**Validation of a Simple, Ethologically Relevant Paradigm for Assessing
Anxiety in Mice**

A Thesis submitted to the Department of Psychology and Faculty of Graduate Studies
and Research of Carleton University in partial fulfillment of the requirements for the
degree of

Master of Psychology
Specialization in Neuroscience

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Department of Psychology
Carleton University (2003)

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acceptance of the thesis:

**“Validation of a Simple, Ethologically Relevant Paradigm for Assessing
Anxiety in Mice”**

Submitted by

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In partial fulfillment of the requirements for
the degree of Master of Psychology

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Abstract

Although there are numerous behavioral tests that assess anxiety, the present investigation introduces a simplified version of a previously established test that is exquisitely sensitive and reliable. When offered a highly palatable snack (graham wafer crumbs) in a familiar environment (home cage), mice readily approach and consume it. However, when the same snack is offered in a novel environment (i.e., a cage with fresh bedding), the latencies to approach the snack increase by about 10-fold. A similar outcome was observed in the home cage when predator scent (rat feces) was introduced, while the same manipulation in a novel environment provoked a still more dramatic effect. The test can be used repeatedly, especially when novel environment tests are interposed between home cage tests, permitting assessment of anxiolytic and anxiogenic treatments in a within-subject design. Treatment with known anxiolytics, including acute treatment with diazepam, chlordiazepoxide, or propranolol, or chronic treatment with buspirone or the antidepressant, desmethyylimipramine, attenuated the effects of the novel environment, without affecting home cage responding. In contrast, pharmacological treatments that ordinarily do not affect anxiety (e.g., haloperidol, amphetamine, acute buspirone or acute desmethyylimipramine) failed to affect novel environment-induced prolongation of the latency to approach the snack, attesting to the specificity of the test. The simplicity of design, the absence of food deprivation or the use of neurogenic stressors, the reliability and magnitude of response, the possibility of using the paradigm in a repeated measures design, and the specificity and sensitivity to anxiolytic drugs, makes this an ideal preparation with which to assess anxiety and anxiety-altering manipulations.

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Introduction

In the face of threatening or potentially harmful situations, animals engage in numerous defensive behaviors that are accompanied by various neurochemical alterations (Adamec et al., 1998; Belzung et al., 2001; Blanchard et al., 2001a,b; File et al., 1993a; Hayley et al., 2001). Predictably, stressor experiences are associated by anxiety, characterized by increased autonomic nervous system activity, inhibition of ongoing behaviors, increased vigilance and scanning activities, and elevated behavioral reactivity (Blanchard & Blanchard, 1989; Carrive, 2000; Dielenberg et al., 2001; van den Berg et al., 1998; van den Buuse et al., 2001). In humans, anxiety states may be manifested in the form of pathologies including generalized anxiety disorders, phobias, panic and obsessive-compulsive disorders, acute stress disorder, and posttraumatic stress disorder, and may be a comorbid feature of other illnesses such as depression (Bryant & Panasetis, 2001; Chantarujikapong et al., 2001; Diefenbach et al., 2001; Melartin et al., 2002; Otto et al., 2001).

Numerous animal models have been developed in an effort to delineate the mechanisms underlying anxiety states and hence the development of efficacious treatment strategies (Belzung & Griebel, 2001; Lister, 1990; Rodgers et al., 1997; Shekhar et al., 2001). These have ranged from paradigms that assess the behavioral responses to neurogenic or painful stimuli (e.g., activity levels following footshock, restraint, exposure to cold; startle response to strong acoustic stimuli) or to cues that have previously been associated with painful stimuli (e.g., fear-potentiated startle response, conditioned emotional response; CER), through paradigms that involve the use of psychogenic stressors (e.g., open-field emergence, open-field activity, light-dark box,

elevated plus maze, social interaction test) (Cabib et al., 1988; Costall et al., 1989; Davis, 1989; Davis et al., 1993; de Angelis & File, 1979; Lane, 1992; Lister, 1987; Nakamura & Kurasawa, 2001; Paré et al., 2001; Sutoo et al., 1991; van Dijken et al., 1992a). In addition, recent investigations have focused on the use of paradigms that assess the response of animals to naturalistic or ethologically relevant stimuli (e.g., exposure to a novel environment, a live predator or to predator odor) (Adamec & Shallow, 1993; Anisman et al., 2001; Belzung et al., 2001; Blanchard et al., 1998; File et al., 1993a; Morrow et al., 2000a,b).

The stressors employed in most animal models of anxiety elicit several common neurochemical changes, including altered CRH activity within the amygdala and paraventricular nucleus of the hypothalamus (Anisman et al., 1998; Hsu et al., 1998; Kalin et al., 1994) and increased monoamine turnover in hypothalamus and limbic regions including the prefrontal cortex, nucleus accumbens and amygdala nuclei (Belzung et al., 2001; Dazzi et al., 2001; Hayley et al., 2001; Ihalaenen et al., 1999; Jones et al., 1996; Morrow et al., 2000a,b). Yet, in several respects the impact of different stressors can be distinguished from one another. For instance, it appears that the patterns of central neurochemical activity elicited by systemically administered cytokines (immune challenge) may be distinguished from those elicited by neurogenic stressors (Herman & Cullinan, 1997; Merali et al., 1997). Likewise, it has been demonstrated that conditioned aversive stimuli (i.e., a light or tone paired with shock) and predator-related cues (presentation of fox urine odor to rats or mice) may involve different region-specific neurochemical mechanisms (Hayley et al., 2001; Morrow et al., 2000a).

Although anxiety is often used as a general term to describe a response to stressors, it seems that subtypes of anxiety exist that may either be stimulus-bound or independent of extraneous stimuli (Davis, 1998; Fyer et al., 1989; Lang et al., 2000), tied to contextual stimuli or to discrete stimuli associated with the primary aversive stimulus (Baas et al., 2002), comorbid or secondary to other psychological states (e.g. depressogenic factors) (Furukawa et al., 2001) and can reflect trait or state factors (Belzung et al., 1994; Griebel et al., 1996). Moreover, the effects of anxiolytic agents may be dependent on the nature of the anxiety and hence on the particular anxiety model being employed (Berton et al., 1998; Blanchard et al., 1990a; Zangrossi & File 1992). Taking these findings into account, the availability of a wide variety of different approaches may be very useful in the assessment of anxiety-related states.

The ensuing section provides an overview of animal models suitable for investigating the pharmacological treatment of anxiety disorders. Commonly employed animal models of anxiety are either neurogenic or psychogenic in nature, and involve either conditioned or unconditioned responses. Recent developments in our understanding of mammalian defensive behavior suggest a need for more ethologically relevant paradigms that involve spontaneous or natural reactions to stimuli, without pain or discomfort. The advantages and disadvantages of both the traditional and newer ethological models will be discussed.

Finally, the present report will introduce a modification of a previously established technique that was occasionally used to assess anxiety. This model is predicated on an animal's reluctance, when placed in a novel environment, to approach a favored snack. Moderately threatening situations (e.g. placement in an unfamiliar

environment) induce behavioral patterns like those elicited by predator scent, including inhibition of non-defensive behaviors (e.g., reluctance to ingest food) (Blanchard & Blanchard, 1989) and these effects are antagonized by anxiolytic agents (Bodnoff et al., 1989a,b; Rex et al., 1996; Stout & Weiss, 1994). Although assessing food intake in an unfamiliar environment is practical, its use has been limited by methodological problems. For instance, the anxiogenic attributes of the unfamiliar environment may be confounded by the effects of food or water deprivation (concurrent systemic stressors) or presentation of a novel food (which may produce neophobia). As well, presentation of regular chow yields low basal feeding levels (floor effect) making further reductions of food intake less discernible. We observed that offering animals highly palatable, familiar snacks in a novel environment obviates these problems, allowing for an unadulterated appraisal of anxiety (Muller-Gass et al., 2000).

Neurogenic Models

The Acoustic Startle Response (ASR) and Fear-Potentiated Startle Paradigms

The acoustic startle response (ASR) comprises of a fast twitch of facial and body muscles elicited by a sudden and intense acoustic stimulus. The precise pattern of the ASR, consisting of eye-lid closure, muscle contraction, accelerated heart rate and the arrest of ongoing behaviors, may be indicative of a protective function of the startle response against injury from a predator or blow (Koch, 1999). The amplitude of the ASR is modified by various pathological conditions or experimental manipulations. For example, the ASR is enhanced in individuals suffering from panic disorder (Grillon et al., 1994) and PTSD (American Psychiatric Association, 1994), and in laboratory animals

subjected to electric footshock (Davis, 1989), bright illumination (Walker & Davis, 1997a) or predatory stress (Adamec et al., 1999). Moreover, the amplitude of the ASR is reliably enhanced in both humans and animals in the presence of a cue predicting an aversive event (i.e., fear-potentiated startle). For example, elevations in ASR amplitude are observed in rats exposed to a light or tone that has been previously paired with footshock (Davis et al., 1993).

Lesion and drug infusion experiments have revealed that the ASR is mediated by a simple neuronal circuit within the brainstem consisting of the auditory nerve, the ventral cochlear nucleus, the dorsal nucleus of the lateral lemniscus and caudal pontine reticular nucleus (Davis et al., 1982; Koch et al., 1992; Carlson & Willott, 1998). Further studies suggest that an anatomic connection between the amygdala and the acoustic startle pathway is involved in the sensitization of the startle response by aversive stimuli or by conditioned fear. For instance, lesions or chemical inactivation of the central nucleus of the amygdala completely block expression of fear-potentiated startle but had no effect on startle enhanced by bright light or by the administration of corticotropin-releasing hormone (CRH) (Hitchcock & Davis, 1986; Kim et al., 1993; Lee & Davis, 1997; Walker & Davis, 1997b; Davis et al., 1997). Alternatively, lesions or chemical inactivation of the bed nucleus of the stria terminalis (BNST) significantly attenuated light enhanced or CRH-enhanced startle without having any effect on FPS (Gewirtz et al., 1998; Hitchcock & Davis, 1991; Lee & Davis, 1997; Walker & Davis, 1997b; Davis et al., 1997). Taken together, these studies suggest that while the bed nucleus of the stria terminalis may be a system related to general anxiety, the central nucleus of the amygdala is a system more closely aligned with fear (Davis, 1998; Davis & Shi, 1999).

The ASR and fear-potentiated startle paradigms provide a unique opportunity to examine the biological similarities and differences between conditioned fear and unconditioned anxiety, yet these models have several limitations. For example, while the fear-potentiated startle paradigm permits precise experimenter control over behavioral baselines, it often requires the use of electric shock as well as considerable time investment in the training of subjects. The ASR, a model based on an unconditioned response, results in more variable baselines, but offers a higher degree of ecological validity and is less susceptible to confounds arising from interference with learning and memory that may occur with repeated testing (Lister, 1990; Rodgers et al., 1997). In addition, both models involve the activation of the acoustic startle pathway, and hence do not necessarily represent anxiety responses unrelated to this pathway. Furthermore, not all animals exhibit the fear-potentiated response, and in most studies subjects are selected on the basis of those who show the response. As such, the population of rats studied is restricted.

The Conditioned Emotional Response (CER) Paradigm

The conditioned emotional response (CER), first described by Estes and Skinner (1941), was long used as a prototype of behavioral anxiety. In the CER paradigm, a food-deprived rat or mouse is first trained to press a lever for food using intermittent reinforcement. Once a stable rate of bar pressing is established, a neutral stimulus such as a light or tone is paired with a shock. After several pairings, presentation of the light or tone alone is sufficient in disrupting the lever-pressing behavior. This phenomenon has been termed conditioned suppression and has become widely used as an index of anxiety

in laboratory animals. Although the model is sensitive to drug effects, for which it has been used most extensively, it requires food or water deprivation, the use of electric shock and considerable time investment in the training of the subjects. Thus, its utility is somewhat limited.

Geller-Seifter Paradigm

In this procedure, rats or mice are trained to press a lever to obtain some form of positive reinforcement, such as a food pellet. The animals are trained using a multiple schedule of reinforcement. In the first component of the schedule (signaled by an auditory or visual stimulus), responding is reinforced at irregular intervals (i.e., a variable interval (VI) schedule of reinforcement). Animals typically show consistent and relatively high rates of responding during this regime (Geller & Seifter, 1960; Geller et al., 1962; Geller, 1964). In the second component of the schedule (signaled by a different stimulus) every lever press results in reward but the animal receives a mild footshock at the same time. In this continuous reinforcement (CRF) or fixed ratio (FR) schedule, the animals learn to reduce their rate of responding so as to avoid punishment. Animals treated with anxiolytic drugs, particularly benzodiazepines, show little change in the rate of responding in the VI schedule but an increased rate of responding in the CRF schedule (Babbini et al., 1982; Geller et al., 1962; Howard et al., 1982; Wiley et al., 1993).

Although the G-S model has figured prominently in behavioral studies of benzodiazepines, it necessitates food or water deprivation, electric footshock and involves extensive, time-consuming training of the subjects. Furthermore, the model does

not reliably detect the anti-conflict/anxiolytic effects of agents that do not act on benzodiazepine receptors such as buspirone or ritanserin (Brocco et al., 1990).

Psychogenic Models

One of the problems associated with paradigms that employ physical stressors, such as footshock or restraint, is that many of these tests are not relevant to the stressors that rodents encounter in their natural environments. Additionally, tests using physical stressors may involve the activation of central pain pathways (Morrow et al., 2000a) that may not be relevant to other forms of anxiety. Thus, increasing attention has been devoted to the development of strategies that assess anxiety in the absence of neurogenic insults. As indicated earlier, however, even among those models that employ psychogenic stressors one can distinguish a range of variables that assess different attributes of the stress response.

The Elevated Plus-Maze Test

The elevated plus-maze takes advantage of the rodent's natural aversion to open spaces (Lister, 1990). The apparatus consists of a plus-shaped maze with two open and two closed arms. The animal is placed in the center of the apparatus and permitted to explore for a short period. Two indices of anxiety are used in the plus-maze: the proportion of entries that are made on to the open arms of the maze, and the time spent on the open arms expressed as a percentage of the total time spent on both the open and closed arms. Mice taken directly from their home cages show a behavioral pattern characterized by open-arm avoidance (Lister, 1987; Pellow et al., 1985). This tendency to

avoid the naturally aversive open arms is suppressed by anxiolytics and potentiated by anxiogenic agents (Cole & Rodgers, 1995; Pellow et al., 1985; Lister, 1987).

The popularity of the elevated plus-maze is due largely to its practical effectiveness, rather than theoretical reasons. Indeed, the plus-maze permits rapid screening of anxiety-modulating drugs or mouse genotypes without training or involvement of complex schedules. However, research confirms that both mice (Rodgers & Shephard, 1993) and rats (File et al., 1990; 1993b; Lister, 1987) are insensitive to anxiolytic drug treatment if the animal has had prior experience with the plus-maze apparatus. Furthermore, it has been demonstrated that the behavioral parameters in the mouse plus-maze provide measures of two independent factors: anxiety and motor activity (Lister, 1987). Hence, in the assessment of behavioral activity in the plus-maze, it is important to control for such factors. Further, it seems that the plus-maze test is sensitive to an wide array of intra- and extra-maze stimuli, and the construction of the maze itself, and considerable efforts are necessary to establish a baseline of responding that permit both the anxiogenic and anxiolytic actions of various treatments (Fernandes & File, 1996; Hogg, 1996).

The Light-Dark Exploration Test

A brightly lit white environment is aversive to mice and inhibits their exploratory behavior (Crawley & Goodwin, 1980). The light-dark paradigm is a simple and rapid model that employs the natural aversion of mice to bright light. Mice are placed individually into a test box that is partitioned into two compartments: one compartment is painted white and brightly illuminated, while the other is painted black and dimly

illuminated. A small opening in the center of the partition connects the two compartments. The model demonstrates pharmacological specificity for benzodiazepines and 5HT-modulating compounds. For example, both diazepam and chlordiazepoxide and the 5-HT_{1A} agonist buspirone have been shown to enhance exploratory activity (i.e., rearing and ambulation) in the brightly lit compartment of the light-dark box (Crawley & Goodwin, 1980; Costall et al., 1989). In contrast, administration of anxiogenic drugs such as yohimbine and mCPP reduce exploratory activity in this paradigm (Bilkei-Gorzo et al., 1998; Merlo Pich & Samanin, 1989). Further investigation has revealed that animals exposed to various forms of neurogenic and psychogenic stress (i.e., electric footshock, forced swimming, predatory stress) exhibit suppressed exploratory behavior in this paradigm (Adamec et al., 2001; Sanchez, 1997). The light-dark test pits two tendencies against one another: one related to aversion of a brightly lit environment, and the other to explore a novel environment (Costall et al., 1989; Belzung & Le Pape, 1994). However, there are several problems with this procedure. Firstly, as the light-dark test is heavily dependent on locomotor activity, it does not allow for the dissociation between the anxiolytic effect of a particular drug and its action on activity (Belzung & Le Pape, 1994). Therefore, controls for changes in locomotor activity and light sensitivity should be included (Costall et al., 1989; File, 2001). Secondly, it is questionable whether or not the latency of a rat or mouse to enter the dark component of the light dark box describes a neophobic reaction. In the light-dark box, there is no contrast between a familiar and novel place as both sides of the test apparatus are novel (Belzung & Le Pape, 1994). Finally, several studies have demonstrated that rats and mice exhibit insensitivity to

anxiolytic agents in the light dark test in repeated trials, suggesting that reusing animals in the light-dark test should be avoided (Lister 1990; Rodgers & Shephard, 1993).

The Open Field Activity Test

Neophobia is a reaction characterized by hesitancy to engage novel objects, places or conspecifics. A standard procedure for assessing neophobia in rodents is the illuminated open field test. The apparatus for the open-field test consists of a large circular arena under a high level of illumination. The rodent is placed in the arena for a fixed period of time and its behavior is noted. The animal typically freezes when exposed to a noxious stimulus and the autonomic system is activated resulting in defecation. An 'emotional' or 'anxious' animal is one that has little ambulatory activity, tends to avoid the central area of the field, adopting instead a thigmotaxic response. The open-field test requires minimal apparatus and is easily evaluated. However, the use of locomotor activity in the open field as a measure of anxiety is questionable, as it is difficult to dissociate it from exploration and from stimulant/depressant actions of drugs (File, 1985; Lister, 1990). Indeed, several agents (i.e., motor stimulants) have been shown to increase locomotor activity in the open field although they are not anxiolytic (Cunha & Masur, 1978). It has also been shown that defecation in the open field can be altered by factors such as food intake or by drugs that affect gut motility by a direct action on the gut rather than via changes in emotionality (Silva & Calil, 1975). Finally, it need be noted that the effects of a drug can be qualitatively altered if the animals have prior experience with the apparatus (Christmas & Maxwell, 1970).

The Open Field Emergence Test

The emergence test is a modified version of the open field activity test, used for assessing neophobia in laboratory animals. The testing apparatus is generally comprised of a large testing arena with no ceiling, and a circular hole in one wall giving access to a smaller compartment with a ceiling. The rodent is placed in the smaller, darker compartment and its latency to emerge into the larger, open-field compartment is determined (Anisman et al., 2001; Meert et al., 1997; Stone et al., 1995). According to Paré et al (2001), the illumination and handling required by the experimenter may be stressful to the animals and mask individual differences in stress reactivity. In their version of the open field emergence test the testing arena is not illuminated, nor is the animal handled. The home cage, with the rat inside is placed on its side in the corner of the testing arena, and the latency of the rat to walk out into the testing arena is recorded. This particular version of the emergence test offers a more ethological meaningful method of assessing anxiety-like behavior as compared to other paradigms that require handling and in which the animals are forced into brightly lit environments. Yet again, the test relies on locomotor activity and it may be difficult to dissociate the anxiolytic effect of a particular drug from its effect on activity.

The Social Interaction Test

The social interaction test developed by File et al (File & Hyde, 1978, 1979; File et al., 1982) uses a combination of novelty and high illumination as anxiogenic stimuli. The time spent by pairs of male rats in active social interaction is measured in a test arena under high or low lighting conditions and in a familiar or unfamiliar testing apparatus

(i.e., high light unfamiliar, low light unfamiliar, high light familiar, low light familiar). Ordinarily, rats spend more time interacting in the low light familiar condition and exhibit intermediate periods of interaction in the other two conditions. Once more, it is essential to measure locomotor activity in order to determine whether any change in social interaction is due to stimulant or sedative effects. A further disadvantage of the test is that it involves pairs of animals and therefore it is difficult to examine individual differences in behavior (Lister, 1990).

The Use of Defensive Behaviors to Study Anxiety

The Visible Burrows System

Defensive behaviors are the reactions of animals to life threatening dangers (predator or conspecific attack, threatening features of the environment) in the natural environment. Blanchard and Blanchard (1989) examined the defensive behaviors of rats in a visible burrow system (VBS) following the presentation of a cat. The VBS is a semi-natural habitat comprised of a tunnel/burrow system (under constant red light) and an adjacent open surface area (maintained under a 12:12 light-dark cycle) with food and water. Activity of the animals is videotaped within the burrows and on the surface using low light cameras (described in Blanchard et al., 1993). A 15-minute presentation of a cat in the open space changes the behavior of the mixed-sex rats within the burrow system for up to 24 hours. Rats typically exhibit defensive behaviors such as flight to the burrows, freezing and emission of ultrasonic vocalizations (approximately 22 kHz) throughout the first 30 minutes following the exposure, followed by avoidance of the open area for up to 4 hours. Furthermore, risk assessment behaviors (gradual approach to

tunnel openings, extension of the head out to the surface, scanning, stretch-attend postures) are observed for up to an additional 20 hours. Stretch-attend postures are associated with the gathering of information concerning threat sources (Blanchard et al., 1993). Inhibition of non-defensive behaviors (eating, drinking, sexual and aggressive behaviors) is also seen for up to 24 hours.

Blanchard and colleagues (Blanchard & Blanchard, 1989; Blanchard et al., 1993) compared the defensive behaviors exhibited by rats in the presence of a cat to those exhibited in the presence of stimuli associated with the cat (i.e., cat odor). While the former behaviors were suggested to reflect fear, the latter behaviors were believed to reflect anxiety. Two test batteries were developed as a result of the research carried out by the Blanchards: the fear/defense test battery (F/DTB), and the anxiety/defense test battery (A/DTB). The F/DTB assesses the defensive reactions (flight, freezing, defensive attack) of rats to an approaching and contacting predator, the A/DTB measures risk assessment and inhibition of non-defensive behaviors to a potential threat source such as a cloth impregnated with the odor of a cat (Blanchard et al., 1990b). Recently, the mouse defense test battery was created, which combines features of the F/DTB and A/DTB into the same procedure (Blanchard et al., 1998; Griebel et al., 1995a). In a mouse-scaled oval runway, the defensive behaviors of Swiss-Webster mice are assessed in response to both present and anticipated threat (i.e., a hand-held, deeply anesthetized rat versus the hand alone, in a rat-grasping position). In addition, by closing off the runway, a straight alley is formed so as to measure the reactions of mice to an inescapable threat. In this particular situation, mice exhibit defensive threat and attack behaviors (not seen in the VBS as the subject can always escape from close contact with the predator) (Blanchard et al., 1998).

While the F/DTB and A/DTB are ethological justified designs (i.e., the VBS provides an environment in which the animals are able to interact freely and express their own defense patterns with minimal contrivance by the experimenter), the disadvantages of using defense test batteries are that they require specialized equipment and a great deal of space. In addition, the behavioral analyses are more extensive than those involved in conventional tests of fear or anxiety. Furthermore, some of the defensive behaviors observed in these tests are not affected by anxiolytic drug treatment. For example, benzodiazepines including diazepam and chlordiazepoxide have no effect on flight in either the F/DTB or A/DTB (Blanchard et al., 1993).

The Search for Ethologically Relevant Paradigms of Anxiety

As alluded to earlier, the focus on “ethologically-relevant” animal models of anxiety has contributed to the behavioral and neurochemical characterization of anxiety, particularly since these typically are not confounded by the effects of painful stimuli, and usually are relatively simple to conduct. For instance, the social interaction test is at first blush relatively simple to undertake, does not require complex training procedures, and can be accomplished with limited expense. Yet, the test involves the use of pairs of animals, making it difficult to examine individual differences in behavior. Furthermore, although the test has been widely established in rats, it may be more difficult to incorporate in mice as pairs of mice are much more aggressive than pairs of rats, and attacks and bites are commonly observed (Lister, 1990).

Consumption of a favored snack in a novel environment

When food deprived animals are placed in a novel environment, they ordinarily exhibited some reluctance to approach and eat lab chow that may be available. As animals habituate to the moderately threatening environment active responding animals begin to explore, eventually consuming the food. Thus, in this situation, it is typically observed that the latency to initiate feeding is increased, relative to that observed in the home cage or a familiar environment (Bodnoff et al., 1988, 1989a,b; Fletcher & Davies, 1990; Rex et al., 1998; Shepherd & Broadhurst, 1982) and food intake is reduced (Britton & Thatcher-Britton, 1981; Shepherd & Broadhurst, 1982). Indeed, placement in a novel environment may promote behavioral responses like those elicited by predator scent, including inhibition of non-defensive behaviors such as approach to food (among food deprived animals) (Blanchard & Blanchard, 1989) and these effects are antagonized by anxiolytic agents (Bodnoff et al., 1989a,b; Fletcher & Davies, 1990; Rex et al., 1998; Stout & Weiss, 1994).

Although assessing food intake in an unfamiliar environment is practical, its use has been limited by methodological problems. For instance, the anxiogenic attributes of the unfamiliar environment may be confounded by the effects of food or water deprivation (concurrent systemic stressors), while presentation of regular chow in non-deprived animals yields low basal feeding levels (floor effect), making further reductions of food intake less discernible. Recent experiments in our laboratory demonstrated that offering animals highly palatable, familiar snacks in a novel environment obviates these problems, allowing for an unadulterated appraisal of anxiety (Muller-Gass et al., 2000). Importantly, the increased consumption induced by anxiolytics was not evident in the

home cage, whereas orexigenic agents (e.g., central administration of neuropeptide Y which stimulate food consumption) increased food consumption in both the novel and the home cage tests (Merali et al., 2002). As well, this exceedingly simple behavior could be used on repeated occasions before animals adapted to the novel environment, thus permitting within-subject analyses of various anxiolytic treatments.

Experimental Prospectus

The present investigation was undertaken to characterize in greater detail the utility of using this paradigm to evaluate anxiety and the effects of anxiolytic agents. To this end a series of experiments is presented in which it is demonstrated that (a) mice tested in a novel environment, defined primarily by the fact that novel bedding is introduced into a cage (Muller-Gass et al., 2000) increases response latencies, (b) the procedure can be used repeatedly owing to slow habituation, thereby making the paradigm useful for within-subjects designs, (c) the anxiogenic response, even in a habituated animal, can be re-elicited or augmented by the introduction of other naturalistic stressors, such as predator odors, (d) the anxiety response in this situation can be attenuated by known anxiolytic agents (e.g., diazepam, chlordiazepoxide, propranolol), (e) reluctance to approach the novel food is attenuated, to some degree, by antidepressants which also have some anxiolytic actions, and finally (f) the specificity of the test was shown in that the behavior was not attenuated by non-anxiolytic agents (e.g., haloperidol).

General Methods

Subjects

Subjects were male, CD-1 mice, approximately 10 weeks of age, obtained from Charles River Canada Inc. (St Constant, Québec), or C57BL/6ByJ and BALB/cByJ mice obtained from the Jackson Laboratories (Bar Harbor, Maine). Upon arrival in the laboratory, mice were housed (by strain) in groups of 4 in standard polypropylene cages and were permitted 2-3 weeks to acclimatize to the laboratory setting. Before the experiment commenced mice were transferred to individual cages where they remained over a 7-10 day adaptation period. Throughout, mice were maintained on a 12 hr light-dark cycle (lights on: 0800-2000 hr) in a temperature controlled vivarium, and were provided free access to food and water.

Procedures

All procedures met the guidelines set out by the Canadian Council on Animal Care, and were approved by the University Ethics Committee. Mice were individually housed in clear plastic cages and on each day a dish containing approximately 10 gm of “Honey Maid Graham Crumbs”® was placed in the corner of the home cage. The latency to commence consumption of the palatable snack was recorded (to a maximum of 15 minutes). Once the latencies to approach the food had stabilized (less than 20% variability between days over 3 successive days) the experimental procedures began. This procedure was adapted from Khan et al (1999) and Muller-Gass et al (2000), which showed that latency to respond and the amount of food consumed were sensitive indices of the anxiogenic response, with latency being the more reliable of the two.

Testing occurred in either the home cage or an unfamiliar cage. The home cage

test was the same as baseline training, wherein mice were presented with the food dish and the latency to consume was recorded. Testing in the unfamiliar environment comprised placing mice in an opaque cage with fresh beta chips on the floor. The cage contained a Graham crumb filled dish, and once again the latency to approach and eat was recorded. If the test involved drug administration, then the active compound or vehicle was administered 20 min prior to testing (unless otherwise specified), during which mice remained in their home cages. All testing was conducted between 1000 and 1400 hr.

Experiments 1-4: Response inhibition associated with an unfamiliar environment and predator odor: Attenuation by diazepam.

The initial series of experiments was conducted to establish the utility of the paradigm by (a) examining latencies to consume a familiar snack upon transferring mice to a novel cage, (b) assessing the viability of repeated testing of mice, (c) determining whether the response inhibition in a familiar or unfamiliar environment would be augmented by the introduction of predator scents, and (d) assessing whether the response inhibition associated with the unfamiliar environment or rat scent would be attenuated by diazepam treatment.

Methods

Experiment 1: Influence of diazepam on approach to snack in an unfamiliar environment:

Following baseline training to approach the snack, CD-1 mice (n=20) were placed in the unfamiliar environment and response latencies determined. On the ensuing 3 days this procedure was repeated, but 20 min prior to testing mice were injected intraperitoneally

(i.p.) with either diazepam (dissolved in 40% propylene glycol, 10% alcohol and 50% water) or vehicle in a volume of 10 ml/kg. The latency to begin snack consumption was recorded.

Experiment 2: Alternating tests in a familiar and unfamiliar environment: In the preceding study, tests in the unfamiliar environment were conducted on successive days, permitting rapid habituation to this environment. To assess the utility of repeatedly testing animals, Experiment 2 assessed whether the course of habituation to the test environment would be attenuated by alternating tests between the unfamiliar and home cage. Following establishment of stable baseline consumption, mice (n=17) received testing on 7 consecutive days in which mice were alternately tested in the home cage, followed by the unfamiliar environment. The procedure was otherwise the same as in the preceding study.

Experiment 3: Response inhibition associated with an unfamiliar environment: exacerbation by predator scent. Following the establishment of stable response latencies, mice (n=20) were tested in the home cage after either introduction of rat feces (one bolus placed at the end of the cage opposite the food) or no treatment. The following day mice were tested in the home cage without the presence of predator stimuli to ascertain whether any carry-over effects persisted.

To assess the effects of the introduction of predator scents in the unfamiliar environment, the same mice received 3 further test days. On the first and second test days all mice were test in the home cage and then the unfamiliar environment, respectively. On the third day all mice were tested in the unfamiliar environment, but for half the mice the test environment contained rat feces, while for the remaining mice no such stressor cues

were presented.

Experiment 4: Response inhibition associated with predator scent and the effects of diazepam: In order to assess the effects of diazepam on response inhibition associated with predator scent, CD-1 mice (n=40) were presented with Graham crumbs daily until stable baseline approach latencies were evident. On the ensuing day half the mice received diazepam (0.25 mg/kg, i.p.) while the remaining mice received vehicle. Rat feces were introduced to the mouse cage, followed immediately afterward by the snack dish, and the latency to begin consumption was determined. The procedure was repeated the next day in the absence of any rat scent or diazepam administration in order to evaluate carry-over effects of the treatments.

Results:

Experiment 1: Influence of diazepam on approach to snack in an unfamiliar environment:

The latency to approach the palatable food in the home cage, the unfamiliar cage, and the unfamiliar cage following diazepam or vehicle treatment are shown in Figure 1. The analysis of variance indicated that latencies varied significantly over days, $F(4, 72) = 6.65$, $p < 0.01$. The post hoc tests indicated that on the first test day in the novel environment, the latencies were markedly longer (10-fold) relative to baseline, and declined over successive tests. The analysis also indicated that response latencies were shorter among diazepam than among vehicle-treated mice, $F(1, 18) = 4.50$, $p < 0.05$. Although the interaction between Drug and Days was not significant, *a priori* predictions had been made in this respect, and hence post hoc tests of the simple effects were conducted. As shown in Figure 1, and confirmed by the post hoc tests, prior to drug or vehicle treatment, differences of response latencies were not evident between the groups.

On the first Drug test day in the unfamiliar environment the response latencies of the vehicle treated mice significantly exceeded those of the diazepam treated mice. Thereafter the response latencies declined in both groups, and the effects of the drug treatment were no longer evident.

Insert Figure 1 about here

Experiment 2: Alternating tests in a familiar and unfamiliar environment: When performance was assessed alternately in the home cage and unfamiliar environments, response latencies varied over the test days, $F(6,96) = 38.16$, $p < 0.01$. The post hoc tests confirmed that home cage latencies were shorter than those in the novel environment. As shown in Table 1, in CD-1 mice this effect was relatively persistent over the course of the 3 tests in the novel environment, as there was little indication of response latencies declining over successive tests.

Insert Table 1 about here

Experiment 3: Response inhibition associated with an unfamiliar environment: exacerbation by predator scent: The effects of exposing mice, while in the home cage, to predator (rat) scent is shown in Figure 2 (upper panel). The analysis of variance indicated that the interaction between Test session x Rat scent was significant, $F(2, 36) = 24.37$, $p < 0.01$. The post hoc tests confirmed that among mice not exposed to the rat scent the

Figure 1. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack in the home cage (first set of bars) and in an unfamiliar environment (next four set of bars). On the first of the tests in the unfamiliar environment no drug treatment was administered, whereas on the remaining 3 test days mice received either diazepam (0.25 mg/kg) or vehicle. * denotes increased latencies ($p < 0.05$) relative to home cage responding. ** denotes increased latencies relative to home cage performance, but decreased latencies relative to vehicle treated mice tested in the unfamiliar environment.

Test Condition

Table 1. Response latencies on alternate tests within the home cage and unfamiliar environment

Home cage 1	21.47 ± 2.33
Unfamiliar test 1	$236.59 \pm 27.79^*$
Home cage 2	20.47 ± 2.97
Unfamiliar test 2	$223.70 \pm 26.43^*$
Home cage 3	23.23 ± 2.26
Unfamiliar test 3	$312.94 \pm 47.39^*$
Home cage 4	23.35 ± 3.98

$p < .01$ relative to the preceding home cage test

response latencies were stable over the 3 tests. However, in those mice that were exposed to the rat scent a significant increase of response latencies occurred and then declined upon later retest in the absence of the scent.

Insert Figure 2 about here

In the second phase of this particular experiment, when testing was conducted in the novel environment, consumption again varied over tests Sessions, $F(5, 80) = 22.31$, $p < 0.01$ and as a function of the Scent, $F(1,16) = 4.49$, $p < 0.05$, irrespective of the animal's previous stressor experiences. As seen in Figure 2 (lower panel), and confirmed by the multiple comparisons, relative to baseline the approach to the food was markedly slower when mice were tested in the unfamiliar environment relative to that seen in the home cage. Upon introduction of rat feces the next day a further retardation of responding was apparent (2-fold increase of the already slow response latencies). Over ensuing sessions in the novel environment, without rat odor present, the latencies decline significantly, but still exceed those evident within the home cage.

Experiment 4: Response inhibition associated with predator scent and the effects of diazepam: Response latencies to approach the snack varied as a function of the Session x Drug treatment interaction, $F(2, 76) = 3.37$, $p < 0.05$. As shown in Figure 3 and confirmed by the multiple comparisons, baseline latencies (prior to treatment) to consume were rapid and comparable across groups. On the ensuing day, when rat feces were introduced into the animal's home cage, latencies increased markedly among vehicle-treated mice, whereas the increase was markedly attenuated and non-significant in mice

Figure 2. The upper panel depicts the mean ($\pm$ SEM) latencies to approach and begin to consume the snack in the home cage. On the second test day rat feces (predator scent) was introduced for half the mice. No scent was present on the third test day. The lower panel depicts the mean ($\pm$ SEM) latencies to approach and begin to consume the snack in the home cage, the unfamiliar environment, or the unfamiliar environment when rat feces were present. * denotes significant differences ($p < 0.05$) relative to responding in the home cage test. ** denotes differences in responding relative to animals tested in the unfamiliar environment in the absence of the predator scent.

treated with diazepam. Indeed, the response latencies of the diazepam treated mice were significantly shorter than among vehicle treated mice. Upon retesting in the absence of any treatments, comparable response latencies were evident indicating the absence of any carry-over effects.

Insert Figure 3 about here

Experiment 5: Influence of unfamiliar environment and predator scent in C57BL/6ByJ and BALB/cByJ mice.

It has repeatedly been shown that strains of rats and mice differ appreciably in their anxiety responses to stressors. In this respect it appears that BALB/cByJ mice tend to exhibit more pronounced anxiety than do C57BL/6ByJ or C57BL/6J mice (Anisman et al., 2001; Belzung & Griebel 2001; Crawley et al., 1997; DeFries & Hegmann, 1970; Griebel et al., 1993, 2000; Logue et al., 1997; Peeler & Nowakowski, 1984) as well as greater variations of CRH within the paraventricular nucleus and median eminence (Anisman et al., 1998), elevations of plasma ACTH and corticosterone (Shanks et al., 1990; Zaharia et al., 1996), and increased monoamine turnover in several stressor-sensitive brain regions (Herve et al., 1979; Shanks et al., 1991, 1994a,b). However, it appeared that the differences between BALB/cByJ and other strains (such as C57BL/6J and C57BL/6ByJ) were stressor-specific, being prominent in response to neurogenic stressors (e.g., in response to footshock or loud noise), but either modest or absent in response to psychogenic stressors (e.g., cat feces, odor of fox urine, exposure to a rat) (Anisman et al., 2001; Belzung et al., 2001; Roy et al., 2001). In fact, the behavioral

Figure 3. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack at baseline (last home cage test) upon the introduction of rat scent, and again in the home cage without scent. Prior to the second test (in the presence of rat scent) mice were treated with either diazepam (0.25 mg/kg) or vehicle. * $p < 0.05$ relative to the preceding home cage test.

effects of stressors involving predator-cues were more prominent in C57BL/6ByJ mice (Anisman et al., 2001; Belzung et al., 2001), as were the monoamine increases within the central amygdala (Hayley et al., 2001). Experiment 4 was conducted to assess whether the effects of the novel environment and predator scent would be greater in C57BL/6ByJ than in BALB/cByJ as observed in other tests of anxiety following exposure to predator scents.

Methods

Mice (n=20 male BALB/cByJ; 20 male C57BL/6ByJ) were obtained from the Jackson Laboratory, Bar Harbor Maine, at 7 weeks of age and were acclimatized to the laboratory for 3 weeks before serving as experimental subjects. Housing and experimental conditions were the same as described earlier. Following the establishment of stable baseline snack consumption, mice received 9 days of testing in which the approach to the snack was assessed, on alternate days, within the home cage and the unfamiliar cage. On the last test day (after some adaptation had taken place), mice were placed in the unfamiliar cage, but in this instance rat feces were present within the cage. The latency to begin to consume the snack was recorded.

Results

Experiment 5: Influence of unfamiliar environment and predator scent in C57BL/6ByJ and BALB/cByJ mice: Figure 4 shows the latency to approach and begin consumption of the Graham crumbs snack in BALB/cByJ and C57BL/6ByJ mice tested in the home cage and in the unfamiliar environment, as well as a test (on the last day) in the unfamiliar environment in which rat feces was introduced. The latencies were found to vary as a function of the Strain x Test day interaction, $F(9, 342) = 2.21$, $p < 0.05$. The post hoc

tests indicated that in both strains consumption latencies on the initial test in the unfamiliar environment were longer than in the home cage test. With successive tests in the unfamiliar environment the latencies were reduced from that seen during the first such test, but still exceeded that evident within the home cage. Finally, on the last test day, where rat feces was placed in the novel environment, latencies again were longer in both strains than those evident on the preceding tests in either the home cage or the novel environment. However, the increase was greater in C57BL/6ByJ than in BALB/cByJ mice.

As indicated earlier, BALB/cByJ mice are typically more stress-reactive than C57BL/6ByJ mice in response to neurogenic and systemic stressors, but in tests that involve predator odors this strain effect was reversed (Anisman et al., 2001). Thus, it was deemed propitious to assess again the response to the rat scent in a new environment. In a partial replication of this study (n=13/strain) it was again found that following habituation to an unfamiliar environment, the introduction of rat feces resulted in C57BL/6ByJ mice exhibiting significantly longer latencies ($X \pm \text{SEM} = 318.58 \pm 56.77$) to approach food relative to BALB/cByJ mice ($X \pm \text{SEM} = 132.46 \pm 20.81$).

Insert Figure 4 about here

***Experiment 6-9.** Effects of chlordiazepoxide, propranolol, haloperidol and d-amphetamine.*

The preceding studies indicated that diazepam effectively attenuated the response inhibition engendered by an unfamiliar environment. To assess the utility of the test

Figure 4. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack between BALB/cByJ and C57BL/6ByJ mice. On alternate days mice were tested in the home cage (H) or the unfamiliar cage (U). On the last test day in the unfamiliar cage rat feces was introduced (U/S). * $p < 0.05$ relative to the preceding home cage test. ** $p < 0.05$ relative to BALB/cByJ mice.

preparation, it was necessary to demonstrate that the behavioral inhibition associated with the unfamiliar environment could be attenuated by other compounds with known anxiolytic properties, and conversely, would not be affected by agents that ordinarily do not act in this capacity.

To this end, two experiments assessed the effects of chlordiazepoxide and propranolol on response inhibition in the unfamiliar environment. The anxiolytic effects of chlordiazepoxide, like that of diazepam, have been demonstrated across numerous test situations including the response to psychogenic types of stressors (Bruijnzeel et al., 2001; Griebel et al., 1993). Likewise, the β -NE antagonist, propranolol, has anxiolytic effects (Emilien & Maloteaux, 1998) that are particularly evident with respect to the somatic or autonomically mediated features of anxiety (Turner, 1991) and the behavioral effects of stressors (Gorman & Dunn, 1993; Rex et al., 1998; Walker & Davis, 2002). However, at least some of the effects of propranolol can be distinguished from those elicited by benzodiazepines (Gorman & Dunn, 1993), and in contrast to diazepam and chlordiazepoxide, its presumed action does not involve GABA neuronal changes. Thus, it was of particular interest to ascertain whether chlordiazepoxide and propranolol would have anti-anxiety effects, like those of diazepam, in a novel environment. Finally, to assess the specificity of the test, the effects of haloperidol and d-amphetamine were assessed in the home cage and the unfamiliar environment, as neither of these compounds ordinarily has anxiolytic effects.

Methods

The procedure of each experiment was essentially the same. Following the establishment of stable responding to Graham crumbs, mice ($n = 10/\text{group}$) received three

successive tests 20 min following i.p. injection of either the active compound (chlordiazepoxide hydrochloride, 3.0 mg/kg; dissolved in 50% propylene glycol, 50% saline; propranolol hydrochloride, 2.0 mg/kg; haloperidol hydrochloride, 0.3 mg/kg dissolved in 0.1N HCl and water added to make up the final volume; d-amphetamine sulfate, 0.25 mg/kg) or its respective vehicle in a volume of 10 ml/kg. On the 1st and 3rd test days the procedures were undertaken in the unfamiliar environment, while on the intermediate day the test was conducted within the home cage.

Results

Experiments 6-9: Effects of chlordiazepoxide, propranolol, haloperidol and d-amphetamine: Analysis of the chlordiazepoxide test revealed a significant Drug x Test interaction, $F(3, 54) = 8.39$, $p < 0.01$. The post hoc tests indicated that among vehicle treated mice, tests in the unfamiliar environment resulted in significantly longer response latencies than in the familiar environment. As seen in Figure 5 (upper left panel) pretreatment with diazepam in the unfamiliar environment significantly reduced the response latencies in the unfamiliar environment, but had no effect in the home cage tests. While the drug treatment markedly reduced the inhibition associated with the unfamiliar environment, the latencies to eat the snack were still longer than those seen at baseline or during the intervening home cage test.

Insert Figure 5 about here

The effects of the propranolol treatment were very similar to those elicited by diazepam and chlordiazepoxide (see Figure 5, upper right panel). The analysis of

Figure 5. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack at baseline (home cage) in an unfamiliar environment, another test in the home cage, followed by a second test in the unfamiliar environment. Drug treatments were only applied during the latter 3 tests. The four panels depict the effects of chlordiazepoxide, propranolol, haloperidol and amphetamine. * $p < 0.05$ relative to baseline. ° $p < 0.05$ relative to vehicle.

variance indicated that response latencies varied as a function of the Drug treatment x Test interaction, $F(3,54) = 3.33$, $p < 0.05$. The post hoc tests once again indicated that response latencies among vehicle treated mice tested in the unfamiliar environment were markedly longer than those observed in the home cage. Treatment with propranolol significantly reduced the response latencies, although performance still differed significantly from that observed in the home cage.

The effects of haloperidol were clearly distinguishable from those of the anxiolytic agents (Figure 5, lower left panel). Specifically, it was again observed that approach to the snack varied as a function of the test environment, $F(3,51) = 9.71$, $p < 0.01$, with longer latencies occurring in the unfamiliar than the familiar environment. There was no indication, however, of haloperidol treatment attenuating this effect. In contrast to the other drug treatments, d-amphetamine prolonged the animal's latency to approach and consume the snack (Figure 5, lower right panel). Indeed, while saline treated animals exhibited latencies of 110.4, 20.6 and 69.4 sec in the unfamiliar, home cage and unfamiliar tests, respectively, the d-amphetamine treated mice exhibited latencies of 900 sec on all 3 trials. Importantly, irrespective of whether mice were tested in the unfamiliar environment or the home cage, none of the mice consumed any of the snack throughout the test period.

Experiment 10a-b: Effects of acute and repeated buspirone.

To further assess the paradigm the effects of acute and chronic buspirone and desmethylinipramine treatment were assessed. Buspirone, a 5-HT_{1A} agonist has potent anxiolytic effects (Lucki, 1996), which are more pronounced following repeated than

after acute treatment (Cole & Rodgers, 1994; Yamashita et al., 1995). While the effects of this compound appear to involve 5-HT processes, it also has actions on DA release at the prefrontal cortex, and neuropeptide Y at various limbic sites, and might thereby affect the response to stressors (Krysiak et al., 2000; Wedzony et al., 1996). It has been suggested that despite its anti-anxiety actions, inconsistent effects of buspirone have been seen in various animal models of anxiety (Cole & Rodgers, 1994). Indeed, it has been reported that in some paradigms buspirone, in contrast to benzodiazepine treatment, does not promote an anxiolytic effect (e.g., elevated plus-maze, conditioned emotional response, attenuation of punished responding) (Martin et al., 1993; Rodgers et al., 1997; Stanhope & Dourish, 1996). Other investigators, however, indicated that in some instances, notably with respect to the prophylactic action in attenuating the effects of subsequently applied stressors, buspirone was more effective than benzodiazepine treatment (Tsuji et al., 2000).

Methods

Following the establishment of stable baseline responding for the Graham crumbs, mice ($n=7/8$ per group) were treated with either buspirone hydrochloride (4.0 mg/kg, subcutaneously; 50% propylene glycol, 50% saline) or vehicle for 17 successive days. On the last three days, 20 min after injection, mice were tested with the drug or vehicle in the home cage, the unfamiliar environment, and then again in the home cage, respectively. In a second experiment, the effects of acute buspirone were assessed ($n=10$ /group). The procedure was the same as in the preceding study, except that the drug or vehicle treatments were only administered on the 3 test days.

Results

Experiment 10a-b: Effects of acute and repeated buspirone: The effects of buspirone varied as a function of the Drug condition x Test day interaction, $F(2,26) = 4.27$, $p < 0.05$. As in the preceding studies, when vehicle-treated mice were placed in the unfamiliar environment latencies to respond to the snack were longer than those observed in the familiar environment. Among mice that had received repeated buspirone treatment the increased latencies evident in the unfamiliar test were markedly attenuated, so that latencies in the buspirone-treated animals were shorter than in vehicle treated mice (Figure 6, upper panel). In contrast to the effects of repeated buspirone treatment, as depicted in Figure 6 (lower panel), in the study assessing the effects of acute treatment, the increased latencies to approach the novel food in the unfamiliar environment, $F(2, 28) = 38.30$, $p < 0.01$, were unaffected by the buspirone treatment ($F < 1$). Indeed, the latencies to respond were slightly longer than among the vehicle treated mice.

Insert Figure 6 about here

Experiment 11a-b: Effects of acute and repeated desmethylinipramine:

Antidepressant compounds may diminish anxiety, particularly in the case of generalized anxiety disorder, where depression is a frequent comorbid feature (Davidson, 2001). As well, paroxetine (and other SSRIs) has been found effective across a number of other anxiety-related disorders (Wagstaff et al., 2002). Paralleling these human reports, studies in rodents indicated that chronic desmethylinipramine treatment may influence

Figure 6. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack within the home cage, in an unfamiliar environment, and again in the home cage. The upper panel depicts the performance of mice that received repeated treatment with buspirone (4.0 mg/kg) over 17 days (drug or vehicle were administered on each of the 3 test days). The lower panel depicts the performance of mice that received the treatments only on the 3 test days.

anxiety responses, but these effects are dependent on the specific test paradigms being used (Rodrigues-Filho & Takahashi, 1999). Thus, in the present investigation we assessed whether repeated and acute desmethylinipramine treatment would influence approach to a palatable snack in the home cage and in a novel environment.

Methods

Following the establishment of stable responding, mice received desmethylinipramine hydrochloride (7.50, 10.0 or 12.50 mg/kg, sc) or saline on each of 21 successive days ($n=8/\text{group}$). On the last 3 consecutive days, 30 min after the drug or vehicle injection, mice were tested in the home cage, the novel environment, and then the home cage, respectively. In a second study that assessed the effects of acute antidepressant treatment, the procedure was repeated except that mice received the desmethylinipramine treatment (10.0 mg/kg) or saline only on the last three test days, 30 min before behavioral assessments ($n = 8/\text{group}$).

Results

Experiment 11a-b: Effects of acute and repeated desmethylinipramine: The latency to begin consumption of the Graham crumbs varied as a function of the chronic Drug treatment x Test session interaction, $F(6, 56) = 2.37$, $p < 0.05$. As previously observed, when saline-treated mice were tested in an unfamiliar environment, response latency increased markedly. Among mice that received the chronic desmethylinipramine treatment, irrespective of the dosage, the latency to approach the snack was significantly shorter than in saline treated mice, although latencies still differed from those evident within the home cage (Figure 7, upper panel). The latencies to respond did not differ as a function of the desmethylinipramine dose used. In contrast to the effects of the chronic

treatment, the increased response latencies associated with an unfamiliar environment, $F(3, 42) = 42.03$, $p < 0.01$, was unaffected by acute treatment with the antidepressant, ($F < 1$) (Figure 7, lower panel).

Insert Figure 7 about here

Discussion

Animals ordinarily exhibit disruptions in feeding behavior following exposure to various neurogenic and psychogenic stressors. For example, decreased food consumption is observed in rodents subjected to footshock or restraint stress (Paré et al., 1999; van dijken et al., 1992b) and following exposure to a predator or predator-related cue (Blanchard & Blanchard, 1989; Calvo-Torrent et al., 1999; Hendrie et al., 1999). Disruptions of feeding behavior also occur when rats or mice are presented with a novel food (i.e., food neophobia) (Fletcher & Davies, 1990; Shephard & Estall, 1984) or placed in an unfamiliar environment (i.e., hyponeophagia) (Soubrié et al., 1975; Britton & Thatcher-Britton, 1980). Novelty-induced suppression of feeding is attenuated by anxiolytic agents (e.g., diazepam, chlordiazepoxide, buspirone), as reflected by shortened latencies to approach the food (Bodnoff et al., 1988; Fletcher & Davies 1990; Shephard & Broadhurst, 1982) and increases in the total amount eaten (Britton & Thatcher-Britton, 1981; Shephard & Estall., 1984).

Previous investigations concerning novelty-suppressed feeding involved food-depriving the animals prior to experimental testing. However, food deprivation per se is a

Figure 7. Mean ($\pm$ SEM) latencies to approach and begin to consume the snack within the home cage, in an unfamiliar environment, and again in the home cage. The upper panel depicts the performance of mice that received repeated treatment with desmethylinipramine (7.5, 10.0 or 12.5 mg/kg) over 21 days (drug or vehicle were administered on each of the 3 test days). The lower panel depicts the performance of mice that received the desmethylinipramine (10.0 mg/kg) only on the 3 test days. * $p < 0.05$ relative to the home cage test. ** $p < 0.05$ relative to the home cage test and relative to vehicle treated mice during the unfamiliar test.

stressor, which is typically associated with altered hypothalamic-pituitary-adrenal (HPA) activity (Hwang & Guntz, 1997; Kiss et al., 1994) and anxiety-like behavior in the elevated plus-maze (Jaiswal et al., 1996). As the effects of food deprivation might interfere with the anxiogenic attributes of the novel environment (thereby presenting an experimental confound), a more recent investigation was carried out in non-food-deprived animals. Unfortunately, in non-deprived animals, the food intake was so small and variable, that clear drug effects were hard to discern (floor effect) (Khan et al., 1999; Muller-Gass et al., 2000). In the present investigation, these difficulties were overcome by using a familiar, highly palatable snack (Christie® Graham crumbs), in non-deprived mice. Under such conditions, it was observed that when placed in an unfamiliar environment, the latencies to approach and consume the highly palatable snack were markedly increased. This effect was exceptionally robust, and was evident over repeated tests, particularly when intervening tests were conducted within the home cage.

Earlier studies in our laboratory (Khan et al., 1999; Muller-Gass et al., 2000) revealed that the fundamental element distinguishing the approach to food in the unfamiliar and home cage environments comprised the bedding in the test area. If animals were placed in a test cage with novel bedding or no bedding, then latencies to approach and consume the palatable snack were markedly prolonged. In the present investigation, it was observed that the inhibition of responding could also be provoked in the home cage in the presence of a predatory stressor. It has been repeatedly demonstrated that the rat is a natural predator of the mouse (Johansson & Peder, 1984; Kemble et al., 1985) and that exposure of mice to rat-related sensory stimuli is accompanied by a variety of anxiety-like behaviors (Calvo-Torrent et al., 1999; Griebel et

al., 1995b). Hence, when rat feces were placed within the home cage, the latency of the mice to begin feeding was increased. Likewise, if animals had habituated to a novel test cage, so that long latencies were no longer evident, the introduction of rat feces inhibited responding.

The introduction of predator odors does not necessitate handling animals or transferring them to a new venue. This is particularly advantageous as such procedures have been associated with behavioral and neurochemical variations that potentially interfere with the outcome of anxiolytic drug administration. For example, both handling and transferring animals from one cage to another promotes increased sensitivity to benzodiazepines in the open-field exploration and elevated-plus maze tests (De Kock & Meert, 1997; Hogg, 1996; Schmitt & Hiemke, 1998). Additional studies demonstrate that handling of animals is accompanied by increased release of catecholamines (e.g., dopamine, noradrenaline) within the prefrontal cortex and nucleus accumbens (Feenstra & Blotterblom, 1996; Feenstra et al., 1998; Ihalaenen et al., 1999). The method used in the present investigation is not confounded by the anxiety-inducing effects of experimental handling, and may prove useful in pharmacological studies assessing anxiety processes.

The effectiveness of the unfamiliar environment in inhibiting the response to a palatable snack was evident not only in CD-1 mice but was apparent in other strains as well, including the inbred BALB/cByJ and C57BL/6ByJ strains.

BALB/cByJ and C57BL/6ByJ mice typically exhibit differences in their responsivity to stressors. While BALB/cByJ mice display more pronounced signs of anxiety in stressful situations, C57/6ByJ mice tend to be stressor resilient. For example,

behavioral reactivity is more pronounced in BALB/cByJ mice with respect to the open-field activity and light-dark exploration tests (Anisman et al., 2001; Crawley et al., 1997; Defries and Hegmann, 1970; Logue et al., 1997; Peeler & Nowakowski, 1987) and following exposure to various neurogenic stimuli such as footshock or forced swim stress (Griffiths et al., 1992; Zaharia et al., 1996). In response to neurogenic stressors, BALB/cByJ mice also exhibit more pronounced HPA activation (Anisman et al., 1998, 2001; Shanks et al., 1990; Zaharia et al., 1996) and larger increases in monoamine turnover in several anxiety-associated brain regions (Shanks et al., 1991; 1994a,b).

Curiously, while BALB/cByJ mice display greater behavioral and neurochemical variations upon exposure to neurogenic stressors, C57/6ByJ mice appear to be more responsive to predatory stressors. For instance, C57/6ByJ mice exhibit greater elevations in plasma corticosterone and monoamine turnover following exposure to a live predator such as a rat, or to a predator-related cue such as a cloth impregnated with fox odor (Anisman et al., 2001; Belzung et al., 2001). Furthermore, in studies using the free-exploration test, BALB/cByJ mice exhibit anxiety-like responses only when exposed to both cat and, subsequently, to cat feces, while C57/6ByJ are responsive to cat feces alone (Belzung et al., 2001).

Paralleling the latter findings, the present investigation demonstrated that although the latencies to begin eating were comparable among the strains upon exposure to a novel environment, exposure to rat scent evoked larger eating latencies in C57/6ByJ mice relative to BALB/cByJ mice. However, it remains unclear whether the absence of a strain difference is related to psychogenic stressors generally, or to stressors involving specific olfactory stimuli. Taken together, these findings suggest that strain-specific

alterations in behavior and neurochemistry may be dependent upon the characteristics of the particular stressor employed.

Consistent with the view that the latency to approach food in a novel environment is related to anxiety, acute treatment with benzodiazepines including diazepam and chlordiazepoxide, reduced the latency of mice to begin eating the palatable snack. Propranolol, which has been shown to attenuate anxiety in a number of paradigms including the elevated plus-maze and light-dark tests (Gorman & Dunn, 1993; Kshama et al., 1990), was also effective in reducing the latency of mice to begin eating in the novel environment. These findings are consistent with those of an earlier investigation in which both diazepam and propranolol increased the amount of food eaten by food-deprived rats in a brightly lit, novel open field (Rex et al., 1998).

Previous investigations have demonstrated that agents such as the 5HT_{1A} receptor agonist, buspirone, and the tricyclic antidepressant, desmethylinipramine, acquire anxiolytic properties chronic rather than acute administration. For example, chronic but not acute administration of either buspirone or desmethylinipramine decreases exploratory activity in the open-field and elevated plus-maze tests (Angrini et al., 1998; Cole and Rodgers, 1994; D'Aquila et al., 2000). Likewise, in the present investigation, chronic (21-day) but not acute treatment with either buspirone or desmethylinipramine attenuated novelty-suppressed feeding in satiated mice.

Haloperidol is a neuroleptic agent commonly used in the treatment of psychotic disorders such as schizophrenia. While haloperidol elicits a variety of locomotor effects, it lacks anxiolytic properties and is ineffective in anxiety paradigms including the light-dark exploration, elevated plus-maze and social interaction tests (Bilkei-Gorzo et al.,

1998; Emerich et al., 1991; Graeff et al., 1998; Sakamoto et al., 1998). Consistent with previous studies, haloperidol did not affect the latency of mice to begin eating in the novel environment. Likewise, in studies conducted in rats, we observed that other agents that ordinarily do not reduce anxiety (e.g., caffeine) were ineffective in augmenting responding in this paradigm (Muller-Gass et al., 2000). Hence, specificity exists with respect to the treatments that attenuate behavioral inhibition. Furthermore, diazepam, which reduced response latencies in the novel environment, did not influence performance in the home cage. These effects could not readily be attributed to motoric factors associated with the treatment. Specifically, the approach to food involved the initiation of an active response, and thus motor side effects or lethargy elicited by the drugs in question would have resulted in slower response latencies.

Increasing evidence has accumulated suggesting that anxiogenic responses and feeding may be linked to one another. While benzodiazepines reduced the latencies to approach the snack (due to anxiolytic action), such compounds could potentially enhance the palatability or hedonic value of food (Berridge et al., 1995; Higgs & Cooper, 1996a,b; Pecina & Berridge, 1996; Stratford & Kelley, 1997). Additional studies have demonstrated the ability of benzodiazepines to increase the food intake of non-deprived animals in either a familiar or unfamiliar environment, suggesting an appetitive action of benzodiazepines (Cooper & Moores, 1985a,b; Cooper et al., 1985; Soubrié et al., 1975). On the contrary, the present investigation demonstrated that while benzodiazepines reduced the latency of satiated mice to begin eating in the novel environment, these treatments had no effect in the home cage. This particular finding indicates that benzodiazepines act by means of an anxiolytic rather than an appetitive mechanism and is

consistent with the results of previous studies carried out in food-deprived rats (Bodnoff et al., 1988, 1989a,b; Britton & Thatcher-Britton, 1981; Fletcher & Davies, 1990; Rex et al., 1998; Shephard & Broadhurst, 1982; Shephard & Estall, 1984). Moreover, previous investigations in the lab demonstrated that a potent orexigenic agent with anxiolytic properties, namely neuropeptide Y (NPY) administered centrally to rats, increased snack intake both in the home cage and the novel environment (Merali et al., 2002). As indicated earlier, anti-anxiety treatments that do not act via central GABAergic mechanisms, and likely do not have orexigenic properties (e.g., propranolol) also decreased the latency to approach the snack in the unfamiliar environment.

Finally, the results from the *d*-amphetamine treatment, also attest to the specificity of this paradigm. The dopamine agonist, *d*-amphetamine, not only stimulates motor activity, but also has anorexic effects (Bendotti et al., 1982; Chen et al., 2001; Gilbert & Cooper, 1985). In parallel with studies carried out by other investigators, *d*-amphetamine increased the latency of animals to approach the snack and decreased the amount consumed (i.e., none of the animals ate the snack), in both the home and novel cages. This finding suggests that *d*-amphetamine produced anorexic effects in this paradigm and provides support for the utility of this paradigm in differentiating anxiety-dependent and –independent drug effects.

Taken together, it appears that snack consumption in an unfamiliar environment is a sensitive and reliable index of anxiety. Because of its simplicity, this paradigm lends itself to the concurrent assessment of dynamic neurotransmitter changes that occur in association with anxiety and anxiolytic drug treatment. However, it should be emphasized that the effectiveness of anxiolytics may be situation-specific, and likewise

the neurochemical concomitants of anxiety may be related to the specific conditions eliciting the anxiogenic response. For instance, it was previously mentioned that the central nucleus of the amygdala may be associated with cue-specific anxiety (i.e., fear), while the bed nucleus of the stria terminalis may be more closely aligned with generalized anxiety (Davis & Shi, 1999). In this respect, previous studies in our laboratory revealed that the reluctance to consume a snack in a novel environment was accompanied by increased *in vivo* amygdala CRH release (Khan et al., 1999). However, while pre-treatment with benzodiazepines were highly effective in attenuating novelty-suppressed feeding, these drugs were ineffective in reversing novelty-associated CRH release. Furthermore, the suppression of feeding was not produced by the central administration of CRH, nor was it attenuated by the administration of CRH antagonists (Merali et al., 2002). As such, it is likely that CRH does not subserve the provocation of the anxiety response, but likely involves the co-activation of some other neurotransmitter working independently or in conjunction with CRH. In this respect, however, Merali et al (2002) indicated that the mechanisms supporting the anxiety response may be stimulus- or situation-specific. It may be that novelty or predator odors engage pre-wired circuits to elicit anxiety, whereas other systems are operative when learned fear/anxiety responses are introduced.

In sum, these experiments suggest that when animals are introduced to a novel environment or exposed to predator-related cues in either a familiar or unfamiliar environment, the latency to approach and consume a familiar palatable snack is markedly prolonged. The effects of novelty or predatory stress can be blocked or attenuated by various pharmacological treatments known to alleviate anxiety. Moreover, these effects

are specific in that drugs devoid of anxiolytic properties fail to show a similar profile, and that potentially confounding variables can be detected by assessing snack consumption both in the home and novel (stressful) environments. We believe that this model is more akin to other models of 'spontaneous' anxiety (e.g. elevated plus maze, open-field emergence), where conflict is engendered by the drive to explore (and seek out new sources of food) and the response inhibition imposed by the potential threat (e.g. the scent of a predator) of the new milieu. The major advantages of this paradigm include the simplicity of the design, the reliability and magnitude of the response (i.e., there is a 10-fold increase in the approach latency following exposure to novelty or predatory stress), the specificity and sensitivity to anxiolytic drugs, and the possibility of testing animals repeatedly. With the advent of transgenic mouse models, it has become imperative to carefully evaluate diverse phenotypic changes in mice with specific genetic deletions. A model such as the one described here would be useful in assessing several effects simultaneously, including effects on anxiety, anorexia and motor performance.

References

- Adamec, R.E. & Shallow, T. (1993) Lasting effects on rodent anxiety of a single exposure to a cat. *Physiol. Behav.*, **54**, 101-109.
- Adamec, R.E., Kent, P., Anisman, H., Shallow, T. & Merali, Z. (1998) Neural plasticity, neuropeptides and anxiety in animals – implications for understanding and treating affective disorder following traumatic stress in humans. *Neurosci. Biobehav. Rev.*, **23**, 301-318.
- Adamec, R.E., Burton, P., Shallow, T. & Budgell, J. (1999) Unilateral block of NMDA receptors in the amygdala prevents predator stress-induced lasting increases in anxiety-like behavior and unconditioned startle – effective hemisphere depends on the behavior. *Physiol. Behav.*, **65**, 739-751.
- Adamec, R.E., Blundell, J. & Collins, A. (2001) Neural plasticity and stress induced changes in defense in the rat. *Neurosci. Biobehav. Rev.*, **25**, 721-744.
- American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)*. American Psychiatric Press. Washington, D.C.
- Anisman, H., Lacosta, S., Kent, P., McIntyre, D.C. & Merali, Z. (1998) Stressor-induced corticotropin-releasing hormone, bombesin, ACTH and corticosterone variations in strains of mice differentially responsive to stressors. *Stress*, **2**, 209-220.

- Anisman, H., Hayley, S. & Kelly, O. (2001) Psychogenic, neurogenic, and systemic stressor effects on plasma corticosterone and behavior: Mouse strain-dependent outcomes. *Behav. Neurosci.*, **115**, 443-454.
- Angrini, M., Leslie, J.C. & Shephard, R.A. (1998) Effects of propranolol, buspirone, pCPA, reserpine, and chlordiazepoxide on open-field behavior. *Pharmacol. Biochem. Behav.*, **59**, 387-397.
- Babbini, M., Gaiardi, M. & Bartoletti, M. (1982) Benzodiazepine effects upon Geller-Seifter conflict test in rats: analysis in individual variability. *Pharmacol. Biochem. Behav.*, **17**, 43-48.
- Belzung, C. & Le Pape, G. (1994) Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiol. Behav.*, **56**, 623-628.
- Belzung, C., Pineau, N., Beuzen, A. & Misslin, R. (1994) PD135158, a CCK-B antagonist, reduces "state" but not "trait" anxiety in mice. *Pharmacol. Biochem. Behav.*, **49**, 433-436.
- Belzung, C., El Hage, W., Moindrot, N. & Griebel, G. (2001) Behavioral and neurochemical changes following predatory stress in mice. *Neuropharmacology*, **41**, 400-408.

- Belzung, C. & Griebel, G. (2001) Measuring normal and pathological anxiety-like behavior in mice: a review. *Behav. Brain Res.*, **125**, 141-149.
- Bendotti, C., Borsini, F., Cotecchia, S., De Blasi, A., Mennini, T. & Samanin, R. (1982) *d*-amphetamine-induced anorexia and motor behavior after chronic treatment in rats: relationship with changes in the number of catecholamine receptor sites in the brain. *Arch. Int. Pharmacodyn. Ther.*, **260**, 36-49.
- Berridge K.C., Pecina S. (1995) Benzodiazepines, appetite, and taste palatability. *Neurosci. Biobehav. Rev.*, **19**, 121-131.
- Berton, F., Vogel, E. & Belzung, C. (1998) Modulation of mice anxiety in response to cat odor as a consequence of predators diet. *Physiol. Behav.*, **65**, 247-254.
- Bilkei-Gorzo, A., Gyertyan, I. & Levay, G. (1998) mCPP-induced anxiety in the light-dark box in rats – a new method for screening anxiolytic activity. *Psychopharmacology*, **136**, 291-298.
- Blanchard, R.J. & Blanchard, D.C. (1989) Antipredator defensive behaviors in a visible burrow system. *J. Comp. Psychol.*, **103**, 70-82.

- Blanchard, D.C., Blanchard, R.J., Tom, P. & Rodgers, R.J. (1990a) Diazepam changes risk assessment in an anxiety/defense test battery. *Psychopharmacology*, **101**, 511-518.
- Blanchard, R.J., Blanchard, D.C., Weiss, S.M. & Meyer, S. (1990b) The effects of ethanol and diazepam on reactions to predatory odors. *Pharmacol. Biochem. Behav.*, **35**, 775-780.
- Blanchard, R.J., Yudko, E.B., Rodgers, R.J. & Blanchard, D.C. (1993) Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behav. Brain Res.*, **58**, 155-165.
- Blanchard, R.J., Hebert, M.A., Ferrari, P., Palanza, P., Figueira, R., Blanchard, D.C. & Parmigiani, S. (1998) Defensive behaviors in wild and laboratory (Swiss) mice: the mouse defense test battery. *Physiol. Behav.*, **65**, 201-209.
- Blanchard, D.C., Griebel, G. & Blanchard, R.J. (2001a) Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. *Neurosci. Biobehav. Rev.*, **25**, 205-218.
- Blanchard, R.J., McKittrick, C.R. & Blanchard, D.C. (2001b) Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol. Behav.*, **73**, 261-271.

- Bodnoff, S.R., Suranyi-Cadotte, B., Aitken, D.H., Quirion, R. & Meaney, M.J. (1988) The effects of chronic antidepressant treatment in an animal model of anxiety. *Psychopharmacology*, **95**, 298-302.
- Bodnoff, S.R., Suranyi-Cadotte, B., Quirion, R. & Meaney, M.J. (1989a) A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacology*, **97**, 277-279.
- Bodnoff, S.R., Suranyi-Cadotte, B., Quirion, R. & Meaney, M.J. (1989b) Role of the central benzodiazepine receptor system in behavioral habituation to novelty. *Behav. Neurosci.*, **103**, 209-212.
- Britton, D.R. & Thatcher-Britton, K. (1981) A sensitive open field measure of anxiolytic drug activity. *Pharmacol. Biochem., Behav.*, **15**, 577-582.
- Brocco, M.J., Koek, W., Degryse, A.D. & Colpaert, F.C. (1990) Comparative studies on the anti-punishment effects of chlordiazepoxide, buspirone and ritanserin in the pigeon, Geller-Seifter and Vogel conflict procedures. *Behav. Pharmacol.*, **1**, 403-418.
- Bruijnzeel, A.W., Stam, R. & Wiegant, V.M. (2001): Effect of a benzodiazepine receptor agonist and corticotropin-releasing hormone receptor antagonists on long-term foot-shock-induced increase in defensive withdrawal behavior. *Psychopharmacology*, **158**, 132-139.

- Bryant, R.A. & Panasetis, P. (2001) Panic syndromes during trauma and acute stress disorder. *Behav. Res. Ther.*, **39**, 961-966.
- Cabib, S., Kempf, E., Schleef, C., Mele, A. & Puglisi-Allegra, S. (1988) Different effects of acute and chronic stress on two dopamine-mediated behaviors in the mouse. *Physiol. Behav.*, **43**, 223-227.
- Calvo-Torrent, A., Brain, P.F. & Martinez, M. (1999) Effect of predatory stress on sucrose intake and behavior on the plus-maze in male mice. *Physiol. Behav.*, **67**, 189-196.
- Carlson, S. & Willott, J.F. (1998) Caudal pontine reticular formation of C57BL/6J mice: response to startle stimuli, inhibition by tones, and plasticity. *J. Neurophysiol.*, **79**, 2602-2614.
- Carrive, P. (2000) Conditioned fear to environmental context: cardiovascular and behavioral components in the rat. *Brain Res.*, **858**, 440-445.
- Chantarujikapong, S.I., Scherrer, J.F., Xian, H., Eisen, S.A., Lyons, M.J., Goldberg, J., Tsuang, M. & True, W.R. (2001) A twin study of generalized anxiety disorder symptoms, panic disorder symptoms and post-traumatic stress disorder in men. *Psychiatry Res.*, **103**, 133-145.

- Chen, T.Y., Duh, S.L., Huang, C.C., Lin, T.B. & Kuo, D.Y. (2001) Evidence for the involvement of dopamine D(1) and D(2) receptors in mediating the decrease of food intake during repeated treatment with amphetamine. *J. Biomed. Sci.*, **8**, 462-466.
- Cheeta, S. Kenny, P.J. & File, S.E. (2000) Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.*, **24**, 1053-1067.
- Christmas, A.J. & Maxwell, D.R. (1970) A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behavior in mice and rats. *Neuropharmacology*, **9**, 17-29.
- Cole, J.C. & Rodgers, R.J. (1994) Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacology*, **114**, 288-296.
- Cole, J.C. & Rodgers, R.J. (1995) Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behavior of mice in the elevated plus-maze. *Pharmacol. Biochem. Behav.*, **52**, 473-478.

- Cooper, S.J., Barber, D.J., Gilbert, D.B. & Moores, W.R. (1985) Benzodiazepine receptor ligands and the consumption of a highly palatable diet in non-deprived male rats. *Psychopharmacol.*, **86**, 348-355.
- Cooper, S.J. & Moores, W.R. (1985a) Chlordiazepoxide-induced hyperphagia in non-food-deprived rats: effects of RO15-1788, CGS 8216 and ZK 93 426. *Eur. J. Pharmacol.*, **112**, 39-45.
- Cooper, S.J. & Moores, W.R. (1985b) Benzodiazepine-induced hyperphagia in the nondeprived rat: comparisons with CL 218,872, zopiclone, tracazolate and Phenobarbital. *Pharmacol. Biochem. Behav.*, **23**, 169-172.
- Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J. & Tomkins, D.M. (1989) Exploration of mice in a black and white test box: Validation as a model of anxiety. *Pharmacol. Biochem. Behav.*, **32**, 777-785.
- Crawley, J. & Goodwin, F.K. (1980) Preliminary report of a simple animal behavioral model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.*, **13**, 167-170.
- Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains:

- implications and recommendations for molecular studies. *Psychopharmacology*, **132**, 107-124.
- Cunha, J.M. & Masur, J. (1978) Evaluation of psychotropic drugs with a modified open field test. *Pharmacology*, **16**, 259-267.
- Davidson, R.J. (2001) Toward a biology of personality and emotion. *Ann. N.Y. Acad. Sci.*, **935**, 191-207.
- Davis, M., Gendelman, D.S., Tischler, M.D. & Gendelman, P.M. (1982) A primary acoustic startle circuit: lesion and stimulation studies. *J. Neurosci.*, **2**, 791-805.
- Davis, M. (1989) Sensitization of the acoustic startle reflex by footshock. *Behav. Neurosci.*, **103**, 495-503.
- Davis, M., Falls, W.A., Campeau, S. & Kim, M. (1993) Fear-potentiated startle: a neural and pharmacological analysis. *Behav. Brain. Res.*, **58**, 175-198.
- Davis, M., Walker, D.L. & Lee, Y. (1997) Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex. Possible relevance to PTSD. *Ann. N.Y. Acad. Sci.*, **821**, 305-331.

- Davis M (1998) Anatomic and physiologic substrates of emotion in an animal model. *J. Clin. Neurophysiol.*, **15**, 378-387.
- Davis, M. & Shi, C. (1999) The extended amygdala: Are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? *Ann. N.Y. Acad. Sci.*, **877**, 281-291.
- D'Aquila, P.S., Peana, A.T., Carboni, V. & Serra, G. (2000) Exploratory behavior and grooming after repeated restraint and chronic mild stress: effect of desipramine. *Eur. J. Pharmacol.*, **399**, 43-47.
- Dazzi, L., Serra, M., Spiga, F., Pisu, M.G., Jentsch, J.D. & Biggio, G. (2001) Prevention of the stress-induced increase in frontal cortical dopamine efflux of freely moving rats by long term-treatment with antidepressant drugs. *Eur. Neuropsychopharmacology*, **11**, 343-349.
- De Angelis, L. & File, S.E. (1979) Acute and chronic effects of three benzodiazepines in the social interaction anxiety test in mice. *Psychopharmacology*, **64**, 127-129.
- Diefenbach, G.J., McCarthy-Larzelere, M.E., Williamson, D.A., Mathews, A., Mangun-Mire, G.M. & Bentz, B.G. (2001) Anxiety, depression, and the content of worries. *Depress. Anxiety*, **14**, 247-250.

- Dielenberg, R.A., Carrive, P. & McGregor, I.S. (2001) The cardiovascular and behavioral response to cat odor in rats: unconditioned and conditioned effects. *Brain. Res.*, **897**, 228-237.
- DeFries, J.C. & Hegmann, J.P. (1970): Genetic analysis of open-field behavior. In: Lindzey, G. & Thiessen, D.D., editors. *Contributions to Behavior Genetic Analysis: The Mouse as a Prototype*, New York: Appleton-Century-Crofts, pp 23-56.
- De Kock, M. & Meert, T.F. (1997) Alpha 2-adrenoreceptor agonists and stress-induced analgesia in rats: influence of stressors and methods of analysis. *Pharmacol. Biochem. Behav.*, **58**, 109-117.
- Emerich, D.F., Zanol, M.D., Norman, A.B., McConville, B.J. & Sanberg, P.R. (1991) Nicotine potentiates haloperidol-induced catalepsy and locomotor hypoactivity. *Pharmacol. Biochem. Behav.*, **38**, 875-80.
- Emilien, G. & Maloteaux, J.M. (1998) Current therapeutic uses and potential of beta-adrenoreceptor agonists and antagonists. *Eur. J. Clin. Pharmacol.*, **53**, 389-404.
- Estes, W.K. & Skinner, B.F. (1941) Some quantitative aspects of anxiety. *J. Exp. Psychol.*, **29**, 390-400.

- Feenstra, M.G. & Blotterblom, M.H. (1996) Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. *Brain Res.*, **742**, 17-24.
- Feenstra, M.G., Blotterblom, M.H. & Van Uum, J.F. (1998) Local activation of metabotropic glutamate receptors inhibits the handling-induced increased release of dopamine in the nucleus accumbens but not that of dopamine or noradrenaline in the prefrontal cortex: comparison with inhibition of ionotropic receptors. *J. Neurochem.*, **70**, 1104-1113.
- File, S.E. & Hyde, J.R. (1978) Can social interaction be used to measure anxiety? *Br. J. Pharmacol.*, **62**, 19-24.
- File, S.E. & Hyde, J.R. (1979) A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilizers and of stimulants. *Pharmacol. Biochem. Behav.*, **11**, 65-9.
- File, S.E., Lister, R.G. & Nutt, D.J. (1982) The anxiogenic action of benzodiazepine antagonists. *Neuropharmacology*, **21**, 1033-1037.
- File, S.E. (1985) What can be learned from the effects of benzodiazepines on exploratory behavior? *Neurosci. Biobehav. Rev.*, **9**: 45-54.

- File, S.E., Mabbutt, P.S. & Hitchcott, P.K. (1990) Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus maze. *Psychopharmacology*, **102**, 98-101.
- File, S.E., Zangrossi, H. Jr. & Andrews, N. (1993a) Novel environment and cat odor change GABA and 5-HT release and reuptake in the rat. *Pharmacol. Biochem. Behav.*, **45**, 931-934.
- File, S.E., Zangrossi, H. Jr., Viana, M. & Graeff, F.G. (1993b) Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology*, **111**, 491-494.
- File, S.E. (2001) Factors controlling measures of anxiety and responses to novelty in the mouse. *Behav. Brain Res.*, **125**, 151-157.
- Fernandes, C., File, S.E. (1996) The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacol. Biochem. Behav.*, **54**, 31-40.
- Fletcher, P.J. & Davies, M. (1990) Effects of 8-OH-DPAT, buspirone and ICS 205-930 on feeding in a novel environment: comparisons with chlordiazepoxide and FG 7142. *Psychopharmacology*, **102**, 301-308.

- Furukawa, T., Streiner, D.L. & Young, L.T. 2000. Antidepressant plus benzodiazepine for major depression (Cochrane Review). *Cochrane Database System Review*, **4**, CD001026.
- Geller, I. & Seifter, J. (1960) The effects of meprobamate, barbituates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia*, **1**, 482-492.
- Geller, I., Kulak J.T. & Seifter, J. (1962) The effects of chlordiazepoxide and chlorpromazine on punishment discrimination. *Psychopharmacologia*, **3**, 374-385.
- Geller, I. (1964) Relative potencies of benzodiazepines as measured by their effects on conflict behavior. *Arch. Int. Pharmacodyn. Ther.*, **149**: 243-247.
- Gewirtz, J.C., McNish, K.A. & Davis, M. (1998) Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **22**, 625-648.
- Gilbert, D.B. & Cooper, S.J. (1985) Analysis of dopamine D1 and D2 receptor involvement in *d*- and *l*-amphetamine-induced anorexia in rats. *Brain Res. Bull.*, **15**, 385-389.

- Gorman, A.L. & Dunn, A.J. (1993) Beta-adrenergic receptors are involved in stress-related behavioral changes. *Pharmacol. Biochem. Behav.*, **45**, 1-7.
- Graeff, F.G., Netto, C.F. & Zangrossi, H. Jr. (1998) The elevated T-maze as an experimental model of anxiety. *Neurosci. Biobehav. Rev.*, **23**, 237-246.
- Griebel, G., Belzung, C., Misslin, R. & Vogel, E. (1993) The free-exploratory paradigm : an effective method for measuring neophobic behavior in mice and testing potential neophobia-reducing drugs. *Behav. Pharmacol.*, **4**, 637-644.
- Griebel, G., Blanchard, D.C., Agnes, R.S. & Blanchard, R.J. (1995a) Differential modulation of antipredator defensive behavior in Swiss-Webster mice following acute or chronic administration of imipramine and fluoxetine. *Psychopharmacology*, **120**, 57-66.
- Griebel, G., Blanchard, D.C., Jung, A. & Blanchard, R.J. (1995b) A model of 'antipredator' defense in Swiss-Webster mice: effects of benzodiazepine receptor ligands with different intrinsic activities. *Behav. Pharmacol.*, **6**, 732-745.
- Griebel, G., Sanger, D.J. & Perrault, G. (1996) Further evidence for differences between non-selective and BZ-1 (omega 1) selective benzodiazepine receptor ligands in murine models of "state" or "trait" anxiety. *Neuropharmacology*, **35**, 1081-1091.

- Griebel, G., Belzung, C., Perrault, G. & Sanger, D.J. (2000) Differences in anxiety-related behaviors and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology*, **148**, 164-170.
- Griffiths, J., Shanks, N. & Anisman, H. (1992) Strain-specific alterations in consumption of a palatable diet following repeated stressor exposure. *Pharmacol. Biochem. Behav.*, **42**, 219-227.
- Grillon, C., Ameli, R., Goddard, A. (1994) Baseline and fear-potentiated startle in panic disorder patients. *Biol. Psychiatry*, **35**, 431-439.
- Hayley, S., Borowski, T., Merali, Z. & Anisman, H. (2001) Central monoamine activity in genetically distinct strains of mice following a psychogenic stressor: effects of predator exposure. *Brain Res.*, **892**, 293-300.
- Hegmann, J.P. & Defries, J.C. (1970) Are genetic correlations and environmental correlations correlated? *Nature*, **226**, 284-286.
- Hendrie, C.A. & Neill, J.C. (1991) Exposure to the calls of predators of mice activates defensive mechanisms and inhibits consummatory behavior in an inbred mouse strain. *Neurosci. Biobehav. Rev.* **15**, 479-482.

- Herman, J.P. & Cullinan, W.E. (1997) Neurocircuitry of stress: Central control of hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci.*, **20**, 78-84.
- Herve, D., Tassin, J.P., Barthelemy, C., Blanc, G., Lavielle, S. & Glowinski, J. (1979): Difference in the reactivity of the mesocortical dopaminergic neurons to stress in the BALB/C and C57 BL/6 mice. *Life Sci.*, **25**, 1659-1664.
- Higgs, S. & Cooper, S.J. (1996a) Effects of the benzodiazepine receptor inverse agonist Ro 15-4513 on the ingestion of sucrose and sodium saccharin solutions: a microstructural analysis of licking behavior. *Behav. Neurosci.*, **110**, 559-566.
- Higgs, S. & Cooper, S.J. (1996b) Hyperphagia induced by direct administration of midazolam into the parabrachial nucleus of the rat. *Eur. J. Pharmacol.*, **313**, 1-9.
- Hitchcock, J.M. & Davis, M. (1986) Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behav. Neurosci.*, **100**, 11-22.
- Hitchcock, J.M. & Davis, M. (1991) Efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behav. Neurosci.*, **105**, 826-842.

- Hogg, S. (1996) A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol. Biochem. Behav.*, **54**, 21-30.
- Howard, J.L., Rohrbach, K.W. & Pollard, G.T. (1982) Cumulative dose-effect curves in a conflict test with incremental shock. *Psychopharmacology*, **78**, 195-6.
- Hsu, D.T., Chen, F.L., Takahashi, L.K. & Kalin, N.H. (1998) Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: an in situ hybridization analysis. *Brain Res.*, **788**, 305-310.
- Hwang, B.H. & Guntz, J.M. (1997) Downregulation of corticotropin-releasing factor mRNA, but not vasopressin mRNA, in the paraventricular hypothalamic nucleus of rats following nutritional stress. *Brain Res. Bull.*, **43**, 509-514.
- Ihalainen, J.A., Riekkinen, P. Jr. & Feenstra, M.G.P. (1999) Comparison of dopamine and noradrenaline release in mouse prefrontal cortex, striatum and hippocampus using microdialysis. *Neurosci. Lett.*, **277**, 71-74.
- Jaiswal, A.K., Upadhyay, S.N., Satyan, K.S. & Bhattacharya, S.K. (1996) Behavioral effects of prenatal and postnatal undernutrition in rats. *Indian J. Exp. Biol.*, **34**, 1216-1219.

- Johansson, G.G. & Peder, M.W. (1984) Mouse-killing in Srague-Dawley rats: effects of lighting conditions. *Physiol. Behav.*, **32**, 153-154.
- Jones, B.C., Hou, X. & Cook, M.N. (1996) Effect of exposure to novelty on brain monoamines in C57/6 and DBA/2 mice. *Physiol. Behav.*, **59**, 361-367.
- Kalin, N.H., Takahashi, L.K. & Chen, F.L. (1994) Restraint stress increases corticotropin-releasing hormone mRNA content in the amygdala and parventricular nucleus. *Brain Res.*, **656**, 182-186.
- Kemble, E.D., Flannelly, K.J., Sally, H. & Blanchard, R.J. (1985) Mouse-killing, insect predation, and conspecific attack by rats with differing prior aggressive experience. *Physiol. Behav.*, **34**, 645-648.
- Khan S, Michaud DS, Anisman H, Merali Z (1999): Diazepam Reverses Novel Environment-Induced Feeding Suppression Without Blocking Amygdaloid CRH Release. *Soc. Neurosci. Abst.*, **25**, 1126.
- Kim, M., Campeau, S., Falls, W.A. & Davis, M. (1993) Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. *Behav. Neural Biol.*, **59**, 5-8.

- Kiss, A., Jezova, D. & Aguilera, G. (1994) Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Res.*, **663**, 84-92.
- Koch, M., Lingenhohl, K. & Pilz, P.K. (1992) Loss of the acoustic startle response following neurotoxic lesions of the caudal pontine reticular formation: possible role of giant neurons. *Neuroscience*, **49**, 617-625.
- Koch, M. (1999) The neurobiology of startle. *Prog. Neurobiol.*, **59**, 107-128.
- Krysiak, R., Obuchowicz, E. & Herman, Z.S. (2000) Conditioned fear-induced changes in neuropeptide Y-like immunoreactivity in rats: the effect of diazepam and buspirone. *Neuropeptides*, **34**, 148-157.
- Kshama, D., Hrishikeshavan, H.J., Shanbhogue, R. & Munonyedi, U.S. (1990) Modulation of baseline behavior in rats by putative serotonergic agents in the ethoexperimental paradigms. *Behav. Neural Biol.*, **54**, 234-253.
- Lane, J.D. (1992) Neurochemical changes associated with the action of acute administration of diazepam in reversing the behavioral paradigm conditioned emotional response (CER). *Neurochem. Res.*, **17**, 497-507.

- Lang P.J., Davis, M. & Ohman, A. (2000) Fear and anxiety: animal models and human cognitive psychophysiology. *J. Affect. Disord.*, **61**: 137-59.
- Lee, Y. & Davis, M. (1997) Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone in the acoustic startle reflex. *J. Neurosci.*, **17**, 6434-6446.
- Lister, R.G. (1987) The use of the plus maze to measure anxiety in the mouse. *Psychopharmacology*, **92**, 180-185.
- Lister, R.G. (1990) Ethologically-based animal models of anxiety disorders. *Pharmacol. Ther.*, **46**, 321-340.
- Logue, S.F., Owen, E.H., Rasmussen, D.L. & Wehner, J.M. (1997) Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: implications of genetic background for single gene and quantitative trait loci analyses. *Neuroscience*, **80**, 1075-1086.
- Lucki, I. (1996) Serotonin receptor specificity in anxiety disorders. *J. Clin. Psychiatry.*, **57**, 5-10.

- Martin, J.R., Moreau, J.L., Jenck, F. & Cumin, R. (1993) Acute and chronic administration of buspirone fails to yield anxiolytic-like effects in a mouse operant punishment paradigm. *Pharmacol. Biochem. Behav.*, **46**, 905-910.
- McGregor, I.S., Schrama, L., Ambermoon, P. & Dielenberg, R.A. (2002) Not all 'predator odors' are equal: cat odor but not 2,4,5 trimethylthiazoline (TMT; fox odor) elicits specific defensive behaviors in rats. *Behav. Brain Res.*, **129**, 1-16.
- McNish, K.A., Gewirtz, J.C. & Davis, M. (2000) Disruption of contextual freezing, but not contextual blocking of fear-potentiated startle, after lesions of the dorsal hippocampus. *Behav. Neurosci.*, **114**, 64-76.
- Meert, T.F., Melis, W., Aerts, N. & Clincke, G. (1997) Antagonism of meta-chlorophenylpiperazine-induced inhibition of exploratory activity in an emergence procedure, the open field test, in rats. *Behav. Pharmacol.*, **8**, 353-363.
- Melartin, T.K., Rytsala, H.J., Leskela, U.S., Lestela-Mielonen, P.S., Sokero, T.P. & Isometsa, E.T. (2002) Current comorbidity of psychiatric disorders among DSM-IV major depressive disorder patients in psychiatric care in the Vantaa Depression Study. *J. Clin. Psychiatry*, **63**, 126-34.

- Merali, Z., Lacosta, S. & Anisman, H. (1997) Effects of interleukin-1 beta and mild stress on alterations of norepinephrine, dopamine and serotonin neurotransmission : a regional microdialysis study. *Brain Res.*, **761**, 225-235.
- Merali, Z., Khan, S., Michaud, D.S., Shippy, S. & Anisman, H. (2002) Release of corticotropin-releasing hormone or glutamate at the amygdala is not necessary for the expression of anxiety-like behaviors evoked by an unfamiliar environment. *J. Neuroscience*, Submitted.
- Merlo Pich, E. & Samanin, R. (1989) A two-compartment exploratory model to study anxiolytic/anxiogenic effects of drugs in the rat. *Pharmacol. Res.*, **21**, 595-602.
- Methot, C. & Deutsch, R. (1984) The effects of diazepam on a conditioned emotional response in the rat. *Pharmacol. Biochem. Behav.*, **20**, 495-499.
- Minor, T.R., Dess, N.K., Ben-David, E. & Chang, W.C. (1994) Individual differences in vulnerability to inescapable shock in rats. *J. Exp. Psychol. Anim. Behav. Process*, **29**, 402-412.
- Morrow, B.A., Redmond, A.J., Roth, R.H. & Elsworth, J.D. (2000a) The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. *Brain Res.*, **864**, 146-151.

- Morrow, B.A., Roth, R.H. & Elsworth, J.D. (2000b) TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. *Brain Res. Bull.*, **52**, 519-523.
- Muller-Gass, A., Porath, A., Anisman, H. & Merali, Z. (2000) Novelty-induced delay in snack approach: A robust and rapid naturalistic model for anxiety assessment. *Soc. Neurosci. Abst.*, **26**, 1773.
- Nakamura, K. & Kurasawa, M. (2001) Anxiolytic effects of aniracetam in three different mouse models of anxiety and the underlying mechanism. *Eur. J. Pharmacol.*, **420**, 33-43.
- Otto, M.W., Pollack, M.H., Maki, K.M., Gould, R.A., Worthington, J.J. 3rd, Smoller, J.W. & Rosenbaum, J.F. (2001) Childhood history of anxiety disorders among adults with social phobia: rates, correlates, and comparisons with patients with panic disorder. *Depress. Anxiety*, **14**, 209-13.
- Padovan, C.M. & Guimaraes, F.S. (2000) Restraint-induced hypoactivity in an elevated plus-maze. *Braz. J Med. Res.*, **33**, 79-83.
- Paré, W.P., Blair, G.R., Kluczynski, J. & Tejani-Butt, S. (1999) Gender differences in acute and chronic stress in Wistar Kyoto (WKY) rats. *Integr. Physiol. Behav. Sci.*, **34**, 227-41.

- Paré, W.P., Tejani-Butt, S. & Kluczynsky, J. (2001) The emergence test: effects of psychotropic drugs on neophobic disposition in Wistar Kyoto (WKY) and Sprague Dawley rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **25**, 1615-1628.
- Pecina, S. & Berridge, K.C. (1996) Brainstem mediates diazepam enhancement of palatability and feeding: microinjections into fourth ventricle versus lateral ventricle. *Brain Research*, **727**, 22-30.
- Peeler, D.F. & Nowakowski, R.S. (1987) Genetic factors and the measurement of exploratory activity. *Behav. Neural Biol.*, **48**, 90-103.
- Pellow, S., Chopin, P., File, S.E. & Briley, M. (1985) Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods*, **14**, 149-167.
- Rex, A., Stephens, D.N. & Fink, H. (1996) "Anxiolytic" action of diazepam and abecarnil in a modified open field test. *Pharmacol. Biochem. Behav.*, **53**, 1005-1011.
- Rex, A., Voight, J.P., Voits, M. & Fink, H. (1998) Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. *Pharmacol. Biochem. Behav.* **59**, 677-683.
-

- Rodgers, R.J., Cao, B.J., Dalvi, A. & Holmes, A. (1997) Animal models of anxiety: an ethological perspective. *Braz. J. Med. Biol. Res.*, **30**, 289-304.
- Rodrigues-Filho, R. & Takahashi, R.N. (1999) Antinociceptive effects induced by desipramine and fluoxetine are dissociated from their antidepressant or anxiolytic action in mice. *Int. J. Neuropsychopharmacol.*, **2**, 263-269.
- Roy, V., Belzung, C., Delarue, C. & Chapillon, P. (2001) Environmental enrichment in BALB/c mice: effects in classical tests of anxiety and exposure to a predatory odor. *Physiol. Behav.*, **74**, 313-320.
- Sakamoto, H., Matsumoto, K., Ohno, Y. & Nakamura, M. (1998) Anxiolytic-like effects of perospirone, a novel serotonin-2 and dopamine-2 antagonist (SDA)-type antipsychotic agent. *Pharmacol. Biochem. Behav.*, **60**, 873-878.
- Sanchez, C. (1997) Acute stress enhances anxiolytic-like drug responses of mice tested in a black and white test box. *Eur. Neuropsychopharmacol.*, **7**, 283-288.
- Sanger, D.J. (1990) Effects of buspirone and related compounds on suppressed operant responding in rats. *J. Pharmacol. Exp. Ther.*, **254**, 420-426.

- Schmitt, U. & Hiemke, C. (1998) Combination of open field and elevated plus-maze: a suitable test battery to assess strain as well as treatment differences in rat behavior. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **22**, 1197-215.
- Shanks, N., Griffiths, J., Zalcman, S., Zacharko, R.M. & Anisman, H. (1990) Mouse strain differences in plasma corticosterone following uncontrollable footshock. *Pharmacol. Biochem. Behav.*, **36**, 515-519.
- Shanks, N., Zalcman, S., Zacharko, R.M. & Anisman, H. (1991) Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. *Pharmacol. Biochem. Behav.*, **38**, 69-75.
- Shanks, N., Griffiths, J. & Anisman, H. (1994a) Central catecholamine alterations induced by stressor exposure: analyses in recombinant inbred strains of mice. *Behav. Brain Res.*, **63**, 25-33.
- Shanks, N., Griffiths, J. & Anisman, H. (1994b) Norepinephrine and serotonin alterations following chronic stressor exposure: mouse strain differences. *Pharmacol. Biochem. Behav.*, **49**, 57-65.
- Shekhar, A., McCann, U.D., Meaney, M.J., Blanchard, D.C., Davis, M., Frey, K.A., Liberzon, I., Overall, K.L., Shear, M.K., Tecott, L.H. & Winsky, L. (2001) Summary

of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. *Psychopharmacology*, **157**, 327-339.

Shephard, R.A. & Broadhurst, P.L. (1982) Hyponeophagia and arousal in rats: effect of diazepam, 5-methoxy-N, N-dimethyltryptamine, d-amphetamine and food deprivation. *Psychopharmacology*, **78**, 368-378.

Shephard, R.A. & Estall, L.B. (1984) Anxiolytic actions of chlordiazepoxide determine its effects on hyponeophagia in rats. *Psychopharmacology*, **82**: 343-347.

Silva, M.T. & Calil, H.M. (1975) Screening hallucinogenic drugs: systemic study of three behavioral tests. *Psychopharmacology*, **42**, 163-171.

Soubrié, P. Kulkarni, S. Simon, P. & Boissier, J.R. (1975) Effects des anxiolytiques sur la prise de nourriture de rats et de souris places en situation nouvelle ou familière. *Psychopharmacologia*, **45**, 203-210.

Stanhope, K.J. & Dourish, C.T. (1996) Effects of 5-HT_{1A} receptor agonists, partial agonists and a silent antagonist on the performance of the conditioned emotional response test in the rat. *Psychopharmacology*, **128**, 293-303.

- Stone, E.A., Najima, M. & Quartermain, D. (1995) Potentiation by propranolol of stress-induced changes in passive avoidance and open-field emergence tests in mice. *Pharmacol. Biochem. Behav.*, **51**, 297-300.
- Stout, J.C. & Weiss, J.M. (1994) An animal model for measuring behavioral responses to anxiogenic and anxiolytic manipulations. *Pharmacol. Biochem. Behav.*, **47**, 459-465.
- Stratford, T.R. & Kelley, A.E. (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. *J. Neurosci.*, **17**, 4434-4440.
- Sutoo, D., Akiyama, K. & Takita, H. (1991) Behavioral changes in cold-stressed mice related to a central calcium-dependent-catecholamine synthesizing system. *Pharmacol. Biochem. Behav.* **40**, 423-428.
- Tsuji, M., Takeda, H. & Matsumiya, T. (2000) Different effects of 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics on the emotional state of naïve and stressed mice: a study using the hole-board test. *Psychopharmacology*, **152**, 157-166.
- Turner, P. (1991) Clinical psychopharmacology of beta-adrenoreceptor antagonism in the treatment of anxiety. *Ann. Acad. Med. Singapore*, **20**, 43-45.

- van den Berg, C.L., Lamberts, R.R., Wolterink, G., Wiegant, V.M. & Van Ree, J.M. (1998) Emotional and footshock stimuli induce differential long-lasting behavioral effects in rats: involvement of opioids. *Brain Res.*, **799**, 6-15.
- van den Buuse, M., Van Acker, S.A., Fluttert, M. & De Kloet, E.R. (2001) Blood pressure, heart rate, and behavioral responses to psychological “novelty” stress in freely moving rats. *Psychopharmacology*, **38**, 490-499.
- van Dijken, H.H., Van der Heyden, J.A., Mos, J. & Tilders, F.J. (1992a) Inescapable footshocks induce progressive and long-lasting behavioral changes in male rats. *Physiol. Behav.*, **51**, 787-794.
- van Dijken, H.H., Mos, J., Van der Heyden, J.A. & Tilders, F.J. (1992b) Characterization of stress-induced long-term behavioral changes in rats: evidence in favor of anxiety. *Physiol. Behav.*, **52**, 945-951.
- Wagstaff, A.J., Cheer, S.M., Matheson, A.J., Ormrod, D. & Goa, K.L. (2002) Paroxetine: an update of its use in psychiatric disorders in adults. *Drugs*, **62**, 655-703.
- Walker, D.L. & Davis, M. (1997a) Anxiogenic effects of high illumination levels assessed with the acoustic startle response in rats. *Biol. Psychiat.*, **42**, 461-471.

- Walker, D.L. & Davis, M. (1997b) Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci.*, **17**, 9375-9383.
- Walker, D.L. & Davis, M. (2002): Light-enhanced startle: further pharmacological and behavioral characterization. *Psychopharmacology*, **159**, 304-310.
- Wedzony, K., Mackowiak, M., Fijal, K. & Golembiowska, K. (1996) Evidence that conditioned stress enhances outflow of dopamine in rat prefrontal cortex: a search for the influence of diazepam and 5-HT_{1A} agonists. *Synapse*, **24**, 240-247.
- Wiley, J.L., Compton, A.D. & Porter, J.H. (1993) Effects of four antipsychotics on punished responding in rats. *Pharmacol. Biochem. Behav.*, **45**, 263-267.
- Yamashita, S., Oishi, R. & Gomita, Y. (1995) Anticonflict effects of acute and chronic treatments with buspirone and gepirone in rats. *Pharmacol. Biochem. Behav.*, **50**, 477-479.
- Zaharia, M.D., Kulczycki, J., Shanks, N., Meaney, M.J. & Anisman, H. (1996) The effects of early postnatal stimulation on Morris water-maze acquisition in adult mice: genetic and maternal factors. *Psychopharmacology*, **128**, 227-239.

Zangrossi, H. & File, S.E. (1992) Chlordiazepoxide reduces the generalized anxiety, but not the direct responses, of rats exposed to cat odor. *Pharmacol. Biochem. Behav.*, **43**, 1195-1200.