1
Introduction

Fear and anxiety are complex concepts. Both terms have been used to describe a set of highly orchestrated neural events that involve sensory processing and motor responses triggered by threatening situations. These events are mediated by central neural circuitries and peripheral neuroendocrine pathways and clearly have adaptive value. Sensory systems function as alarm signals to warn of real or potential danger, producing a shift to a state of high vigilance that prepares the individual to avoid or escape from a wide variety of dangerous situations. Most of these reactions are not exclusive to our species. Because of their importance for survival, fear and anxiety traits are believed to have been selected in animal evolution and shaped by natural selection for their crucial role in protecting individuals who face adverse environments (Coutinho et al., 2010; Gross & Hen, 2004; Marks & Nesse, 1994).

However, these highly adaptive events can be disabling when the individual experiences them excessively or when they occur in the absence of any threatening stimuli. In these cases, they represent a pathological condition termed an anxiety disorder. Often chronic in nature, these disorders are among the most prevalent mental health problems across the individual life span, producing severe impairments in social and occupational functioning.

According to an evolutionary perspective, an anxiety disorder reflects a malfunctioning of the neural circuits responsible for detecting, organizing, or expressing adaptive defense reactions (Jacobson & Cryan, 2010). Humans and nonhuman mammals share approximately the same behavioral defense strategies, reflected by activation of similar underlying neural circuitry. Therefore, animal models of anxiety can be extremely helpful for better understanding the behavioral, neural, and genetic substrates involved in these pathologies. The purpose of this thesis is to present two new rat lines that might be a useful model of generalized anxiety disorder (GAD). Before we discuss this model, defining how anxiety disorders are currently classified is important.

1.1
Clinical Aspects of Anxiety Disorders

The concept of anxiety disorders has changed dramatically over the years as more clinical and experimental evidence has been collected. In the clinical setting, anxiety disorders have departed from a single construct that ranged in intensity from normal to pathological or neurotic levels. A major shift in this view occurred with Klein's pioneering work (Klein, 1964; Klein & Fink, 1962), which showed that imipramine had a selective effect in the treatment of panic disorder. Moreover, certain anxiety disorders have been suggested to differ from each other in the primary object or specificity of threat. Fear of a circumscribed and well-defined object is a characteristic of specific phobias, whereas diffuse and chronic sustained anxiety is the main feature of GAD.

The 3rd edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III; American Psychiatric Association, 1980) introduced the current descriptive symptom-based approach to mental disorders with well-defined, explicit diagnostic criteria. This new classification incorporated distinct nosological entities, such as panic disorder, specific and social phobias, GAD, posttraumatic stress disorder, and obsessive-compulsive disorder. In the DSM-III, GAD was left as a residual diagnosis of worry, to be made only in the absence of other anxiety and depressive syndromes. Consequently, this residual category carried low diagnostic reliability.

With the publication of the DSM-IV (American Psychiatric Association, 1994) and International Classification of Diseases and Related Health Problems (ICD-10; World Health Organization, 1992), these anxiety disorder categories remained basically the same. However, the diagnosis of GAD shifted from a residual category in the DSM-III to an independent anxiety disorder type in the DSM-IV. Free-floating anxiety was associated with the worry construct, which in turn produced several symptoms, such as muscle tension, fatigue, restlessness, concentration difficulties, and irritability. According to the DSM-IV, excessive and unrelenting worry is generally associated with impairments in academic, social, and personal functioning and related to multiple domains or activities. To

be considered a pathological feature of GAD, worry must occur more days than not for a period of at least 6 months.

1.2
Animal Models of Anxiety

In the experimental setting, most of the studies that investigate the etiological mechanisms that underlie anxiety disorders have been performed using animal models. Defensive reactions of the laboratory rat (*Rattus norvegicus*) have been employed as the main system for modeling human anxiety. In his classic work, Calvin Hall (1934) employed the word "emotionality" to describe a set of defensive reactions that an animal presents in a potentially dangerous situation, such as an open field. Since then, several other animal models of anxiety were developed. An important issue regarding these models is the fact that most of them depend on the animal's locomotor activity. This is why a pure measure of emotionality, devoid of non-emotional confounding factors, such as locomotor activity, might be a difficult task to achieve (Ramos, 2008). Therefore, using different animal models of anxiety might help to exclude non-emotionality factors that might interact with an anxiety-related response. Moreover, examining whether a given result in one model can be generalized to other models, and thus estimating the extent to which the expression of different defensive responses might be mediated by a single emotional trait, is also possible.

As in the clinical setting, the traditional view that highlighted these experimental studies was that animal defensive responses were mediated by a single and general anxiety construct (Broadhurst, 1975; Gray, 1979; Hall, 1934). Nevertheless, as new data were collected, it became clear that animal defensive behavior is mediated by a complex and multidimensional construct (Aguilar et al., 2002; Belzung & Le Pape, 1994; Ramos et al., 1997; Torrejais et al., 2008). These diverse dimensions found in animal models of anxiety may indicate that clinically defined anxiety disorders could be associated with a particular animal model. However, the adoption of descriptive and operational criteria from the modern classification systems imposed a validity problem among the several anxiety disorder categories. The DSM-IV and ICD-10 are not primarily based upon

etiology, neurobiology, epidemiology, genetics, or responses to medications, but rather on phenomenological descriptions of clinical data that have imprecise similarity or correlate with each other within and between individuals (Gould & Gottesman, 2006). Therefore, unsurprising are the several problems that are encountered when attempting to use the current systems of mental disorder classification as a guide for developing viable animal models.

These models basically consist of exposing an animal to an innate or learned aversive environment while assessing one or a set of defensive behaviors. Typically, each animal model has been validated with pharmacological agents with well-know anxiolytic or anxiogenic properties in clinical settings. Below we summarize 11 models classified according to the innate or learned characteristics regarding a threatening situation.

1.3
Innate Aversive Paradigms

1.3.1
Open field test

The open field is one of the most popular animal models of anxiety, probably because of the simplicity of the apparatus and the easily identifiable and well-defined defensive reactions observed in animals in this situation. The apparatus consists of a large square or circular arena surrounded by walls so that the animal cannot escape. The floor of the arena is marked with squares or concentric lines to quantify the animal's locomotion. This test was first employed by Hall (1934), who used defecation in the open field as a measure of timidity or emotionality because of its relationship with the autonomous nervous system. General locomotor activity, especially locomotion in the central illuminated area of the arena (which is aversive to the animal), became another index of emotionality. In addition to ambulation, other behavioral measures include grooming, freezing, and rearing on the walls or in the space. The main definition of emotional reactivity in this model is the association between low ambulation

and a high rate of defecation. The effect of anxiolytic and anxiogenic drugs in the open field has been widely demonstrated (e.g., Prut and Belzung, 2003).

1.3.2
Elevated plus-maze

Inspired by an earlier elevated Y-maze (Montgomery, 1955), the elevated plus maze was first introduced in 1984 (Handley and Mithani, 1984) and subsequently validated for use with rats (Pellow et al., 1985). The elevated plus maze is based on the natural fear of rodents for open and elevated alleys. The apparatus consists of four elevated arms that are arranged in a "cross-like" pattern, with two opposite open arms with a minimal lip and two closed arms with high walls. At their intersection, a central platform allows access to all four arms. This region is also called the "decision making" area. The rat is placed on the central platform, and total exploration or locomotion is measured as the total number of entries in the open and closed arms for 5 min. The percentage of open arm entries and percentage of time spent in the open arms are used as anxiety indices, whereas the number of closed arm entries is used as an index of locomotor activity. In fact, rats confined in the open arms show more physiological and behavioral signs of fear, such as higher defecation rates and decreased locomotion, than when confined in the closed arms. Moreover, factor analysis studies indicated that this paradigm reliably dissociates the anxiety-like (open arm entries) from locomotor (close arm entries) effects of several anxiolytic and anxiogenic agents (Cruz et al., 1994). Because of its ability to dissociate the emotional effects from motor effects of drugs, the elevated plus maze is one of the most widely used animal models for screening anxiolytic drugs (Pellow et al., 1985).

1.3.3
Light-Dark box test

The light-dark box test, also known as the light-dark transition test, was first described by Crawley and Goodwin (1980) to investigate the anxiolytic properties of drugs in mice. The model is based on the innate aversion that rodents have to

brightly illuminated areas. The apparatus consists of equally sized compartments connected by a small door. One compartment is brightly illuminated, and the other is dark. Usually, the animal is placed in the dark compartment, and several parameters, such as the distance traveled in each side of the box, total number of transitions between the light and dark compartment, latency to enter the light compartment, and time spent in each compartment, can be measured. The total number of transitions appears to be an index of locomotor activity, whereas the latency to first enter the light compartment or total time spent in the light compartment are considered emotional measures. The anxiolytic effects of benzodiazepines and 5-hydroxytryptamine-1A ($5\text{-}HT_{1A}$) receptor agonist compounds have been detected in this animal model (Bourin and Hascoët, 2003).

1.3.4
Social interaction test

Initially developed by File and Hyde (1978), the social interaction test is based on the fact that the occurrence of pairs of male rats that perform social behaviors depends on the aversiveness (i.e., high light level or novelty of the situation) of the experimental condition. The frequency of and time spent by pairs of males engaging in social interaction can be divided into two categories: aggressive behaviors (e.g., kicking, jumping on, wrestling, and boxing) and non-aggressive behaviors (e.g., sniffing, following, grooming). Because the behavior of one rat influences the behavior of the other, the pair of rats is treated as a unit, and only one score for the pair is used. In a typical protocol, the pair of animals is placed in an arena with the floor divided into squares so that general activity can be measured. All of the animals are individually acclimated to the arena at least 1 day prior to testing. An increase in social interaction without a concomitant increase in motor activity is indicative of an anxiolytic-like effect, whereas a specific decrease in social interaction indicates an anxiogenic-like effect (File and Seth, 2003). The social interaction test has been widely validated with anxiolytic and anxiogenic drugs and is able to distinguish between anxiolytic and sedative effects (File, 1985).

1.3.5
Ultrasonic vocalization

Zippelius and Schleidt (1956) observed that infant mice produced ultrasonic vocalizations (USVs) when separated from their mothers and littermates. These USVs, which can trigger rodent maternal search and retrieval behaviors (Brunelli, 2005), are whistle-like sounds characterized by frequencies ranging between 30 and 90 kHz, with duration of 10-200 ms and intensity of 60-100 dB. Maternal isolation is a stressful event for rodent pups, producing cardiovascular changes, increased autonomic nervous system activity, and activation of the hypothalamic-pituitary-adrenal axis. Ultrasonic vocalizations may also be emitted during other stressful events, such as frustrated non-reward, opiate withdrawal, and cold ambient temperatures.

In this paradigm, when a rat pup between 4 and 16 days of age is separated from its mother and littermates for a brief period of time, it typically emits a so-called 40-kHz vocalization. The test can be performed under two different stress conditions. Pups are placed in isolation in either a warm (37°C) or cold (18°C) environment for 5 min. The total number and duration of ultrasonic calls emitted by the pups during this period is used as an index of anxiety. Anxiolytic compounds reduced the number and cumulative duration of USV (Portfors, 2007). Pharmacological and behavioral studies have also indicated that USV in isolated rat pups might represent an important model of separation anxiety in early development (Ditcher et al., 1996; Insel and Winslow, 1991).

1.4
Learned aversive paradigms

1.4.1
Habituation and sensitization of the acoustic startle response

The acoustic startle response is a reflex characterized by a short-latency sequence of facial and skeletal muscle contractions following an unexpected and intense acoustic stimulus. This is a defensive response because its behavioral

pattern consists of reactions that are likely to prevent a predator attack or other possible injury from the environment. The acoustic startle response is mediated by neural circuitry located in the lower brainstem. Auditory stimuli are processed by cochlear nuclei, which send ascending projections to the caudal pontine nucleus, with descending projections to motor neurons in the spinal cord (Lee et al., 1996).

The amplitude of the acoustic startle response can be measured automatically with special sensors beneath the rat cage and can be modified by various non-associative and associative learning processes. Habituation and sensitization represent two forms of non-associative learning that can bidirectionally modulate the amplitude of this response. Habituation refers to a decrease of the startle reflex magnitude as a function of repeated presentation of the acoustic stimulus (Prosser and Hunter, 1936). Sensitization refers to an increase of the startle reflex amplitude in response to repeated presentation of the acoustic stimulus caused by presentation of an aversive stimulus, such as a footshock (Davis, 1989). An increase of the startle reflex appears to reflect a state of diffuse fear associated with arousal and vigilance produced by the footshock that is presented without any relationship to the acoustic stimuli (Groves and Thompson, 1970). Several reports indicated that limbic structures, such as the amygdaloid complex, play an important role in the sensitizing effects of electric footshocks (Hitchcock et al., 1989). Moreover, this sensitization effect appears to be mediated by γ-aminobutyric acid (GABA) receptors in the basolateral amygdala (Van Nobelen and Kokkinidis, 2006).

1.4.2
Fear-potentiated startle

In addition to sensitization, the amplitude of the acoustic startle response can be enhanced when it is elicited in the presence, rather than absence, of a fear-eliciting conditioned stimulus (CS), such as a light, that was previously associated with an aversive unconditioned stimulus (US), such as a footshock. According to this associative learning paradigm, developed by Brown et al. (1951), a rat is initially exposed to the CS-US pairing. Rats are later tested for fear responses to the CS by eliciting the startle reflex with a series of brief and intense acoustic

stimuli presented in the presence or absence of the CS. An increase in the amplitude of the startle reflex in the presence of the CS has been termed a fear-potentiated startle response. This effect has been replicated using either an auditory or visual CS, when the startle reflex is elicited by either an acoustic or air puff stimulus (Davis, 1986). Several studies indicate that anxiolytic drugs can block fear-potentiated startle in rats (e.g., Davis, 1986, 1993), suggesting that this is an adequate animal model of anxiety.

1.4.3
Avoidance Responses

Avoidance learning involves the acquisition of a response that prevents the occurrence of a future aversive event. There are two forms of avoidance learning: active and passive. In both situations, the animal is required to perform (active avoidance) or suppress (passive avoidance) a response to prevent an aversive event that was scheduled to occur.

1.4.3.1
Active Avoidance

The active avoidance learning procedure has numerous variations. One of the first studies, Mowrer and Lamoreaux (1942, 1946) employed an apparatus known as a shuttle box (Mowrer, 1940; Mowrer and Miller, 1942), which consists of a box divided into two equal compartments by a hurdle, over which the subject can jump to shuttle from one compartment to the other. Variations of the shuttle box replace the hurdle with a doorway between the compartments so the animal can cross from one side of the box to the other. An electric footshock (US) can be delivered to the animal's paws through the grid floor of the box, and a lamp or speaker can present a warning signal (CS).

In the two-way shuttle box avoidance procedure, the animal is placed in one of the compartments. After a predetermined length of time, a CS is presented, and the animal must go to the other compartment before the occurrence of the US. After a short period of time, the CS is presented again, and the subject must return

to the original compartment to avoid the aversive event. If the shuttle response does not occur in the presence of the CS, then the US remains on until an escape response of going to the other compartment occurs. Therefore, in each trial, the onset of the CS precedes the onset of the US. An avoidance response during the CS terminates the CS and cancels the US, whereas an escape response after the onset of the US terminates both the CS and US.

Subjects first learn to escape from the US. As training continues, the escape response begins to occur in the presence of the CS, which turns off the CS and prevents the delivery of the aversive event. Notice that the subject can avoid the footshock by shuttling either way, from the left to right compartment or vice versa. For this reason, the procedure is called two-way avoidance.

The primary measure of learning in this task is an increase in avoidance responses. The acquisition of high rates of this response might require 100 or more trials because of the complex nature of this type of learning. The two-way avoidance procedure involves a conflict situation, given that both compartments have aversive and safety functions. Typically, animals that are less emotionally reactive to this aversive procedure exhibit better learning than animals that are more "afraid" of this situation. Indeed, higher levels of electrical footshock are associated with lower two-way avoidance performance (Levine, 1966; McAllister et al., 1971). Accordingly, anxiolytic drugs enhance, whereas anxiogenic compounds impair, the acquisition of two-way avoidance (Fernández-Teruel et al., 1991; Savić et al., 2005).

The fact that no one side of the shuttle box is always safe can be overcome by testing the animal in a one-way avoidance task. In this paradigm, the animal is always placed in the same compartment where the CS and US occur. In the other compartment, neither the CS nor US appears. An avoidance response is defined when the animal shifts from the danger compartment to the safe compartment in the presence of the CS, whereas an escape response is defined when the animal shuttles from one side to the other in the presence of the US. In the one-way avoidance apparatus, contextual cues associated with the start or dangerous compartment are clearly different from the goal or safe compartment. Although the acquisition of the one-way avoidance response is rapidly observed, this

paradigm involves confounding variables, such as handling stimuli necessary to move the animal from the safe to dangerous compartment between trials.

In the case of one-way avoidance learning, the anxiety index is exactly the opposite from two-way avoidance. The more "afraid" the animal is when learning the situation, the better the acquisition of the one-way avoidance response. For example, one-way avoidance is generally better with higher footshock intensities (Dieter, 1976), in contrast to the acquisition of two-way avoidance (Levine, 1966; McAllister et al., 1971). The effects of anxiolytic drugs on one-way avoidance performance have been inconsistent. Although classic anxiolytic drugs, such as benzodiazepines, did not cause any effect in one-way active avoidance acquisition (Gray and McNaughton, 2000), Sanger et al. (1989) found that 5-HT_{1A} receptor agonists (but not imipramine) impaired the acquisition of one-way avoidance.

1.4.3.2
Passive Avoidance

The passive avoidance response is a rapid learning process that involves single training and test sessions. This paradigm has two versions: step-through and step-down. In step-through passive avoidance, the animal is placed in a bright compartment, and the latency to enter the dark compartment is recorded. After entering this compartment, the animal receives an electric footshock. During the test session, generally 24 h after the training session, the animal is returned to the bright compartment, and the latency to enter the dark compartment, which at this point is not electrified, is measured. In step-down passive avoidance, the animal is placed on a small platform located approximately 4 to 8 cm above the grid. When the animal steps down from the platform with its four paws on the grid, it receives an electric footshock. In the test session, the animal is placed back on the platform, and the step down latency is measured.

Aversive learning is inferred from the delay of the step-through or step-down responses that were made before training. Because delaying a response is an active process, this paradigm has also been termed inhibitory, rather than passive, avoidance. An animal that is more "afraid" has a longer latency (i.e., better inhibitory avoidance). Pharmacological results corroborate this premise.

Benzodiazepines and various 5-HT_{1A} receptor agonist compounds with anxiolytic properties impair passive avoidance performance (Anglade et al., 1994; Misane et al., 1998).

1.4.4
Conditioned emotional response

Initially developed by Estes and Skinner (1941), the conditioned emotional response was one of the first animal models that measured the learned aspects of fear. In a typical experiment, food-deprived rats are initially trained to lever press for food for intermittent reinforcement. After giving a sufficient number of training sessions to establish stable lever pressing, a CS, such as a light or tone, is paired with a US, such as an electric footshock. After a small number of pairings, the animal returns to the appetitive operant condition, and the CS is presented. A variation of this procedure is to measure the disruptive effects of the CS on some consummatory response, such as a thirsty rat licking for water on a water tube. Aversive learning is measured by suppression according to the ratio $a/(a + b)$, in which "*a*" represents the number of responses made during the CS, and "*b*" represents the number of responses made during a period that immediately preceded the onset of the CS and had the same duration as the CS. If the CS did not acquire any associative learning, then suppression does not occur, and the ratio is 0.5. The more "afraid" the animal is of the CS, the lower the ratio. Maximal conditioning to the CS produces total suppression of the response, and the ratio is 0.0. Several reports indicate that anxiolytic drugs alleviate the suppressive effect of the CS (e.g., Davis, 1990).

1.5
Contextual fear conditioning as a model of generalized anxiety disorder

Regardless of the variability of animal models available for the study of current clinically defined anxiety disorders, fear conditioning has been historically associated with one of the main causes of pathological anxiety (i.e., neurosis;

Pavlov, 1927; Watson & Rayner, 1920). In a typical fear conditioning experiment, a discrete and emotionally neutral stimulus, such as a light or tone, reliably signals the occurrence of an aversive stimulus (Unconditioned Stimulus – US), such as an electric footshock. After a few pairings between these two stimuli, the previously harmless stimulus becomes a potent conditioned stimulus (CS) and acquires the ability to elicit several fear reactions, including defensive behaviors (freezing), autonomic (i.e., increases in blood pressure and heart rate) and endocrine (hormone release) responses, as well as modifications in pain sensitivity (analgesia) and reflex expression (eyeblink response and fear potentiated startle).

Another form of fear conditioning is to make the aversive stimulus unpredictable. According to this alternative procedure, a rat is exposed to a novel chamber and, after a few minutes, a brief and unsignaled footshock is delivered. When returned to the same chamber in the absence of the aversive stimulus, the animal presents a permanent fear reaction to contextual cues previously associated with the footshock. Contextual fear conditioning represents one of the simplest and most rapid forms of producing aversive learning (Landeira-Fernandez, 1996).

Considerable evidence from animal and human experiments indicates that fear conditioning in response to a discrete CS or to contextual cues are mediated by different neural circuitries (Indovina et al., 2011; Ferreira et al., 2003; Kim & Fanselow, 1992; LeDoux, 2000; Pohlack et al., 2011). These results support the hypothesis of at least two dimensions of fear conditioning, and each dimension might be related to clinically distinct anxiety disorders. Specific phobias, characterized by cue-specific or phasic fear reactivity, might be modeled by aversive conditioning in response to a discrete CS (Grillon, 2002; Grillon and Davis, 1997). GAD, in contrast, is characterized by persistent and diffuse or non-cue-specific anxiety and might be modeled by contextual fear conditioning (Brandão et al., 2008; Grillon and Davis, 1997).

When the CS-US pairings occur in a certain context, aversive conditioning is simultaneously acquired for both the CS and contextual cues. Conditioning to contextual cues can impose measurement problems with regard to the amount of aversiveness to the discrete CS. Therefore, conditioning to the CS is assessed by placing the animal in a context different from training. To prevent generalization from the training context to the context were the CS was tested, fear extinction of

the training context or pre-exposure to the test context is important to guarantee a low level of freezing in response to the context were the discrete CS was tested (Jacobs et al., 2010).

1.5.1
The neurocircuitry of contextual fear conditioning

The neural circuitry responsible for fear conditioning for both auditory, visual, olfactory or contextual stimuli are mapped and very well understood (Maren, 2001; Romanski et al., 1993; LeDoux, 2003). Although different pathways may participate in processing dangerous stimuli, they all seem to converge in the amygdalae (LeDoux, 2000). In this regard, the amygdaloid nuclei can be roughly divided into two main subsystems: the basolateral complex (BLA) (which in turn, is formed by the lateral (LA), basolateral (BL) and basomedial (BM) nuclei) and the central nucleus (CE). The BLA receives and integrates sensory information from a wide variety of sources. These include the medial and ventral subdivisions of the thalamic medial geniculate nucleus (MGm and MGV, for an auditory stimulus); the perirhinal cortex (PRh, for a visual stimuli); primary auditory cortex (TE) and the insular cortex (INS, for gustatory and somatosensory information); the thalamic posterior intralaminar nucleus (PIN, somatosensory information), the hippocampal formation (spatial and contextual information), which include area CA1, the ventral subiculum (vSUB), the entorhinal cortex (ENT) and the piriform cortex (PIR, for olfactory stimulus). As a result, the BLA is a place of sensory convergence and a possible site for CS-US association within the amygdala. Intra-amygdaloid circuitry sends the CS-US association to the CE, where different projections to the hypothalamus and brainstem mediate fear responses such as potentiated acoustic startle (nucleus reticularis pontinis caudalis, RPC), increased hear rate and blood pressure (lateral hypothalamus, LH; dorsal motor nucleus of vagus, DMN), increased respiration (parabrachial nucleus, PB), glucocorticoid release (paraventricular nucleus of the hypothalamus, PVN; bed nucleus of the stria terminalis) and freezing response (periaqueductal gray, PAG). Figure 1 illustrates the circuitry.

More specifically, the neural circuitry responsible for contextual fear conditioning involves multimodal sensory information that reaches the basolateral amygdala (BLA) through direct projections from the hippocampus. Indeed, long-term potentiation (LTP) has been observed along this hippocampal–amygdaloid pathway (Maren and Fanselow, 1995). Moreover, ascending serotonergic projections from the median raphe nucleus to the hippocampus seem to be part of the pathway that regulates contextual fear conditioning (Silva et al., 2002). The ventral portion of the medial prefrontal cortex (Resstel et al., 2006) and the perirhinal and postrhinal cortices (Bucci et al., 2000; Corodimas and LeDoux, 1995; Sacchetti et al., 1999) are also thought to be involved in the contextual fear conditioning. Direct projections from these cortical areas to the hippocampus and to the BLA may provide higher-order processing of polymodal sensory information. The information flow within the amygdaloid region involves projections from the BLA to the central amygdala (CEA), which constitutes the main output region of the amygdala. Efferent projections from the CEA to the brain stem and hypothalamic areas give rise to distinct behavioral and autonomic reactions involved in this type of conditioning. The motor output of the conditioned freezing response is related to efferents from the CEA to the ventral portion of the periaqueductal gray, which in turn sends projections to motoneuron cell groups in the spinal cord.

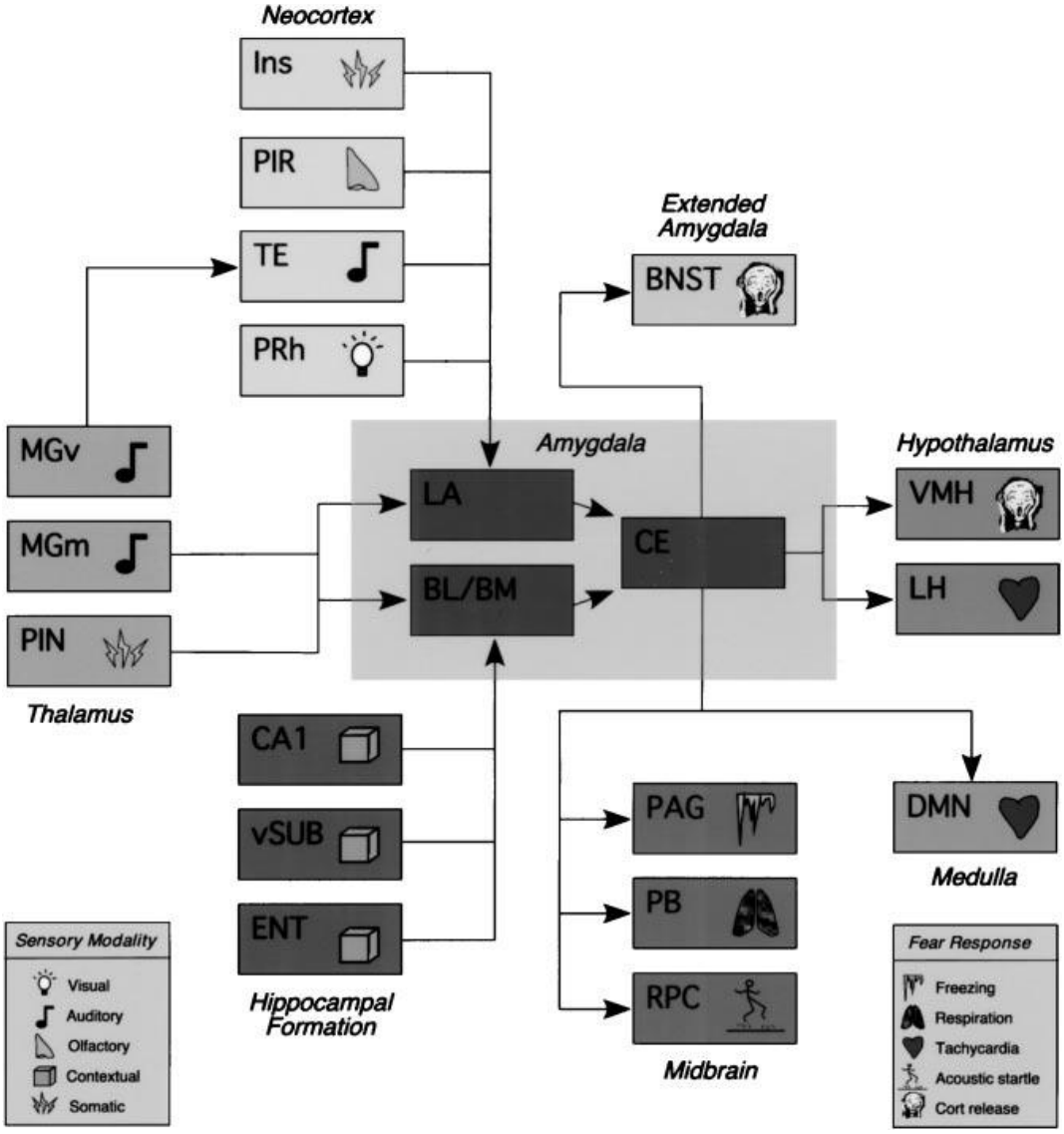

Figure 1: Anatomy of fear conditioning circuits in the brain. To simplify, all projections are drawn as unidirectional connections, although in several cases these connections are reciprocal. LA (lateral amygdala); BL (basolateral amygdala), BM (basomedial amygdala); BLA (basolateral complex); CE (central nucleus); MGm and MGv (thalamic medial and ventral geniculate nucleus); perirhinal cortex (PRh); primary auditory cortex (TE); insular cortex (INS); thalamic posterior intralaminar nucleus (PIN); vSUB (ventral subiculum); ENT (entorhinal cortex); PIR (piriform cortex); PAG (periaqueductal gray); RPC (nucleus reticularis ponits caudalis); LH (lateral hypothalamus); DMN (dorsal motor nucleus of the vagus); PB (parabrachial nucleus); PVN (paraventricular nucleus of the hypothalamus); BNST (bed nucleus of the stria terminalis).(Figure extracted from Maren, 2001) Annu. Rev. Neurosci. 2001.24:897-931. Downloaded from arjournals.annualreviews.org by CAPES

1.5.2
The conditioned freezing response

Freezing behavior is defined as a crouching posture (Blanchard and Blanchard, 1969) and cessation of motor activity, including whisker and nose movements (Bolles and Riley, 1973; Bindra and Anchel, 1963), with the exception of movements necessary for respiration (Bolles and Collier, 1976; Fanselow, 1980). This response is an efficient behavioral defense reaction against predation because predators have difficulty detecting an immobile target, and movement can function as a releasing stimulus that precipitates a predator attack (Fanselow and Lester, 1988).

Several studies showed that freezing is the most reliable measure of aversive contextual conditioning. This defensive response is a direct function of shock intensity (Sigmundi et al., 1980) and depends on the association between the cues of the experimental chamber and the footshock (Landeira-Fernandez et al., 2006). For example, when the footshock is presented simultaneously with the rat's placement in the chamber, no contextual fear conditioning is observed (Landeira-Fernandez et al., 1995). What makes freezing a very attractive index is that fear conditioning can be evaluated directly without any form of food or water deprivation or any form of operant response acquisition. Also, freezing is considered an unconditioned response when triggered by an innate threatening situation. For example, rats freeze when exposed to innately recognized predators, such as a cat (Griffith, 1920).

Conditioned freezing in response to contextual cues previously associated with footshocks has been pharmacologically validated as an adequate model of anxiety disorder. Accordingly, classic anxiolytic benzodiazepines, such as midazolam and diazepam (Fanselow and Helmstetter, 1988), and non-benzodiazepine anxiolytics, such as the serotonin-1A (5-hydroxytryptamine-1A [5-HT_{1A}]) receptor agonist ipsapirone (Inoue, Tsuchiya, Koyama, 1996) and 5-HT reuptake inhibitors citalopram and fluvoxamine (Hashimoto et al., 1996), reduced the amount of conditioned freezing. On the other hand, anxiogenic substances, such the benzodiazepine inverse agonist dimethoxy-β-carboline, produced

freezing behavior similar to that elicited by fear conditioning (Fanselow et al., 1991).

Freezing can also be employed to measure fear conditioning in response to a discrete CS, such as a light or tone (Sigmundi and Bolles, 1983). Freezing in response to contextual cues and a discrete CS previously associated with footshock is mediated by different neural circuitries (Kim and Fanselow, 1992). For that reason, freezing triggered by contextual cues and a discrete CS should be evaluated differently (for a review, see Brandão et al, 2008).

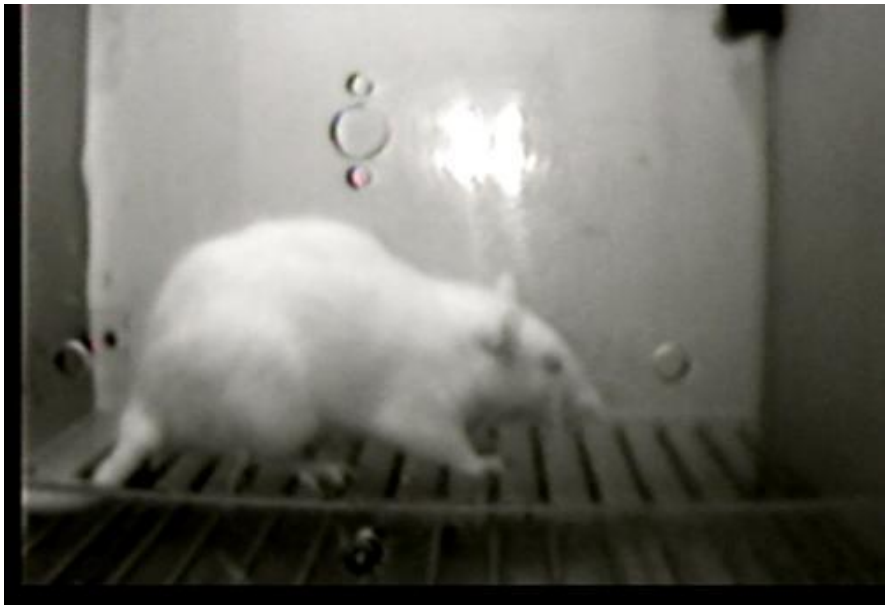

Figure 2: Typical conditioned freezing response in a rat.

1.5.3
Fear Extinction

A behavioral phenomenon that has received considerable interest of late, especially for the use in the treatment of human anxiety disorders, is the fear extinction learning. Extinction can be defined as the decrease of conditional responding (in this case decrease of CRs) following several presentations of a CS in the absence of the US. Extinction was first described by Ivan Pavlov (1927) in

his classic studies employing salivary response in dogs, and since then has received considerable experimental attention. Indeed, there is a large amount of data demonstrating that extinction is itself new learning (in this sense, inhibitory learning) that comes to inhibit or suppress the expression of Pavlovian CRs. Differently from the excitatory memories formed through conditioning, the inhibitory memories established through extinction procedures tend to be relatively volatile, i.e. the extinction weakens over time, promoting the spontaneous recovery of excitatory CRs as time elapses after extinction. Additionally, extinction memories are context-dependent, e.g. CR expression is restricted only in the context in which CS-alone presentations occurred. After extinction, CSs will continue to produce vigorous CRs when they are encountered outside of the extinction context.

Experimental investigation at the behavioral and systems level showed that most forms of extinction learning do not involve the forgetting or reversal of learned fear association (Bouton, 1993 and Rescorla & Heth, 1975). In fact, like other forms of learning, extinction consists of three phases: acquisition, consolidation and retrieval, each of which depends on specific neural structures (amygdala, prefrontal cortex and hippocampus, respectively).

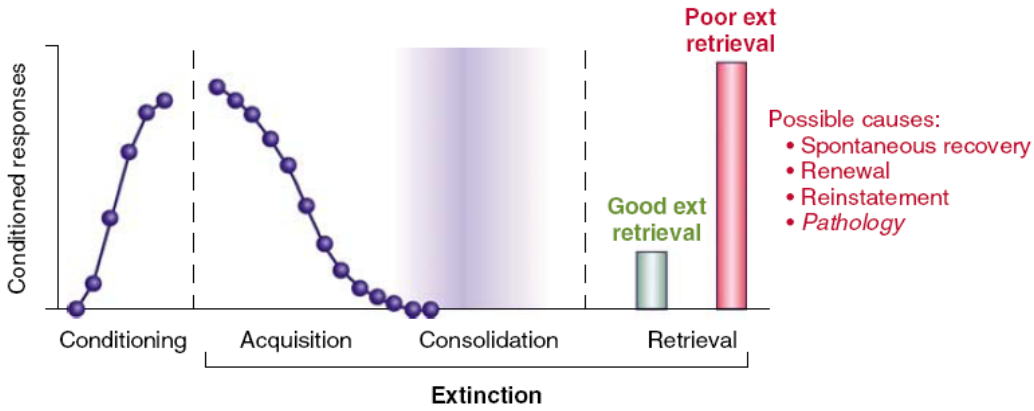

Figure 3: Fear extinction paradigm, with both conditioning, acquisition and consolidation phases (adapted from Quirk, 2008).

1.6
Fear and Anxiety: a genetic approach

Following involvement in a traumatic or stressful incident most people do not develop an anxiety disorder. Individuals differ greatly in their capacity to develop fearful associations, including conditioned fear. In this perspective, it is widely established that, in addition to the neural circuitry responsible for emotional defensive reactions, the environment and genetically determined predisposing factors also play a significant role in the pathogenesis of stress-related disorders (Gordon & Hen, 2004). Notably, the predisposition to develop an anxiety disorder is inherited, meaning that is partially influenced by the genotype of the individual (Stein et al., 2002). A major challenge for the neuroscience of anxiety disorders is to understand why some individuals endure a transition from normal emotional defensive reactions to the pathological and exaggerated patterns characteristics of the generalized anxiety disorders, as well as correlated illness like phobias, obsessive-compulsive disorders, panic disorders, post-traumatic stress disorder (PTSD) and impairments in fear extinction (Gross & Hen, 2004).

Several strategies have been developed in the past 60 years in order to analyze these inter-individual differences and susceptibility to psychiatry disorders, including the GAD. A genetic approach can be employed to investigate and dissect the individual variability between and within populations, searching for the molecular bases (genes and gene products), which underlie such variability. In this sense, these genetic models of anxiety disorders might be a useful tool for understanding why some individuals present adequate emotional reactions and others endure an exaggerated pattern of anxiety responses in the absence of a fear-provoking context (Finn et al, 2003; Grahame, 2000). These strategies include the use of inbred strains, multiple marker strains, animals obtained from gene targeting technologies and quantitative trait locus mapping (QTL), among others (Wood & Toth, 2001; Rudolph & Mohler; 2004; Lesch, 2001; Finn et al, 2003; Clément et al, 2002). Discussing these techniques is beyond the scope of this thesis, but some of those approaches will be considered for future studies. Here, we focused on the so-called Selection Experiments, in particular the “Artificial Selection” procedure.

1.6.1
Selection Experiments

The more newly developed tools of "genetic engineering" (e.g. knockouts, transgenesis) frequently attempt to modify only one or maybe a few gene loci and then analyze the phenotypic outcome. However, in the wild, sexual and natural selection act most directly on complex phenotypes (e.g. life history traits, behavior), which are mostly highly polygenic (affect by a myriad of genes, most of them with relatively small effects). Hence, from an evolutionary perspective, selection experiments may offer a major advantage, being more representative of the kind of genetic changes that occur in nature.

Selection experiments have a long history (Bell, 1997; Falconer, 1992; Falconer and Mackay, 1996; Garland and Carter, 1994; Gibbs, 1999; Hill and Caballero, 1992; Hill and Mackay, 1989; Robertson, 1980; Roff, 1997; Rose et al., 1990; Travisano and Rainey, 2000) and have occurred in non-laboratory contexts since human beings first developed agriculture, including the domestication of several plants and animals (for dog domestication, see Morey, 1994, Vila et al, 1997, Trut, 1999).

Analogously, in a scientific context, evolutionary scientists and behavioral psychologists often use selection experiments. By allowing the alteration of phenotypes at higher and lower levels of certain biological organizations, and then determining quite accurately what other traits change as a result, this is a powerful tool to dissect the basis of such variability.

One interesting example of a selection study is the "Laboratory Natural Selection". In this situation, a freely breeding population is exposed to changed environmental conditions, such as different temperatures or salinities. Assuming that the genetic variance exists for relevant traits, the organisms will then adapt to the new conditions. These kinds of experiments are more common with non-vertebrates, e.g. Drosophila (Gibbs, 1999; Rose, 1984; Rose et al., 1996; Zera and Harshman, 2001), bacteria (Mongold et al., 1996; Travisano and Rainey, 2000; Travisano et al., 1995) and also viruses, but have been employed with vertebrates. A curious variant of laboratory natural selection is termed laboratory culling (Rose et al., 1990). In this type of experiment, a given population is exposed to a

lethal stress until a fraction of the population dies. The remaining is allowed to breed. The majority of experiments employ non-vertebrates, such as Drosophila (Rose et al, 1990), but very rarely with vertebrates, mainly because of ethical considerations.

1.6.2
Artificial Selection

Artificial Selection (or Genetic Selection) is a technique in which every individual of a given population is measured, at each generation, for a particular phenotype trait or a combination of traits (behavior, body size or major component of fitness, e.g. fecundity). The top and bottom fraction of individuals are then chosen as the breeders to produce the next generation. Mating animals within a heterogeneous population, based on the opposite extremes of an observable characteristic, will propagate this particular phenotype in opposite directions over many generations. The selection aggregates increasing and decreasing alleles in the "high" and "low" lines, respectively, leading to two separately and well-contrasted breeding lines. The assumption is that after several generations of selection, the phenotypic contrast between the high and low lines will be maximized based on the effects of the genes that facilitate either the high or the low phenotypes and were polymorphic within the initial founding population. Additionally, genes that do not influence the selected phenotype and are not physically linked to the relevant genes (i.e., do not map in the same chromosome region) will vary randomly within each of the two lines across generations. Due the fact that any finite population will undergo genetic changes caused by random genetic events, an experiment that involves selection in both directions (bidirectional) must involve at least three lines: one selected for high phenotypic values, one randomly bred as a control, and one selected for low phenotypic values (Garland, 2003). Once they have diverged, selected and control lines can be compared with respect to associated traits that are thought to cause differences at the phenotypical level (Rose et al., 1984; Schlager et al., 1983). Differently from laboratory natural selection and laboratory culling studies (see

above), artificial selection allows the researcher to make very detailed choices on what is exactly under selection. In this way, very particular aspects of performance, morphology, physiology and behavior can be targeted (Emlen, 1996, Weber, 1990; Wilkinson, 1993).

Although very useful and relatively simple to perform, some considerations should be made when using the artificial selection technique. Firstly, the consistency of response and replication of experimental lines are crucial to ensuring that differences can be attributed to the effects of selection, instead of founder effects and/or random genetic drift, which can occur in combination with unique mutations. Nevertheless, many early selection studies employed a single line or two, selected according to opposite phenotypes (Falconer, 1992). Still, selection experiments are often performed without replication (Koch and Britton, 2001; Nakamura et al., 1993). Surely, the high costs and intricate logistics involved in a selective breeding program could discourage replication studies, leading to serious implications. Even lines of organisms that are not under divergent selective pressure may be expected to differentiate genetically, and therefore, phenotypically, because of [1] differences in allele frequencies that occurred at the founding lines, [2] random genetic drift and [3] unique mutations (Garland, 2003). The same observations will apply to lines under divergent selective pressure. Thus, if a phenotypic difference between two selected lines is observed (one for “high” values and another “low”) after several generations, perhaps it may not have been caused by the selective procedure that was imposed. Genetic drift and divergence caused by founder effects, as well as limits to selection caused by the exhaustion of additive genetic variance (Weber, 1996), can be diminished by increasing the sample size within each line, which is often difficult to achieve with rodents. Replication of lines under the same selection criterion (e.g. preference for alcohol) can avoid problems of false correlated responses (DeFries et al., 1978; Henderson, 1989, 1997; Rose et al., 1996). In fact, the limitations of selection studies without replication lines are very similar to those of two species comparisons (Garland and Adolph, 1994).

Finally, it is important to emphasize that some behaviors may perhaps not react to artificial selection, and this negative outcome is often difficult to analyze. Besides, the selective breeding procedure might work for only one of the breeding

lines and a loosening of the selective pressure could lead to a regression to the initial behavioral levels of the parental population (Papini, 2002).

1.7
Behavioral Profile of eight rat genetic models

The development of bidirectional lines or strains of rodents with high and low levels of emotional reactions associated with a threatening situation began in the middle of the 20th century and, since then, a relatively large number of different genetic models based on this strategy has been developed (Ramos and Mormède, 2006). These models might represent powerful tools to study the behavioral, neural, and genetic mechanisms that underlie the different types of anxiety disorders. To that end, evaluating whether the phenotypic differences in a bidirectional selected line or strain indeed reflects differential animal emotionality is important. Moreover, still unclear is whether a genetic model of anxiety shares the same emotional system within a unitary construct or reflects a set of qualitatively different emotional dimensions that in turn might recruit distinct neurobiological and genetic mechanisms.

An important issue in validating a genetic model of anxiety is to analyze whether the behavioral differences between two contrasting lines/strains are also present in other animal models of anxiety. Table 1 shows the results of eight bidirectionally selected lines with distinct selection criteria with regard to innate or learned aversive situations across the 11 animals models of anxiety described in the previous section of this study. To facilitate table interpretation, results from rats selectively bred for high anxiety-related responses are always presented first in relation to the counterpart animals. A white cell designates a congruent result (i.e., animals selectively bred for high anxiety-related responses are indeed more emotionally reactive that their counterparts in the particular animal model of anxiety in which they were tested). A cell filled with a dotted pattern indicates a mixed result. Finally, a black cell represents a contradictory result that challenges some aspect of the genetic model, such as the presence of a motor effect, no differences, or differences in opposite directions between the two groups of animals in a particular animal model of anxiety. The behavioral profile of each of

these eight pairs of lines/strains across the 11 models of anxiety is described below:

1.7.1
Maudsley Reactive and Non-Reactive rats

Broadhurst, at the Maudsley Hospital, University of London, London, United Kingdom, began in 1954 the development of two lines of rats based on the procedure by Hall (1934), who used the number of fecal boli excreted in the open field as a measure of emotionality in rats. The lines were named Maudsley Reactive (MR: high-defecating; i.e., high anxiety-related response) and Maudsley Non-Reactive (MNR: low-defecating; i.e., low anxiety-related response).

After only four generations of mating male and female rats with the highest and lowest rates of defecation in the open field, differences between MR and MNR rats were found to be consistent (Broadhurst, 1957, 1958). Defecation scores among MR rats were close to three for both males and females rats, whereas MNR animals displayed scores close to zero. Selection was discontinued in the 15th generation, but the differences regarding defecation were still present when animals were tested in the 20th generation.

In the early 1960s, Broadhurst distributed these lines to investigators in North America, such as Sudak and Maas (1964) at the National Institute of Health (NIH; sublines designated MR/N and MNR/N), who received animals from the 20th generation. Harrington (1972; 1979; 1981), at the University of Northern Iowa, also received animals from the 25th generation. Harrington actually received one reactive (designed MR/Har) and two separate non-reactive (designed MNR/Har and MNRA/Har) sublines from Broadhurst. The MNRA/Har line, originally named MNR-a by Broadhurst, was initiated when an allelic difference was discovered at the agouti locus in the MNR line in the 8th generation. The Harrington colony was later relocated to Lafayette Clinic, Detroit. From this stock, the Maudsley sublines were sent to Blizard (1981) at Wake Forest University and Satinder (1981) at Lakehead University in Canada (sublines designated MR/Har/Lu and MNR/Har/Lu). Notably, Satinder's Non-reactive subline was derived from MNRA/Har and not MNR/Har, as the designation might

suggest. In 1987, the original MR and MNR lines developed in London were terminated but were reimported later with the MR/Har and MRNA/Har lines and have been employed in numerous studies (Blizard and Adams, 2002).

Several experiments have been conducted with MR and MNR rats, the results of which have been analyzed in important papers (e.g., Blizard, 1981; Broadhurst, 1975). Initial behavioral results suggested that these lines could represent an animal model of a general emotional trait. As shown in Table 1, MR rats were less active in the open field than MNR rats (Imada, 1970). Both MR and MR/Har animals presented a greater suppression ratio in the conditioned emotional response paradigm compared with their respective MNR and MNRA/Har counterparts (Commissaris et al., 1986; Singh, 1959). Interestingly, MR/N animals in the first postnatal week presented a higher USV frequency than MNR/N pups when isolated from their mothers and littermates for a brief period of time (Insel and Hill, 1987). These findings are consistent with the adult characteristics of the Maudsley lines because they were selectively bred for adult expression of high and low levels of emotionality.

However, conflicting or even opposite results in other animal models of anxiety imposed a serious threat to the possibility that the Maudsley lines might indeed represent a genetic model of a general emotional trait. For example, Overstreet et al. (1992) reported a congruent result in which the MR rats spent very little time in the open arms of the elevated plus maze compared with MRNA rats. However, Paterson et al. (2001) did not observe any difference between MR/Har and MRNA/Har rats in the open arms. Instead, a motor effect was found, in which MR/Har animals spent more time in the closed arms compared with MNRA/Har animals. Inconsistent results were also found in the two-avoidance learning paradigm;- While Broadhurst and Levine (1963) found that MR animals perform worse in the two-way avoidance procedure compared with MRN rats, Paterson et al. (2001) did not observe any differences between MR/Har and MNR/Har rats.

Results from the acoustic startle response are in the opposite direction; Commissaris et al. (1988) found that MR/Har rats presented within-session habituation to repeated presentation of a brief acoustic stimulus, whereas MNRA/Har rats exhibited virtually no habituation. In another study, Paterson et

al. (2001) found that MR/Har rats exhibited less sensitization of the acoustic startle response compared with MRNA/Har rats. Finally, Paterson et al. (2001) reported the absence of differences between MR/Har and MRNA/Har animals in the fear-potentiated startle paradigm.

1.7.2
Floripa High and Low rats

In 2003, Ramos, at the Federal University of Santa Catarina, Florianópolis, Brazil, reported the development of two new rat lines selectively bred for high and low locomotion in the central aversive area of the open field (Ramos et al., 2003). Initially, they produced a highly heterogeneous population through an intercross of three rat strains (i.e., Wistar, Hooded, and Lewis) and then initiated selective breeding of male and female rats for the lowest and highest scores of central open field ambulation. These lines were named Floripa[1] Low (L: low locomotion in the central area; i.e., high anxiety-related response) and High (H: high locomotion in the central area; i.e., low anxiety-related response) rat lines. After four generations of selection, a difference between the Floripa L and H rat lines in locomotor activity within the center of an open field was observed. As expected, the L line consistently displayed lower locomotion in the central area of the open field than rats of the H line. Floripa L lines also exhibited lower locomotion in the periphery of the open field (i.e., where the animal concentrates most of its activity) compared with the H line.

Several behavioral studies have investigated the Floripa H and L lines. In the black and white box test, Floripa L rats spent less time in the white compartment than Floripa H rats after four generations (Ramos et al., 2003). Although this result is consistent with the view that Floripa L rats are more emotionally reactive that Floripa H rats, other results contest this possibility. For example, although Hinojosa et al. (2006) found that Floripa L animals presented a higher defecation rate in the open field compared with Floripa H animals, this difference was not detected in an early study (Ramos et al., 2003). Anxiety-related

[1] Floripa is short for the city of Florianópolis

responses observed in these two lines of animals in the elevated plus maze have also been confusing. Ramos et al. (2003) found that, after four generations, Floripa L rats spent less time in the open arms than Floripa H rats. However, in a subsequent study, this difference between the Floripa lines in the open arms was found only in females but not in males (Hinojosa et al., 2006). Finally a motor effect has also been detected in these two lines of animals, both in the elevated plus maze and black and white box paradigm (Ramos et al., 2003).

1.7.3
Tsukuba High and Low Emotional rats

In 1975, Fujita reported the development of two new lines of animals with high and low emotional reactivity at the University of Tsukuba, Ibaraki, Japan (Fujita, 1975, 1984). Similar to the Floripa H and L animals, locomotion was also employed as the selection criterion. However, a different perspective was adopted. In its natural habitat, the rat that easily emerges from its burrow and explores its surroundings might be less anxious or emotionally reactive than another animal that prefers its burrow. An apparatus that simulates this situation in a laboratory setting and used for the bidirectional selection of these two lines consists of a dark starting box (7 cm × 7 cm) with a small exit to a bright straight runway (120 cm long × 20 cm wide × 45 cm high). According to this procedure, each animal is placed in the dark starting box, and 30 s later the door is opened so that the animal has access to the runway. Each test lasts for 5 min, and animals are tested for 3 consecutive days. Male and female rats with the lowest and highest ambulatory activity scores in the runway are then mated.

After 34 generations of inbreeding (brother and sister mating), two strains with significant differences in activity in the runway test were defined as Tsukuba High Emotional (THE: low ambulatory activity in the runway; i.e., high anxiety-related response) and Tsukuba Low Emotional (TLE: high ambulatory activity in the runway; i.e., low anxiety-related response). As expected, THE rats showed higher latencies in leaving the start box, taking more time to arrive at the end of the runway. Most initial research with these strains has been performed in the

strain's country of origin, Japan, and a review with the large amount of physiological and behavioral data obtained was published by Fujita et al. (1994).

Several results suggest that THE animals are more emotionally reactive than TLE animals. Accordingly, Kitaoka and Fujita (1991) reported that THE rats presented lower activity and higher defection compared with TLE animals in the open field paradigm. Moreover, Naito et al. (2000) also found that THE pups emitted higher USV rates in response to isolation distress from day 3 to day 18 compared with TLE pups, indicating a consistent defensive response pattern from early development to adulthood. Finally, THE animals showed lower shuttle avoidance acquisition compared with their counterpart strain (Fujita and Katayama, 1981).

Results from the passive avoidance paradigm are confusing. Miyamoto and Fujita (1997) showed that THE animals had better step-down passive avoidance performance compared with TLE rats. However, no differences between these two strains were found in step-through passive avoidance performance (Wada and Makino, 1997). Finally, divergent results argued against the possibility that THE animals represent an animal model of a general anxiety trait. Employing the conditioned suppression paradigm, Fujii et al. (1989) did not find any differences between the Tsukuba strains in the suppression ratios of licking and an operant response.

1.7.4
High and Low Anxiety-related Behavior rats

In 1998, Landgraff and colleagues (Liebsch et al., 1998a, b), at the Max Planck Institute of Psychiatry, Munich, Germany, reported the creation of two lines of Wistar rat based on open arm entries in the elevated plus maze. The percentage of time spent in the open arms was employed as the main criterion for bidirectional selection. Other open-arm parameters were also employed in the following rank order: percentage of entries into the open arms > number of full open arm entries > latency to first open arm entry. Only animals with average activity scores (distance traveled) were selected (Liebsch et al., 1998a). Beginning in 1993, male and female rats with the lowest and highest proportion of open arm

scores were mated to establish the two lines now termed High Anxiety-related Behavior (HAB: low proportion of open arm scores; i.e., high anxiety-related response) and Low Anxiety-related Behavior (LAB: high proportion of open arm scores; i.e., low anxiety-related response).

Henniger et al. (2000) reported a study with female HAB and LAB rats at the 7th generation in the elevated plus maze. The results indicated that HAB animals displayed a lower percentage of entries into and time spent on the open arms as compared with LAB rats. Importantly, HAB and LAB rats did not differ in the number of closed arm entries. Yilmazer-Hanke et al. (2004) also observed the same pattern of results with male HAB and LAB rats at the 9th generation.

The HAB and LAB lines have been evaluated in various behavioral paradigms of anxiety, and some of these results corroborated the hypothesis that HAB animals are more emotionally reactive than LAB animals. Liebsch et al. (1998b) reported that the HAB line showed a decrease in open field ambulation compared with LAB animals. Wigger et al. (2001) also reported that HAB rat pups exhibited an enhanced frequency of USVs in response to isolation distress on postnatal day 11 and lower open arm exploration in the elevated plus maze throughout adulthood compared with LAB animals, suggesting that the differences in emotionality between these two lines are already present in the early phase of development and remain present during adulthood. Finally, Henniger et al. (2000) investigated whether the anxiety-related response differences in HAB and LAB rats were also present in the light-dark box and social interaction tests. The results indicated that HAB animals spent less time in the light compartment and engaged in less active social interaction than LAB rats. Importantly, this study also found a locomotor activity effect in both the light-dark box and social interaction test, with HAB rats being less active than their LAB counterparts.

Results from learned aversive paradigms, however, did not support the possibility that HAB and LAB animals represent a model of general emotionality. For example, Muigg et al. (2008) reported that HAB and LAB animals presented the same freezing response during the acquisition of an aversive conditioning task in response to a tone paired with an electrical footshock, although HAB rats showed a considerable deficit in the ability to extinguish the conditioned freezing

response to the acoustic stimulus. Yilmazer-Hanke et al. (2004) also reported that HAB and LAB animals did not differ in the freezing response to contextual cues previously associated with footshocks. In this study, Yilmazer-Hanke et al. (2004) also reported divergent results when HAB and LAB animals were tested in the acoustic startle paradigm. As expected, HAB animals presented lower scores on the open arms in the elevated plus maze compared with LAB animals. However, an opposite response pattern was observed in the acoustic startle response paradigm, in which HAB rats also displayed lower fear sensitization than their HAB counterparts.

1.7.5
High and Low Ultrasonic Vocalization rats

To investigate generational and developmental variables associated with anxiety, Brunelli et al. (1996), at Columbia University, New York, USA, reported the creation of two lines of rats selected for different rates of USVs in response to isolation. Rat pups were screened at 10 ±1 days of age in a 2 min isolation test. Male and female pups with the highest and lowest rates of USV were selected for later breeding. After only one generation, the High line presented more USVs than the Low line. After three generations, the Low and High lines diverged significantly from each other in their USV responses rates and from control animals that were mated randomly. This selection program was the first successful study that attempted to selectively breed a neonatal phenotype among rats and has been termed USV High (high neonatal isolation-induced USV; i.e., high anxiety-related response) and USV Low (low neonatal isolation-induced USV; i.e., low anxiety-related response).

Few behavioral studies have investigated USV animals. Results from a modified version of the open field suggested that USV High and Low animals might represent an adult genetic model of anxiety (Zimmerberg et al., 2005). In this study, adult USV rats were placed inside a closed and opaque cylinder that was in turn placed in an open field. The results indicated that USV High animals emerged into the open field later and crossed fewer squares in the central area of the open field than USV Low rats.

However, confusing results with male and female (in either proestrus or diestrus) USV rats were also reported in the social interaction test (Zimmerberg et al., 2005). The results indicated that only proestrus female USV High rats engaged in less social interaction compared with proestrus female USV Low rats. No differences were found between male or diestrus female USV High and Low animals. Confusing results were also reported by Ditcher et al. (1996) in the elevated plus maze. This study indicated that although USV High animals presented a lower percentage of time in the open arms than USV Low animals, no difference was observed between USV High and control unselected animals.

Table 1. Behavioral profile of eight rat genetic models (columns) across 11 animal models of anxiety (lines). The results from rats selectively bred for high anxiety-related responses are always presented first in relation to the counterpart animals. White cells indicate that differences between the two groups are in the expected direction. Cells filled with a dotted pattern indicate mixed results. Black cells indicate that the result challenged some aspect of the genetic model (i.e., motor effect, no differences between the two groups, or differences in the opposite direction). Superscript numbers indicate the bibliographic references of the behavioral result. M, male; F, female; FP, female proestrus; FD, female diestrus.

Animal model of anxiety-like behavior	Maudsley Reactive and Non-Reactive rats	Floripa High and Low rats	Tsukuba High and Low Emotional rats	High and Low Anxiety-related Behavior rats	High and Low Ultrasonic Vocalizations rats	Roman High and Low Avoidance rats	Syracuse High and Low Avoidance rats	Carioca High and Low Conditioned Freezing rats
Open field								
Defecation	Parameter employed to create the line	Floripa L = Floripa H[9] Floripa L > Floripa H[10]	THE > TLE[11]	--------	--------	RLA = RHA[31] RLA/Verh > RHA/Verh[32]	SLA/Bru > SLA/Bru[40]	--------
Ambulation	MR < MNR[1]	Parameter employed to create the line	THE < TLE[11]	HAB < LAB[19]	USV High < USV Low[22]	RLA < RHA[31] RLA/Verh < RHA/Verh[32]	SLA/Bru = SHA/Bru[40]	--------
Elevated plus maze								
Open arm	MR < MNRA[5] MR/Har = MNRA/Har[6]	Floripa L < Floripa[9] F: Floripa L < Floripa H[10] M: Floripa L = Floripa H[10]	--------	Parameter employed to create the line	USV High < USV Low[23] USV High = Random[23]	RLA/Verh < RHA/Verh[33] RLA/I < RHA/I[34, 35] RLA/Verh > RHA/Verh[36]	--------	CHF < Control[41]
Closed arm	MR/Har < MNRA/Har[6]	Floripa L < Floripa H[9, 10]	--------	HAB = LAB[17, 18]	--------	RLA/Verh < RHA/Verh[33]	--------	CHF = Control[41]
Light-dark box								
Time in the light compartment	--------	Floripa L < Floripa H[9]	--------	HAB < LAB[17]	--------	RLA/Verh < RHA/Verh[37] RLA/Verh > RHA/Verh[36]	--------	--------
Locomotor activity	--------	Floripa L < Floripa H[9]	--------	HAB < LAB[17]	--------	--------	--------	--------
Social interaction								
Social activity	--------	--------	--------	HAB < LAB[17]	FP: USV High > USV Low[22] DF: USV High = USV Low[22] M: USV High = USV Low[22]	RLA/Verh = RHA/Verh[36, 37]		CHF < Control[41]
Locomotor activity	--------	--------	--------	HAB < LAB[17]		RLA/Verh = RHA/Verh[36, 37] RLA/Verh < RHA/Verh[37]		CHF = Control[41]

Table 1 (*continued*):

Animal model of anxiety-like behavior	Maudsley Reactive and Non-Reactive rats	The Floripa High and Low rats	The Tsukuba High and Low Emotional rats	The High and Low Anxiety-related Behavior rats	The High and Low Ultrasonic Vocalizations rats	The Roman High and Low Avoidance rats	The Syracuse High and Low Avoidance rats	Carioca High and Low Conditioned Freezing rats
Ultrasonic vocalization								
Frequency	MR/N > MNRA/N[4]	--------	THE > TLE[12]	HAB > LAB[20]	Parameter employed to create the line	--------	--------	--------
Acoustic startle response								
Habituation	MR/Har > MNRA/Har[8]	--------	--------	--------	--------	--------	--------	--------
Sensitization	MR/Har < MNRA/Har[6]	--------	--------	HAB < LAB[18]	--------	RLA/Verh > RHA/Verh[24]	--------	--------
Fear-potentiated startle								
Startle amplitude	MR/Har = MNRA/Har[6]	--------	--------	--------	--------	RLA/Verh > RHA/Verh[25]	--------	--------
Active avoidance								
Two-way	MR < MNRA[7] MR/Har = MNRA/Har[6]	--------	THE < TLE[13]	--------	--------	Parameter employed to create the line	Parameter employed to create the line	--------
One-way	--------	--------	--------	--------	--------	Only *1 sec in the safe compartment:* RLA/I < RHA/I[27, 28]	--------	--------
Passive avoidance								
Step-down	--------	--------	THE > TLE[14]	--------	--------	--------	--------	--------
Step-through	--------	--------	THE = TLE[15]	--------	--------	--------	--------	--------

Table 1 (*continued*):

Animal model of anxiety-like behavior	Maudsley Reactive and Non-Reactive rats	The Floripa High and Low rats	The Tsukuba High and Low Emotional rats	The High and Low Anxiety-related Behavior rats	The High and Low Ultrasonic Vocalizations rats	The Roman High and Low Avoidance rats	The Syracuse High and Low Avoidance rats	Carioca High and Low Conditioned Freezing rats
Conditioned emotional response								
Supression ration	MR > MNR[2] MR/Har > MNRA/Har[3]	--------	THE = TLE[16]	--------	--------	RLA/Verh > RHA/Verh[26]	SLA/Bru > SHA/Bru[38, 39]	--------
Conditioned freezing								
Context	--------	--------	--------	HAB = LAB[18]	--------	RLA/Verh > RHA/Verh[25, 29] RLA/I > RHA/I[30]	--------	Parameter employed to create the line
Discrete CS	--------	--------	--------	HAB = LAB[21]	--------	RLA/Verh > RHA/Verh[27, 29]	--------	--------

Note: 1, Imada (1979); 2, Singh (1959); 3, Commissaris et al. (1986); 4, Insel and Hill (1987); 5, Overstreet et al. (1992); 6, Paterson et al. (2001); 7, Broadhurst and Levine (1963); 8, Commissaris et al. (1988); 9, Ramos et al. (2003); 10, Hinojosa et al. (2006); 11, Kitaoka and Fujita (1991); 12, Naito et a(2000); 13, Fujita and Katayama (1981); 14, Miyamoto and Fujita (1997); 15, Wada and Makino (1997); 16, Fujii et al. (1989); 17, Henniger et al. (2000); 18, Yilmazer-Hanke et al. (2004); 19, Liebsch et al. (1998b); 20, Wigger et al. (2001); 21, Muigg et al. (2008); 22, Zimmerberg et al. (2005); 23, Ditcher et al. (1996); 24, Schwegler et al. (1997); 25, López-Aumatell et al. (2009); 26, Ferré et al. (1995); 27, Morón et al. (2010); 28, Torres et al. (2007); 29, Aguilar et al. (2002); 30, Escorihuela et al. (1997); 31, Broadhurst and Bignami (1965); 32, Gentsch et al. (1982); 33, Meyza et al. (2009); 34, Driscoll et al. (1998); 35, Escorihuela et al. (1999); 36, Chaouloff et al. (1994); 37, Steimer and Driscoll (2003); 38, Brush et al. (1988); 39, Gupta and Brush (1998); 40, Brush et al. (1985); 41, Dias et al. (2009).

1.7.6
Roman High and Low Avoidance rats

In 1961, Bignami, at the Istituto Superiore di Sanità, Rome, Italy, started a selective breeding program with Wistar rats for low and high rates of two-way avoidance. The animals were subjected to five daily sessions of 50 trials, with 30 s between trials. Each trial consisted of a visual CS (light) that preceded the onset of a footshock US. The occurrence of a crossing response from one side to the other side of a shuttle box during the CS terminated the CS and avoided the US. If the response occurred after the onset of the US, then both the CS and US were terminated. Male and female rats with the lowest and highest rates of avoidance were selected and mated together while avoiding inbreeding. After five generations, the two selected lines differed markedly (at least threefold differences) in the number of avoidance responses, with no sex differences (Bignami, 1965). The lines were named Roman Low Avoidance (RLA: low rates of two-way avoidance; i.e., high anxiety-related response) and Roman High Avoidance (RHA: high rates of two-way avoidance; i.e., low anxiety-related response).

In 1964, Bignami took a sabbatical to work with Broadhurst and transferred the two lines to England, where they were distributed to various laboratories (Broadhurst and Bignami, 1965). One of the most well-know colonies was established in 1972 at the Institut für Verhaltenswissenschaft, Zürich, Switzerland. The two sublines were named RLA/Verh and RHA/Verh and have been continuously bred since then, initially by Bättig, and later by Driscoll (Driscoll and Bättig, 1982). In parallel with the RLA/Verh and RHA/Verg sublines, an inbreeding program was initiated in 1993, derived through brother and sister matings from the outbred sublines. Since 1997, the inbred RLA/Verh (RLA/I) and RHA/Verh (RLA/I) rats have been maintained at the Universidad Autónoma de Barcelona, Spain, under the direction of Fernández-Teruel (Escorihuela et al., 1999).

Several results from learned aversive paradigms support the hypothesis that RLA rats have a stronger emotional reaction than RHA animals. For example, in the acoustic startle response test, RLA/Verh displayed higher sensitization (Schwegler et al., 1997) and greater fear-potentiated startle (López-Aumatell et al., 2009) compared with RHA/Verh rats. Results from the conditioned emotional response test also indicated that

RLA/Verh rats presented more shock-induced suppression of drinking behavior compared with RHA/Verh rats (Ferré et al., 1995).

Evidence also showed that Roman inbred animals behaved differently during the acquisition of a one-way avoidance response (Morón et al., 2010; Torres et al., 2007). In these experiments, the rats learned to run from a danger compartment, where they received a warning signal followed by an electric footshock, to a safe compartment, where these stimuli were not presented. The results indicated that RLA/I rats exposed for 1 s to the safe compartment showed poorer performance than RHA/I rats. These differences were not observed when the animals were exposed for 30 s to the safe compartment. Because the reinforcing value of the running response among animals that remained in the safe compartment for only 1 s would be very low (fear relief), the one-way avoidance response would be expected to mainly result from the aversive conditioning that occurred in the danger compartment. Accordingly, RLA animals would tend to freeze, whereas RHA animals would tend to flee.

Indeed, conditioned freezing appears to be one of the main differences between the Roman animals. For example, López-Aumatell et al. (2009) found that RLA/Verh animals presented more conditioned freezing in response to contextual cues and to a discrete CS previously associated with electrical footshocks. The same results were also found by Aguilar et al. (2002). Finally, Escorihuela et al. (1997), working with inbred Roman rats, also found that RLA/I rats presented more conditioned freezing in response to contextual cues than RHA/I rats. Therefore, the low two-way avoidance performance of the RLA rats might be attributable to the fact that these animals are more "afraid" of the contextual cues previously associated with footshock. The interaction between conditioned freezing in response to contextual cues and the acquisition of a two-way avoidance response has been recently demonstrated by Vicens-Costa et al. (2011). They found that rats that presented relatively higher levels of context-conditioned freezing during the initial trials of two-way avoidance learning were less likely to acquire this response. Therefore, contextual fear conditioning negatively predicted two-way avoidance acquisition.

The behavioral results from the Roman animals in innate paradigms of anxiety are puzzling. Initial studies in the open field indicated that RLA rats were less active, without any differences in defection, compared with RHA rats (Broadhurst and Bignami, 1965). However, other studies with the Swiss subline indicated that

RLA/Verh rats were less active and defecated more than RHA/Verh rats (Gentsch et al., 1982). Confusing results were also reported in the elevated plus maze. For example, Meyza et al. (2009) reported that RLA/Verh rats entered the open arms less compared with RLA/Verh rats. RLA/I animals also entered the open arms of the elevated plus maze less compared with RHA/I animals (Driscoll et al., 1998; Escorihuela et al., 1999). Surprisingly, opposite results were found by Chaouloff et al. (1994), in which RLA/Verh rats spent more time on the open arms compared with RHA/Verh rats.

Results from the light-dark box test are also confusing. Steimer and Driscoll (2003) reported that RLA/Verh rats were more emotionally reactive, reflected by an increased latency to first enter the light compartment, than their RHA/Verh counterparts. However, Chaouloff et al. (1994) found that RLA/Verh rats were less emotionally reactive, reflected by the time spent in the light compartment, than RHA/Verh rats. Finally, results from the social interaction test are not consistent with the view that Roman animals represent a model of a general anxiety trait since two studies failed to detect differences in anxiety-related response parameters in this paradigm between the two Verh sublines (Chaouloff et al., 1994; Steimer and Driscoll, 2003).

Locomotor activity results from social interaction paradigm are unclear. Chaouloff et al. (1994) did not find any differences between RHA/Verh and RLA/Verh animals. However, Steimer and Driscoll (2003) showed that RLA/Verh rats were less active than RHA/Verh rats. Results from the elevated plus maze indicated that RHA/Verh animals were less active than RLA/Verh rats (Meyza et al., 2009).

1.7.7
Syracuse High and Low Avoidance rats

In 1965, Brush, at the Syracuse University, Syracuse, New York, USA, started a selective breeding program with Long-Evans hooded rats, also based on low and high rates of two-way avoidance (Brush, 1966). Similar to Bignami's Roman lines, Brush's animals were required to cross from one side to the other side of a shuttle box to avoid an electrical footshock. However, Brush's procedure was slightly different from Bignami's and had only a single test session composed of 10 pretest trials, in which the CS was presented alone with an intertrial interval of 120 s. Immediately after the 10

pretest trials were 60 training trails, in which the CS was followed by the US. The warning CS was a compound auditory and visual stimulus that lasted for 5 s, whereas the US was a low-intensity footshock (0.25 mA). Male and female rats with the lowest and the highest avoidance responses during the 60 trials and that met the pretest criteria (response latencies less than 5 s on fewer than five of the 10 pretest trials and on fewer than three of the last five pretest trials) were selected and mated.

In 1979, Brush and colleagues reported the results of 25 consecutive generations (Brush et al., 1979). Similar to the study by Bignami (1965), the two selected strains differed markedly in the number of two-way avoidance responses after five generations, with no sex differences (Bignami, 1965). These strains were named Syracuse Low Avoidance (SLA/Bru: low rates of two-way avoidance; i.e., high anxiety-related response) and Syracuse High Avoidance (SHA/Bru: high rates of two-way avoidance; i.e., low anxiety-related response).

The hypothesis that differences in emotionality might covariate with this selected phenotype received support when the Syracuse animals were tested in the conditioned emotional response paradigm. Brush et al. (1988) and Gupta and Brush (1998), ten years later, found that SLA/Bru rats had greater suppression ratios than SHA/Bru rats. However, results from the open field were incoherent (Brush et al., 1985). SLA/Bru rats showed greater defecation than SHA/Bru animals, but these two lines did not differ in ambulation.

1.7.8
Carioca High and Low Conditioned Freezing rats

Landeira-Fernandez, at the Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, Brazil, was also interested in developing a rat genetic model of extreme phenotypes for learned fear. Instead of the two-way avoidance paradigm, conditioned freezing in response to contextual cues previously associated with footshock was used as the phenotype criterion for developing the two lines.

The breeding program began in 2006. The contextual fear conditioning protocol involved acquisition and test sessions. During acquisition, Albino Wistar rats were placed in the observation chamber. After 8 min, three unsignaled electrical footshocks were delivered. Twenty-four hours later, each animal was placed again in the original

context, but no footshock or other stimulation occurred. Freezing was recorded for 8 min. The total amount of freezing behavior observed during the test session was used as the criterion for animal mating. Male and female rats with the highest and lowest conditioned freezing scores were selected and mated. Gomes and Landeira-Fernandez (2008) found that after three generations, reliable differences between these two lines were already present, indicating a strong heritable component of this type of learning. Males consistently exhibit more conditioned freezing in response to contextual cues than females. The lines were named Carioca[2] High Conditioned Freezing (CHF: high amount of freezing in response to contextual cues previously associated with footshock; i.e., high anxiety-related response) and Carioca Low Conditioned Freezing (CLF: low amount of freezing in response to contextual cues previously associated with footshock; i.e., low anxiety-related response). This model will be further detailed in Study 1 of this thesis.

The first behavioral results from this ongoing selective breeding program were reported by Dias et al. (2009). They performed a battery of behavioral tests with the fourth generation of CHF lines and control unselected rats using the elevated plus maze and social interaction test, among others. In the elevated plus maze, the results indicated that CHF animals were significantly more emotionally reactive than control rats in terms of both the number of entries into the open arms and percentage of time spent on the open arms. Their time spent engaging in social interaction was also significantly decreased. Importantly, no differences were found in locomotor activity, reflected by the number of entries into the closed arms of the elevated plus maze and number of crossings into the social interaction test arena.

1.8
Problem of locomotor activity

One of the main problems of using animal models of anxiety is the possible interaction between the behavioral measurements of emotionality and the animal's locomotor activity. Indeed, motor effects have been found in Maudsley (measured in the elevated plus maze), Floripa (measured in the elevated plus maze and light-dark box),

[2] Carioca is the name given to those born in Rio de Janeiro.

HAB/LAB (measured in the light-dark box and social interaction test), and Roman (measured in the elevated plus maze) animals. In all of these cases, rats with high anxiety-related responses also exhibited a reduction in locomotor activity. These motor effects might represent an important confounding variable because the differences in emotionality among these animals might be at least partially explained by differences in locomotor activity.

Anxiety and locomotor activity are intimately associated. Most defensive reactions involve a decrease in exploratory ambulation and an increase in freezing behavior. Therefore, discarding any reasonable influence of locomotor activity on the occurrence of anxiety-like responses is almost impossible. Even in paradigms in which anxiety and motor indices are relatively well dissociated, such as in the elevated plus maze, it is unclear how these performance variables may in fact interact in this animal model of anxiety. For example, hypoactivity in the elevated plus maze can overcome the detection of anxiogenic-like effects in some experimental manipulations (e.g., Padovan and Guimarães, 2000) but not in others (e.g., Maisonnette et al., 1993). Moreover, a motor effect in the elevated plus maze can be part of the defense response to an anxiogenic compound (e.g. Cruz et al., 2005).

However, a few procedural manipulations can be conducted to estimate the possible modulatory effect of locomotor activity on defensive reactions. For example, Tsukuba (Fujita et al., 1994), HAB/LAB (Liebsch et al., 1998a) and Roman (Meerlo et al., 1997) animals did not exhibit any motor differences when measured under basal conditions in their home cage using a radiotelemetric system. Therefore, the motor effect observed in these genetic models of anxiety is not associated with general spontaneous locomotor activity but is a reaction to a possible threatening situation.

When locomotor activity plays a fundamental role in the expression of defensive behavior and is part of the natural coping reaction that the animal adopts in response to a threatening situation, employing statistical procedures to test whether the difference between the high and low anxiety groups in the emotional index can be explained by differences in locomotor activity is still possible. Accordingly, when the two groups exhibit a significant difference in both emotional and locomotor activity indices, examining these data with an analysis of covariance (ANCOVA) is important. This analysis is performed on the emotional index, including locomotor activity as a covariate variable. This analysis can lead to two possible conclusions. If the difference

previously detected between the two groups is still present after the ANCOVA, then the conclusion can be made that the anxiety-like behavior was not biased by locomotor activity. However, if the significant difference disappears with the ANCOVA, then locomotor activity is an important confounding variable, and the anxiety-like response difference between the two groups might be attributable to differences in motor activity.

None of the studies reviewed in the present work employed an ANCOVA as a possible statistical procedure to assess the extent to which individual variability in locomotor activity contributed to the anxiety-like effect. This is surprising because this analysis can be easily performed in several innate aversive paradigms that derive an emotional and motor variable for the same animal. For example, in the open field, comparing the number of squares visited in the central area of the open field is possible using the number of squares visited on the periphery of the open field as a covariate variable. In the social interaction test, differences in the total amount of social interaction can be controlled by the locomotor activity measures, reflected by the number of squares visited by the animal. Differences in emotional parameters in the light-dark box, such as the latency to first enter the light compartment or total time spent in the light compartment, can be controlled by a locomotor activity parameter such as the total number of transitions. Finally, the ANCOVA can be used in the elevated plus maze to determine whether the differences in the percentage of time spent on or percentage of entries into the open arms can be explained (covariate) by the number of entries into the closed arms.

1.8.1
Anxiety as a multidimensional construct

One of the main goals of the present short review was to investigate whether a genetic model of rats selectively bred for high and low levels of a particular anxiety-like response would display similar results in other experimental paradigms that also require the expression of a different defensive response. A positive answer to this question would support the traditional view that differences in emotionality reflect a continuum of variation within a single general trait that ranges in intensity from normal to pathological levels (Broadhurst, 1975; Gray, 1979; Hall, 1934).

However, the results found in the present work do not support this view. As shown in Table 1, the Maudsley animals presented inconsistent results in the open arm parameter of the elevated plus maze and acquisition of two-way avoidance learning. Divergent results were also detected in the habituation, sensitization, and fear-potentiation of the acoustic startle response. The Floripa lines also had inconsistent results with regard to open field defecation and the open arm parameters of the elevated plus maze. Tsukuba animals also presented opposite results in the acquisition of passive step-through avoidance and the suppression ratio of the conditioned emotional response.

Results that were opposite to the general trait hypothesis were also found in HAB and LAB lines when tested for fear sensitization of the acoustic startle response and conditioned freezing in response to contextual cues and a CS previously associated with footshock. The USV strains presented inconsistent results in the open arm parameter of the elevated plus maze and social interaction test. The Roman strains also presented opposite results in the social interaction test and inconsistent results in several innate aversive models of anxiety, such as defecation in the open field, the open arm parameters in the elevated plus maze, and time spent in the light compartment of the light-dark box. Finally, Syracuse rats also presented opposite results with regard to ambulation in the open field.

These results clearly argue against the early conceptualization of emotional reactivity as a unitary construct and reinforce a more recent approach that proposed that anxiety is a complex, multidimensional, and dynamic phenomenon (Aguilar et al., 2002; Belzung and Le Pape, 1994; Ramos et al., 1997; Torrejais et al., 2008). In these studies, statistical techniques, such as the factor analysis, have been employed to investigate whether different animal models of anxiety measure the same underlying latent factor. These factor analyses studies indicate that different aversive paradigms may assess different forms of anxiety. For example, File (1992) showed that indices of anxiety derived from the elevated plus maze (i.e., number of entries into and time spent on the open arms), Vogel test (i.e., frequency of punished drinking), and social interaction test (i.e., time spent engaged in social interaction), loaded on three independent factors, suggested the existence of different forms of anxiety generated by each of these paradigms. Similarly, Belzung and Le Pape (1994) found a weak correlation between the measures of anxiety in the elevated plus maze and in light-dark box. For a review of

the similarities and differences between the elevated plus maze, light-dark box, and open field, see Ramos (2008).

These diverse dimensions found in animal models of anxiety might reflect the clinical diversity generally found among human patients, in whom pathological anxiety is classified into several categories (American Psychiatric Association, 1994; World Health Organization, 1993). In fact, the treatment of different anxiety disorders might involve a wide range of pharmacological compounds, with distinct mechanisms of action, such as increasing the effects of GABAergic neurotransmission or modulating serotonergic activity (Outhoff, 2011). Pharmacological studies that have used diverse anxiety tests have also detected the multidimensional aspect of anxiety. For example, experimental paradigms that generate behavioral inhibition caused by conflicts between approach and avoidance tendencies are sensitive to benzodiazepine compounds. These animal models also indicated that substances that decrease serotonergic activity increased anxiety, whereas those that increase serotonergic neurotransmission produced an anxiogenic effect. In contrast, other animal models that require vigorous escape responses to proximal aversive stimuli appeared to be resistant to benzodiazepine drugs, whereas substances that increased serotonergic activity produced an anxiolytic effect (Graeff and Zangrossi, 2010).

Different neural circuitries also appear to be involved in distinct dimensions of anxiety. Gray and McNaughton (2000) argued that the septo-hippocampal system contributes to the cognitive component of anxiety (worry), whereas the amygdaloid complex and its projections to the ventral portion of the periaqueductal gray are critically involved in the regulation of defensive freezing behavior in response to innate or conditioned aversive stimuli (Fanselow, 1994). Active defensive responses to proximal stimuli, generally associated with nociception, appear to involve the dorsal portion of the periaqueductal gray and its ascending projections to forebrain structures related to the sensorial processing of aversive stimuli (Vianna et al., 2001a).

The present review also found a remarkable relationship between anxiety-like responses during early development and adulthood. The USV lines were created to produce a developmental-genetic model system. The hypothesis is that autonomic and behavioral temperamental differences in infancy might cause behavioral or autonomic nervous system dysfunction in adulthood (Brunelli, 2005). The results appear to be encouraging because the USV High and Low lines selected for different rates of USV in

response to isolation during infancy and tested during adulthood presented reliable differences in several animal models, such as the open field, social interaction test, and elevated plus maze. Moreover, MR, THE, and HAB pups consistently presented more USV isolation calls than their respective counterpart lines/strains.

The fact that differences in emotionality in adulthood might be already present early in development converges with results from clinical studies, which have indicated that there is an influence of temperamental factors present in childhood on the development of anxious symptoms during adult life (Kagan and Snidman, 1999). These results are also in agreement with the conceptual distinction between trait and state anxiety. State anxiety refers to a transient condition that is only observable at particular moments and varies in intensity over time. Trait anxiety refers to a relatively permanent and stable characteristic that is less susceptible to influences by a particular state or situation (Cattell and Scheier, 1961).

1.8.2
Phenotype comparisons and possible methodological limitations

Importantly, one needs to be extremely careful when interpreting either the presence or absence of correlations/associations between two phenotypic traits (e.g., behavioral, anatomical, biochemical, etc.) in one or several pairs of selected lines. Therefore, a few genetic considerations about the selection method should be clarified. Firstly, two pairs of rat lines that are selected in different laboratories will differ, not only with regard to the behavioral method used to select them, but also in the genetic characteristics of their initial populations. Therefore, even if the foundation rat lines have the same name (e.g., Wistar), which is obviously often not the case, because they are outbred, each sample of animals screened in the first generation (S0) of each study has different polymorphisms for different genes. Selection can only act upon the genes that vary (i.e., are polymorphic) in that specific population. Behaviors are almost always polygenic (i.e., they are influenced by a myriad of genes). Thus, if two genes, A and B, are equally relevant to a trait, but each of them is polymorphic only in one of the two starting populations, future differences between the lines will be related to gene A only in one line pair and related to gene B only in the other line pair. Therefore, if genes A and B act through different neurobiological mechanisms, then the two analogous

genetic models (e.g., Maudsley and Roman) may display emotional similarities that are attributable to different underlying mechanisms. In conclusion, two traits that are correlated in one model and uncorrelated in another model, although they effectively share biological pathways, should not be surprising. Thus, two final lines could be equally fearful, for example, through different biological mechanisms.

Secondly, because of practical reasons, selection experiments in rodents can only be performed in relatively small samples of larger foundation populations. In such small samples, totally avoiding two genetic phenomena, namely genetic drift and inbreeding (Falconer and MacKay, 1996), is virtually impossible. Both these factors can produce significant increases or decreases in allele frequency, possibly leading to fixation, differentially in either the high or low selected line (e.g., 100% of allele "A" in the high line and 100% of allele "a" in the low line), and this may occur in any gene that has absolutely no effect on the selected trait. Consequently, these lines may differ in innumerable behavioral, anatomical, and biochemical traits that have nothing to do with the desired phenotype (e.g., emotionality), similar to any random pair of unselected inbred strains. Thus, significant correlated traits may be spurious unless they are proven to appear in different independent selected studies, which was the case for several behaviors discussed above, or in different replicate lines of the same study (Crabbe, 1999).

Finally, the importance of linked genes should not be overlooked. Because genes lie in chromosomes and because the starting rat populations may not be highly outbred, two neighboring polymorphic genes, if in linkage disequilibrium, tend to pass their alleles on to the following generations as a "package" (i.e., allele "A" together with allele "B" and allele "a" together with allele "b"). If only the A/a variation is relevant to the selected phenotype, then the final high/low lines will differ also for the B/b polymorphism and all of the cascading phenotypes influenced by B/b, thus creating an additional false positive result and possibly leading the neuroscientist to believe that fearfulness somehow relates to all of these accidental phenotypic differences.