavior instead of measures of behavioral inhibition to assess anxiety.
V. Other animal models of anxiety

Let us now apply the principles we have elucidated in the elevated plus-maze to other animal models of anxiety. These turn out to involve, as we might expect, both behavioral inhibition and anxiety-specific elicited behavior.

The elevated plus-maze was popular because it was seen as having many advantages over pre-existing animal models of anxiety. These were mostly based on approach-avoidance conflict. Before the plus-maze era, two main classes of animal model were commonly used: those using response-contingent shock and those using non-contingent shock. For example, “conditioned suppression” is the inhibition of ongoing behavior elicited by conditioned stimuli that predict unavoidable electric shock. “Conditioned punishment” involves the suppression of rewarded responding by concurrent response-contingent electric shock. These tests all require food or water deprivation and administration of shock. (The fixed-interval test, discussed earlier, can be viewed as a form of punishment schedule where, in the early part of the interval, frustration rather than shock acts as the punisher.) The earlier tests also required very extensive pre-training. While their expense was considerable, it can be argued that these tests allow a clear identification of the key anxiolytic-sensitive parameter (behavioral inhibition) and also contain (in their un-shocked baseline periods) controls for non-specific effects on motivation, perception, motor control, etc. By contrast, it has taken considerable analysis – and development of a second-generation test, the elevated T-maze – for us to identify the factors governing behavior in the elevated plus-maze.

Drug results are theoretically clearest with conditioned suppression. Animals are trained to respond for reward and then periods of signaled non-contingent punishment are superimposed – the shock can therefore never be avoided and anxiety is always present. Anxiolytic drugs selectively release the inhibition of responding by shock. Similar results are obtained with the Geller–Seifert punishment schedule (Geller and Seifert, 1960), in which shock is contingent. However, it should be noted that, with well-learned punishment (unlike conditioned suppression), the animal comes to avoid the shock or, when it occurs, can predict its occurrence with complete accuracy. At this point, genuine anxiolytic effects are lost and are replaced by state-dependent changes (McNaughton, 1985).

A simpler, and so cheaper, punishment test – the Vogel conflict test – was later developed that involved administration of shock on an unlearned licking baseline (Vogel et al., 1971). We can thus see a progression from tests where approach and avoidance are tightly controlled, and approach-avoidance conflict is clear; through tests that combine innate and learned components; to tests, such as the elevated plus-maze, where the sources of approach and avoidance were initially unknown – and where, as we have seen, their control is problematic. These are all tests of approach-avoidance conflict, with anxiolytics reducing behavioral inhibition. We would argue that, at present, the elevated T-maze provides the best compromise between control of approach and avoidance and the cost of administering the test.

A range of other “innate”/“ethological” tests have been developed since the advent of the plus-maze (for descriptions see the following chapter by Engin and Treit). The key parameters in the social interaction test, the light–dark exploration test, and the shock-probe burying test include clear measures of behavioral inhibition – and so require no further psychological analysis here (but see the neural analysis below). However, it should be noted that the Blanchard’s analysis of anxiety-related behavior (Blanchard et al., 1990, 1991) focuses equally on specific anxiety-elicited behaviors and on behavioral inhibition.

The shock-probe burying test, as the name implies, has its major measure the extent to which a rat will bury the shock probe. This is an elicited behavior that, consistent with our analysis of fear versus anxiety, will only occur when the animal approaches the source of threat. Shock-probe burying is sensitive to anxiolytic drugs. It also shows how such elicited responses are shaped by the context of the threat (burying the scent or memory of a cat is not a functional defensive option; nor is risk assessment of the environment around a shock probe). It also shows that
approach to threat is critical in determining anxiolytic sensitivity, as opposed to whether threat is actual or potential, since the shock probe is a clear and definite threat – but still one that it can make sense to approach.

Ultrasonic vocalization is less easy to categorize. It occurs in rat pups experiencing separation anxiety (which we have already discussed above) and in adult rats subject to inescapable footshock. It is clear that in neither case is this part of a simple threat avoidance repertoire. But, on the other hand, it is far from clear, particularly in the adult case, that the response is elicited in the context of threat approach (the basis for risk-assessment behaviors or shock-probe burying). In the case of the rat pups it could be argued that they are in a safe place and, for sustenance, need to leave it and so approach threat. In that context vocalization is a means of solving the problem (by bringing their sustenance back to them) and so an appropriate part of a threat-approach repertoire. But not only is this speculative; it does not clearly apply to the adult case.

VI. Models of anxiety and their control by the brain

We have already argued from the clinical profile of drugs used to treat disorders of fear and anxiety (Table 1) that specific models and measures need to be chosen carefully to ensure that the model captures the desired type or component of anxiety. The need for this, and the neural basis for it, is made clear in the following chapter by Engin and Treit.

They review the effects of different anxiolytic drugs injected into different sites in the brain and relate the results to the neuropsychological theory of anxiety we discussed earlier (see Section III.C; Fig. 2).

Their key findings are

1. Anxiolytic drugs can produce changes in unlearned tests of anxiety when injected into any part of the defensive approach hierarchy;
2. Within different parts of the same general neural area (such as the amygdala, see their Section 2.4) the same drug can doubly dissociate measures from two different models (such as the elevated plus-maze and the shock-probe burying test);
3. Within a single part of a structure, a drug can dissociate two measures within a single test (such as burying and probe avoidance within the shock-probe burying test);
4. An anxiolytic effect of a GABA_A drug does not guarantee an effect of a 5HT_1A drugs within the same structure despite the fact that systemic injection is effective in both cases;
5. Effects on multiple structures can combine to produce the systemic profile of the drugs within a single test (GABA_A drugs affect shock-probe avoidance but not probe burying in the amygdala and vice versa in the septum).

VII. Conclusions

We have shown that anxiety should be distinguished from fear; that, less categorically, different anxiety disorders and different fear disorders should be distinguished from each other; and that the components of an apparently unitary type of anxiety can be neurally distinct. Theoretical analysis of animal models is, therefore, required if we are to match a particular measure from a particular model with the clinical entity we desire to model.

We have also shown that face validity of a model is meaningless and we have argued that true predictive validity reduces to construct validity. That is, the models we choose to use should be based on strong theory and should have been tested for their detailed conformation to theoretical constructs.

We believe we have shown that when theory is given such primacy it will not only allow us to determine ways in which particular models are unsatisfactory. In many cases a strong theory will demonstrate that a model is actually valid and that it is our picture of the clinical situation being modeled that requires adjustment. This brings us full circle to the point that theory is required as much to decide what our model should be modeling as which model we wish to use.
Animal models have, in the past, often been constructed ad hoc. However, we would argue that theory, particularly neurally based theory, has now advanced to the point where proper theoretical analysis should be the first step in considering any new model or in assessing the predictive validity of an existing model. Critically, neural theory may allow us to develop tests (such as the reticular activation of hippocampal theta described earlier) that can detect specific actions of drugs in the absence of the confounding side effects that often make behavioral models difficult to use. Conversely, behavioral models have the advantage of capturing all the neural circuitry on which drugs might act, without the need to first determine the specific sites of action.

As we attempt to model the many flavors of defensive disorder demonstrated by variation of drug effectiveness (Table 1), we must use theory to link multiple animal models, neural analysis and pharmacological analysis. This will provide us with a means of developing batteries of test with
clear predictive (i.e., construct) validity that can be used for drug discovery of genuinely new classes of drug as well as development of improved variants of already known classes.

Uncited references

Blanchard et al., 2001; Blanchard et al., 1995; Coop et al., 1992.

References


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