INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

65

This reproduction is the best copy available.

UMI'

**Acoustic Startle and Fear-Potentiated Startle in Rats Selectively Bred for Fast and
Slow Kindling Rates: Relation to Monoamine Activity**

**A Thesis presented to the Faculty of Graduate Studies and Research of Carleton
University in partial fulfillment of the requirements of the degree of Master of Science
in Psychology**

**Owen P. Kelly
Institute of Neuroscience
Carleton University**

July, 2001

© Copyright 2001

Owen P. Kelly

**National Library
of Canada**

**Acquisitions and
Bibliographic Services**

**395 Wellington Street
Ottawa ON K1A 0N4
Canada**

**Bibliothèque nationale
du Canada**

**Acquisitions et
services bibliographiques**

**395, rue Wellington
Ottawa ON K1A 0N4
Canada**

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-66908-4

Canada

The undersigned recommend to the
Faculty of Graduate Studies and Research
Acceptance of the thesis:

*" Acoustic Startle and Fear-Potentiated Startle in Rats Selectively Bred for
Fast and Slow Kindling Rates: Relation to Monoamine Activity "*

submitted by

Owen Kelly

in Partial fulfillment of the requirements for
the degree of Master of Science

Chair
Thesis Supervisor

Carleton University
August, 2001

Abstract

The acoustic startle response, prepulse inhibition, fear-potentiated startle and monoamine activity induced by either a novel stimulus or a cue previously paired with footshock (fear conditioning) were assessed in rats selectively bred for differences in amygdala excitability (Fast vs Slow kindling epileptogenesis). Comorbid differences of anxiety, which were dependent both on the rats' behavioral style and the kind of stressor, also characterized these strains. In the present investigation, Slow rats exhibited a greater startle reflex to noise relative to Fast rats, suggesting differences in generalized anxiety, but similar rates of startle habituation and prepulse inhibition. In contrast, a profound fear-potentiated startle effect was readily observed only in Fast rats, however, this outcome could be influenced by manipulating both the shock and startle stimulus intensity employed, as well as by treatment with diazepam. With respect to stressor induced changes in neurochemistry, it was evident that when movement of the rat was restricted in a new environment, presentation of a novel stimulus (light) increased norepinephrine, dopamine and/or serotonin activity in brain regions typically associated with stressors (e.g., locus coeruleus, paraventricular hypothalamic nucleus). Generally, these effects were more pronounced in Fast rats, and norepinephrine utilization in the central amygdala was particularly highlighted in response to a conditioned fear stimulus. Thus, while generalized anxiety appeared greater in Slow rats, behavioral and central neurochemical reactivity to novel stimuli and to fear-eliciting stimuli was greater in Fast rats. Similarly, basal dopamine activity in the prefrontal cortex was greater in Fast rats, but dopamine utilization elicited by a novel stimulus was more pronounced in Slow rats. This suggested that relative to Slow

rats. dopamine neurons in prefrontal cortex of Fast rats do not react normally to environmental stimuli, possibly leading to disturbances of attention or impulsivity.

Acknowledgments

First, I wish to extend a great deal of thanks to Drs. Hymie Anisman and Dan McIntyre for their time and thoughtful advice on a number of areas, as well as for providing ideal conditions under which to conduct my research. Additionally, my thanks to Dr. Jerzy Kulczycki for performing the various biochemical assays employed in this study. Finally, I would like to acknowledge the support of my colleagues, family, the Dude Ranch, and the members of GordonGartrell throughout the duration of this project. This research was supported by a grant from the Medical Research Council of Canada.

Table of Contents

Title.....	i
Acceptance Sheet	ii
Abstract.....	iii
Acknowledgments.....	v
Table of Contents.....	vi
Figure Captions.....	vii
Introduction.....	1
Materials and Methods.....	5
Results.....	14
Discussion.....	25
Conclusion.....	33
References.....	35
Figures and Figure Captions.....	54
Appendix A.....	86

Figure Captions

Figure 1: Schematic diagram (adapted from Paxinos & Watson, 1998) of the anterior (left) and posterior (right) faces of the brain slices taken from three coronal levels (A,B and C) showing the areas punched or transected for the monoamine assays.

Figure 2: Mean startle amplitude ($\pm$ SEM) in FAST and SLOW rats as a function of startle stimulus intensity. Asterisk (*) denotes a significantly higher mean startle amplitude in the SLOW rats in comparison to FAST rats.

Figure 3: Mean startle amplitude ($\pm$ SEM) in FAST and SLOW rats as a function of prepulse and startle stimulus intensity. Prepulse intensity of zero indicates that the rats received only the main startle stimulus.

Figure 4: Mean startle amplitude ($\pm$ SEM) over 5 consecutive days among Fast and Slow rats exposed to 115 dB noise. Rats received 60 trials on each day, and each point represents the average of 5 trials.

Figure 5: Mean startle amplitude ($\pm$ SEM) for startle alone trials and CS- startle trials in FAST and SLOW. Double asterisks (**) denotes $P < 0.05$ relative to Slow kindling, while (*) denotes $P < 0.05$ relative to acoustic startle alone.

Figure 6: Mean startle amplitude ($\pm$ SEM) in Fast and Slow rats that had previously received 0, 5, 10 or 20 fear-conditioning trials. The startle amplitudes depicted are from the same set of animals, measured during noise alone (No CS trials; upper panel), or during trials where noise was preceded by the CS (middle panel). The lowest panel depicts the amplitude of startle responses during CS trials as a percentage of the No CS trials.

Figure 7: Mean startle amplitude (+SEM) for startle alone trials (startle) and CS + startle trials in Fast and Slow rats pretreated prior to testing with either vehicle or diazepam (0.5 mg/kg and 1.0 mg/kg).

Figure 8: Mean startle amplitude (±SEM) in Fast and Slow rats that had previously received 10 conditioning trials (0.3 mA), and were then pretreated with either vehicle or diazepam (0.25 mg/kg) and tested using a startle stimulus intensity of 100 dB. The startle amplitudes depicted are from the same set of animals, measured during startle alone trials and CS – startle trials.

Figure 9: Mean startle amplitude (±SEM) in Fast and Slow rats (n = 3/group) that had previously received 10 conditioning trials (0.6 mA), and were then administered either vehicle or diazepam (0.25 mg/kg) and tested using a 100 dB startle stimulus. Data are from the same set of animals, measured during noise alone (No CS trials), or during trials where noise was preceded by the CS.

Figure 10: Plasma corticosterone (±SEM) as a function of strain, conditioning and test stimulus. Asterisks (*) denote $P < 0.05$ relative to rats not presented with the test stimulus.

Figure 11: Mean (+SEM) levels of MHPG (upper panel) and 5-HIAA (lower panel) in the PVN among Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 13: Mean (+SEM) levels of MHPG in the central amygdala of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test)

Figure 14: Mean (+SEM) levels of 5-HIAA (lower panel) in the basolateral amygdala of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 15: Mean (+SEM) MHPG concentrations within the perihinal cortex (upper panel) and piriform cortex (lower panel) of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 16: Mean (+SEM) concentrations of MHPG (upper panel), DOPAC (middle panel) and 5-HIAA (lower panel) within the medial prefrontal cortex of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Introduction

Behavioral genetic analysis is a powerful tool to identify the mechanisms underlying pathophysiological states. In addition to congenic, transgenic, or knockout strains, lines of rats and mice selectively bred to exhibit a given phenotype can be used to identify specific biological correlates of the pathology (Crawley et al., 1997; Dudek & Underwood, 1993; Takahashi et al., 1994). Of course, owing to pleiotropic effects or linkage effects, the selection of a particular phenotype does not preclude these lines from differing in other respects (Parmigiani et al., 1999). Moreover, even modest environmental differences can profoundly influence strain-dependent outcomes (Crabbe et al., 1999).

In an effort to identify the mechanisms associated with temporal lobe seizures, we selectively bred two lines (strains) of rats differing in amygdala excitability, as realized by either Fast or Slow kindled seizure development (McIntyre, 1999a; Racine et al., 1999). Interestingly, these Fast and Slow kindling rats also display marked behavioral changes, such as increased anxiety in the Slow kindling rat, and problems with attention and/or impulsivity in the Fast kindling rat (Mohapel & McIntyre, 1998; Anisman et al., 1997; McIntyre & Anisman, 2000). For example, in an elevated plus-maze test, Slow-kindling rats were observed to emit fewer open arm entries than Fast-kindling rats (Mohapel & McIntyre, 1998). Moreover, it was noted that the Slow-kindling rats demonstrate more freezing in response to foot shock and longer step-down latencies in a passive avoidance test (Mohapel & McIntyre, 1998).

In parallel with the divergent behavioural profiles exhibited by these two strains, appreciable differences are present between the two lines in GABA_A subunit expression in several temporal lobe structures. In the amygdala, and adjacent piriform and perirhinal

cortices, but not the dorsal hippocampus. Fast rats over-express several embryonic subunit forms of the GABA_A receptor, including the $\alpha 2$, $\alpha 3$, $\alpha 5$ forms compared to controls and Slow rats (Poulter et al., 1999). In contrast, Slow rats underexpress those same embryonic subunits, while they dramatically overexpress the major adult form, $\alpha 1$ (Poulter et al., 1999). Interestingly, it has been noted (Maric et al., 1999) that the specific combination of these various α subunits with other receptor components (e.g. β , γ subunits) may influence the affinity for the benzodiazepine, diazepam.

Ordinarily, stressors provoke several neuroendocrine (Campeau et al., 1997; Cullinen et al., 1994; Kovacs & Sawchenko, 1996; Sapolsky et al., 2000) and monoamine alterations in several brain regions (Anisman et al., 1991; Deutch & Roth, 1990; Pacak et al., 1995; Stanford, 1995) thought to be part of the circuitry associated with anxiety (Gray, 1991; Swanson & Petrovich, 1998). Predictably, the neurochemical changes are highly individualistic, being dependent upon genetic factors (Anisman et al., 1991; Stanford, 1995) and the defensive style exhibited in response to particular stressors (Anisman et al., 1997, 2000; McIntyre et al., 1999b). Concordantly, plasma ACTH and corticosterone levels in Fast and Slow kindling rats correspond with the defensive style adopted by each strain in response to two separate naturalistic stressor situations (i.e. in general, the more active the defensive posture adopted, the higher the levels of plasma ACTH and corticosterone) (Anisman et al., 1997).

While corticotropin releasing hormone (CRH) within the central amygdala has been associated with anxiety, amine changes within mesolimbic regions may also contribute in this respect (Weiss & Simpson, 1989; Coco et al., 1992; Hashimoto et al., 1999; Kaneyuki et al., 1991; Wedzony et al., 1996). Given their differential behavioral

reactivity, the Fast and Slow strains provide an opportunity to evaluate monoamine changes in response to stressors in several amygdala nuclei and other cortical structures that may be associated with both stressors and seizure susceptibility.

The present investigation assessed levels of generalized anxiety in the Fast and Slow rats as indicated by the magnitude of the acoustic startle response to varying acoustic intensities (Davis, 1999). To ascertain that strain differences in startle were unrelated to attentional factors, prepulse inhibition (i.e. the ability of a weak lead stimulus to inhibit a reflex response) was also measured (Grillon & Davis, 1997). As well, fear-potentiated startle, a variant of the startle paradigm, wherein the amplitude of the startle reflex can be modified by the transient presentation of a conditioned stimulus was examined as this behavior may be aligned with focused anxiety (Davis & Shi, 1999). In parallel with the behavioral studies, it was determined whether novel environmental cues, or cues that had been associated with a stressor in the establishment of a conditioned fear response, differentially influenced plasma corticosterone concentrations and central monoamine activity in the Fast and Slow strains.

Finally, as it has been suggested that differences are present in these lines with respect to both stressor reactivity and GABA_A subunit expression, it was hypothesized that the Fast and Slow-kindling rats would differ in their response to the positive GABAergic modulator diazepam. In that previous studies (Bitsios et al., 1999; Davis, 1986; Melia & Davis, 1991; Patrick et al., 1996) have demonstrated diazepam to be effective in attenuating the fear-potentiated startle response in both rodents and in humans, it was of interest to determine whether diazepam would differentially attenuate the fear-potentiated startle effect across the two rat lines. Specifically, owing to the considerable disparity observed between

the two lines with respect to GABA_A receptor morphology, it was expected that the Slow-kindling rats would be more readily affected by the anxiolytic effects of diazepam than their Fast-Kindling counterparts. However, as subtypes of anxiety exist (e.g., general anxiety vs. anxiety that is stimulus bound), it is possible that diazepam may differentially influence startle and fear-potentiated startle in these two rat lines.¹

---

¹ Note - For the sake of simplicity a brief introduction to the topic is given here. However, the interested reader may wish to read the more detailed review of the literature presented in Appendix A.

Materials and Methods

Startle, Prepulse Inhibition and Fear-Potentiated Startle

Subjects

The Fast and Slow strains of rats were selectively bred from an original parent population comprised of Wistar and Long-Evans Hooded rats. These strains, initially established at McMaster University (Hamilton, Ontario), were selectively bred without brother x sister matings (Racine et al., 1999). Following the F₁₁ generation, the strains were transferred to Carleton University, Institute of Neuroscience, where the strain differences persisted despite relaxation of the selection procedure. The rats used in the present investigation were from the 43rd and 44th generations of the two strains. All rats were at least 4 months of age, and allowed free access to food and water. The rats were maintained on a 12-h light-dark cycle, and housed as pairs in standard opaque plastic cages (32 cm x 22 cm x 20 cm). All testing was conducted between 0800 and 1200 hr to minimize the influence of endocrine and central neurotransmitter variations attributable to diurnal rhythms. The experiments met the guidelines set out by the Canadian Council of Animal Care with respect to the care and use of animals for experimental procedures, and received ethical approval from the Carleton University Animal Care Committee.

Apparatus

Startle measurements were made using an animal startle reflex system (MED Associates, St. Albans, Vermont). This system consisted of two ventilated, sound attenuating startle chambers (30 cm x 55 cm x 50cm), a rack mounted operating station, and a standard IBM compatible PC. In each chamber, a wideband speaker (1 kHz - 16 kHz) provided the audio source for startle stimuli, as well as background noise, while a

startle sensor platform, signal transducer and load cell amplifier served to measure the animals startle response. When downward pressure was exerted by the animal in response to a startle stimulus, the resultant force was converted to an analog signal by the sensor platform, amplified by the load cell amplifier and then digitized on a scale of 0 - 2047 arbitrary units by a Dig-729ADC card. The presentation and ordering of all stimuli were controlled by the startle reflex system software (version 4.21) (MED Associates, St. Albans, Vermont).

Procedures

Startle response as a function of stimulus intensity: Rats ($n = 8/\text{strain}$) were taken from their home cage and placed in a clear, cylindrical acrylic holder (7.6cm x 14cm), which was attached to a rectangular base that could be fastened to the startle platform. The holders were ventilated by a long slot running the length of the tube, as well as by slots placed at both ends of the tube, adjacent to the position of the animal's head. Once the animal had been secured in the holder using two removable panels placed at opposite ends of the tube, the holder was secured to the startle platform, which was positioned approximately 20 cm for the audio source. The chamber was then sealed and each animal allowed to acclimate to the startle chamber for a period of 5 minutes. Throughout the length of the experiment, low levels of ambient light were generated in each chamber by a 3-watt red filtered light. Additionally, background white noise with an intensity of 45 dB was maintained to minimize the impact of acoustic stimuli outside of the chamber environment.

Once the habituation period had elapsed, each animal was presented five baseline startle stimuli (50 ms pulse of white noise at 115dB) at an interstimulus interval of 30

seconds. The purpose of these baseline trials was to familiarize the animal with the startle stimulus in order to facilitate more accurate measurement of the animal's overall startle amplitude. After the final baseline trial had been delivered, each animal was then randomly presented 70 startle stimuli, each of which was 50 ms in duration, but varied in intensity from 55 dB to 115 dB, in 10 dB increments. Once the 70 randomized startle trials had terminated, each animal was removed from the startle chamber and returned to its home cage. Following this, the acrylic holder, as well as all components within the startle chamber, were wiped down with alcohol (70%) to minimize residual olfactory cues.

Prepulse Inhibition: The procedure was identical to that of the initial experiment ($n = 8/\text{strain}$), except that baseline trials were either 115 dB (for the Slow rats) or 125 dB (for the Fast rats). As the Fast and Slow rats differed substantially in their acoustic startle response, these noise intensities were used in order that equivalent startle amplitudes would be achieved in the absence of a prepulse inhibitory stimulus. Preliminary studies indicated that the strain difference was minimal using these particular stimulus intensities. After the last baseline trial, the Slow rats were randomly presented either 70 randomized startle trials using a 115 dB startle stimulus, which was either presented alone or preceded by a prepulse stimulus ranging between 55 dB and 105 dB, in 10 dB increments. The Fast rats were presented with 70 randomized startle trials of a 125 dB startle stimulus, which was either presented alone or preceded by a prepulse stimulus ranging between 65 dB and 115 dB, in 10 dB increments. All prepulse stimuli preceded the startle stimulus by 50 ms, and each stimulus combination was presented 10 times.

Startle Habituation: The general procedure utilized in this component of the investigation was similar to that used in the first experiment; however, on each of 5 consecutive days rats ($n = 5/\text{strain}$) received 60 startle trials (50 ms pulse of white noise at 115dB) at an inter-stimulus interval of 30 seconds. Moreover, baseline trials were omitted in order to more precisely characterize the pattern of habituation exhibited by both strains. Once the 60 startle trials had terminated, animals were returned to their home cage.

Fear-potentiated Startle: One week after the habituation session, the rats of the preceding experiment were placed in a holder (16.5 cm x 7.6 cm x 8.9 cm) constructed of stainless steel rods and PVC ends. The 4.8 mm grid rods at the bottom of the holder could be electrified, while the 2.4 mm side and top rods could not be electrified. The holder was attached to a rectangular base that could be fastened to the startle platform. Once the animal had been secured in the holder, it was secured to the startle platform. Each animal was re-acclimated to the startle chamber for 5 minutes. Following habituation each animal was presented 20 light-shock pairings, at 30 sec intervals. The shock (0.6 mA) was presented during the last 500 ms of the 5 sec light. The light stimulus was delivered via a 3-watt, incandescent light-bulb fastened to the inside wall of the startle chamber. Once the conditioning trials were completed, the animals were removed from the grid holder and returned to their home cages.

The testing procedure was similar to that used in the initial experiment; however, 20 rather than 5 baseline trials were administered prior to testing. Additionally, startle measurements were made in the same grid holder that was used to condition the animals. After the final baseline trial had been delivered, each animal was randomly presented 10 startle stimuli, each of which had an intensity of 115 dB and a duration of 50 ms. On half

the trials. the startle stimulus was presented in conjunction with the conditioned stimulus (CS: light). Startle stimuli paired with the CS were delivered the last 50 ms of the 5.0 sec light presentation. It might be noted that as animals had previously received startle habituation training, the startle amplitude was reduced and was comparable in the two strains, thereby permitting unadulterated evaluation of the effects of the fear conditioning.

Fear-potentiated Startle following differing numbers of CS-shock pairings: It has been reported that if the fear response is too great, then the fear-potentiated startle response may be less profound than that observed following the establishment of a moderate fear response (Walker et al., 1997). The procedure of the preceding experiment involved a fairly large number of CS-shock pairings, and testing was conducted in the same apparatus in which shock had been delivered. In order to assess the effects of fear conditioning under a broader set of conditions in the two rat strains, the effects of differing numbers of CS-shock pairings on fear-potentiated startle was evaluated in a chamber that differed from that in which the initial treatment was administered. Rats received either 0, 5, 10 or 20 trials ($n = 7-8/\text{strain}$) in which the CS and shock were paired, following the identical procedures described in the earlier fear-potentiated startle experiment. Rats were then returned to their home cages and 24 hr later tested in the fear-potentiated startle paradigm, as already described, except that rats were tested in the cylindrical holder described in the first experiment (i.e., no grid floor), rather than in the shock chamber.

Startle and Fear-potentiated Startle following administration of diazepam: Animals ($n = 8-11$ /strain per treatment condition) were first conditioned using the same procedure as that employed in the initial fear-potentiated startle experiment. Following training, each animal was injected with either diazepam (1.0 or 0.5 mg/kg, i.p.), or saline over three consecutive days, in order to diminish motoric effects which could have impeded measurement of the startle reflex. On the fourth day each animal was again administered either diazepam (1.0 or 0.5 mg/kg, i.p.) or saline and then returned to their home cage for 30 minutes. All animals were then tested in the fear-potentiated startle paradigm, as already described, except that rats were again tested in the cylindrical holder described in the first experiment (i.e., no grid floor), as opposed to the shock chamber

Monoamine variations associated with fear conditioning

The fear conditioning procedure was similar to that used in the behavioral studies, although several distinct changes were made. Rats of each strain ($n = 40$ /strain; 10 rats in each experimental condition) were placed in the startle apparatus for a 5 min habituation period. Thereafter rats received either no treatment (no conditioned group) or 20 trials where a light stimulus (3.5 sec duration) was paired with 0.6 mA footshock (conditioned group). The footshock was presented during the last 0.5 sec of the light and terminated with light offset. The intertrial interval was 60 sec. Following the training session, rats were returned to their home cages and then tested 24 hr later. The test procedure consisted of rats of each strain being assigned to one of two conditions. Half the fear-conditioned rats were placed in the apparatus (without the grid floor) and exposed to 10 light (CS) presentations (3.5 sec duration, 1 min apart), while the remaining animals received similar handling, but were not placed in the apparatus and exposed to the light.

Five minutes following stressor termination, rats were decapitated by guillotine. Trunk blood was collected and centrifuged to obtain plasma, and the brain of each rat was quickly removed and placed in ice-cold PBS for 2 min. Using a brain blocker (Carleton University, Science Technology Centre, Ottawa, Canada) that produced slices in the Paxinos & Watson (1986) plane, the brain was dissected into 1.0 mm thick coronal slices. Brain regions were micropunched from the slices in ice-chilled buffered saline and transferred to tubes and frozen on dry ice. At the time of punching, the ice-chilled brain slices were verified against the Paxinos and Watson (1998) atlas, and brain punches were taken according to this atlas and that of Palkovits and Brownstein (1988). Brain structures that were pooled bilaterally included the paraventricular nucleus of the hypothalamus (PVN), locus coeruleus, medial prefrontal cortex (PFC), perirhinal cortex, piriform cortex, hippocampus and three nuclei of the amygdala (central, medial and basolateral). Figure 1 provides a schematic representation of the anterior and posterior faces of the brain slices showing the areas punched or transected for the monoamine assays. Frozen tissue samples were stored at -80°C for determination of amine and metabolite concentrations.

Insert Figure 1 about here

Neurochemical determinations: Plasma corticosterone was determined, in duplicate, using RIA kits (ICN Biochemicals, Costa Mesa, CA). The intra-assay variability was less than 10%, and to eliminate interassay variability, plasma corticosterone levels were determined within a single run. Levels of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) and their respective metabolites, MHPG, DOPAC and 5-HIAA were determined in tissue punches

by high performance liquid chromatography (HPLC) with electrochemical detection using a modification of the method of Seegal et al. (1986), as described in Lacosta et al. (1998). Tissue punches were sonicated in a homogenizing solution comprised of 14.17 g monochloroacetic acid, 0.0186 g disodium ethylenediamine tetraacetate (EDTA), 5.0 ml methanol and 500 ml H₂O. Following centrifugation, the supernatants were used for the HPLC analysis. Using a Waters Corp. (Mississauga, Ontario) M-6000 pump, guard column, radial compression column (5 μ , C₁₈ reverse phase, 8mm x 10cm) and a three cell coulometric electrochemical detector (Model 5100A; ESA Inc., Bedford Mass.), a 20 μ l aliquot of the supernatant was injected into the column at a flow rate of 1.5 ml/min (1400-1600 PSI). The mobile phase used for the separation was a modification of that used by Chiueh et al. (1983); each liter consisted of 1.3 g of heptane sulfonic acid, 0.1 g disodium EDTA, 6.5 ml triethylamine, 35 ml acetonitrile. The mobile phase was then filtered (0.22 μ m filter paper) and degassed following which the pH was adjusted to 2.5 with phosphoric acid. The area and height of the peaks was determined using a Hewlett-Packard (Mississauga, Ontario) integrator. The protein content of each sample was determined using bicinchoninic acid with a protein analysis kit (Pierce Scientific, Rockford, Ill.) and a spectrophotometer (PC800 colorimeter; Brinkman Instruments, Mississauga, Ontario).

Statistical Analyses

The behavioral data in the strains of the two initial experiments (effects of stimulus intensity and prepulse inhibition) were analyzed by a mixed measures analysis of variance (ANOVA) with stimulus intensity and prepulse stimulus intensity as within-group variables, respectively. The data of the third experiment (habituation) were analyzed as a mixed measures ANOVA with both Days and Trials within days as within group variables.

Finally, the data of the fear-potentiation studies were analyzed by mixed measures ANOVAs with Strain, CS-shock pairing and/or drug treatment (in three studies) as between-group variables, and stimulus presentation (startle stimulus vs cue plus startle stimulus) as a within subject variable. Newman-Keuls multiple comparisons ($\alpha = 0.05$) were conducted to evaluate the significant treatment main effects or simple effects of significant interactions.

The neurochemical data were analyzed as three-way factorials with Strain, Conditioning procedure and Test stimulus as independent variables. In several instances, technical problems resulted in amine or metabolite concentrations not being obtained, and hence the degrees of freedom varied across the analyses. For each of the brain regions examined the number of subjects in each condition for each strain was equal to either 9 or 10. When *a priori* predictions had been made concerning specific interactions, comparisons were conducted of simple effects comprising the interaction, irrespective of whether the F value for the interaction reached **statistical significance**. Owing to the multiplicity of between-group comparisons, family-wise error was maintained at 0.05 by using Bonferroni corrections.

Results

Startle as a function of stimulus intensity

In the initial behavioral experiment, the startle response varied as a function of the Strain of rat x Startle intensity interaction $F(7, 98) = 17.70$, $P < 0.01$. There was a significant trend for the startle amplitudes of the Slow rats to increase more quickly as a function of stimulus intensity. The multiple comparisons revealed that in comparison to the Fast rats, the startle amplitude of Slow rats was elevated at the 105 and 115 dB intensities (see Figure 2).

 Insert Figure 2 about here

Prepulse Inhibition

Figure 3 shows the startle amplitude in each strain as a function of the intensity of the prepulse stimulus. As the amplitude of the startle response in the strains was equated using a stimulus of 115 dB in the Slow rats and 125 dB in the Fast rats, the Figure shows the prepulse stimuli as intensities differences from the strain-specific startle stimulus. The ANOVA indicated that startle amplitude was affected by the prepulse stimulus in both the Fast and Slow rats, $F(7, 98) = 46.41$; $P < 0.01$. The multiple comparisons indicated that when the intensity of the prepulse stimulus and the startle stimulus were similar, the inhibition of the startle response was maximal. On trials where the two stimuli were relatively similar (differing from one another by less than 40 dB), the effects of the various prepulse stimuli could not be distinguished from one another. The more the intensity of the prepulse stimulus and the startle stimulus differed, the smaller the inhibition. Neither the Strain main effect nor the Strain x Prepulse intensity interaction approached significance (P

> 0.05). indicating that the prepulse inhibition was similar in the Fast and Slow rats (see Figure 3).

 Insert Figure 3 about here

Startle Habituation

Figure 4 shows the startle response of the two strains, collapsed over 5 trials, on each of the 5 test days. The ANOVA confirmed that the Slow rats exhibited a higher startle amplitude than did the Fast rats, $(1, 8) = 39.94$, $p < 0.01$. While the startle amplitude declined over Days and Trials within a day, the interactions of these variables with the Strain of rat did not reach statistical significance. Nevertheless, it appeared that the marked strain differences evident on the initial test day were greatly diminished on the fifth test day. In fact, during the latter half of this final test session, the startle responses in the two strains were similar.

 Insert Figure 4 about here

Fear-potentiated Startle

Analysis of the fear-potentiated startle data among rats tested in the shock chamber revealed that startle amplitude varied as a function of the Strain x Stimulus interaction, $F(1, 8) = 10.34$, $p < 0.01$. The *post hoc* comparisons indicated that on trials preceded by the CS, the startle amplitude was greater than that elicited by the noise stimulus alone. However, as shown in Figure 5, the magnitude of the fear-potentiated startle was greater

among the Fast than among the Slow rats, such that the fear-potentiated startle response differed between the strains. It will be noted that the two strains of rats had undergone startle habituation previously, and hence in this experiment the startle response in the absence of the CS was similar in the two strains.

 Insert Figure 5 about here

Fear-potentiated Startle following differing numbers of CS-shock pairings

Figure 6 shows the startle amplitude among Fast and Slow rats on trials where the startle stimulus was not preceded by the conditioned fear stimulus (no CS trials), and on trials where the CS was presented (CS trials). As seen in the figure, without the CS, the startle amplitude was greater in Slow than in Fast rats, $F(1, 54) = 19.51$, $p < 0.01$, and did not vary as a function of the previous treatments (number of CS-shock pairings). In contrast, on trials where the CS preceded the startle stimulus, the startle response was augmented in both strains, $F(3, 54) = 2.82$, $p < 0.01$. Among Fast rats that had received prior fear conditioning, the extent of the increase was greater than among Slow rats, such that the amplitude of the response was now similar in the two strains. Indeed, the proportionate change between trials where the CS was not presented and those in which the CS was presented (Figure 6, lowest panel) varied as a function the Strain x Fear conditioning interaction, $F(3, 54) = 4.74$, $p < 0.01$. The multiple comparisons confirmed that the startle amplitude in the Slow rats that had received the fear conditioning was not different from those that had not been fear conditioned. In contrast, the startle response was progressively greater in the Fast rats with increasing number of fear conditioning trials.

 Insert Figure 6 about here

Startle and fear-potentiated startle: Effects of Diazepam

The effects diazepam on startle and fear-potentiated startle among Fast and Slow seizing rats are shown in Figure 7. The analysis of variance indicated that startle responsivity in the absence vs presence of the fear cue varied as a function of rat Line. $F(1, 42) = 6.20, p = 0.01$. The multiple comparisons of the simple effects indicated that in the absence of the CS the startle amplitude in Slow rats was 2-fold greater than in the Fast Line. In the Slow rats the presence of the fear cue (CS) did not significantly increase the startle amplitude, but did so in the Fast rats so that the difference of amplitude between the lines was eliminated. Treatment with Diazepam reduced the startle amplitude in both rat lines and during both CS and non-CS trials. $F(2, 42) = 7.83, p < 0.01$. This effect was evident at both doses. Although the magnitude of the effect appeared to be somewhat greater among Slow rats, the Drug x Rat line interaction was not statistically significant.

 Insert Figure 7 about here

As there was no effect of the drug treatment, and the lines differed with respect to the appearance of the fear-potentiated startle, two additional pilot experiments (using a limited N of 4 and 3/group, respectively) were conducted to ascertain whether the fear conditioning procedures, and the baseline levels of startle intensity were too great to permit expression of a fear-potentiated response in the Slow rats. That is, these animals may

already have been exceptionally anxious, and hence a decline of a startle response would occur in the presence of still greater arousal (Walker et al., 1997, Davis et al., 1999). In the first of these sub-experiments rats received 10 instead of 20 fear conditioning trials using a shock intensity of 0.3 mA rather than 0.6 mA, and during testing the acoustic stimulus was 100 dB rather than 115dB. In the second experiment the procedure was exactly the same except that the shock intensity was 0.6 mA. As well, as the two doses of diazepam elicited approximately equal effects, a still lower dose of diazepam was employed in both sub-experiments (0.25 mg/kg), as the possibility was considered that reducing the anxiety somewhat in Slow rats would lead to an increase of the startle response in the Slow line. Otherwise, the procedures were identical to that of the preceding study.

Startle amplitude as a function of the Rat line and Drug treatment is depicted in Figure 8. Neither the main effect of the Line or Drug treatments, nor the interaction of these variables significantly impacted startle or fear-potentiated startle amplitude. Likely, this stemmed from the small N and relatively great variability associated with the test procedure. Nevertheless, it is of interest that with a smaller number of conditioning trials, reduced shock intensity and lower startle stimulus intensity, a modest increase (~30%) of startle amplitude was apparent in the presence of the fear CS in the Slow rats, whereas no such increase was evident in the Fast line. Diazepam was generally without effect in either strain, although a general increase of fear-potentiated startle was evident in Fast rats. This, however, was attributable to the behavior of a single animal that exhibited a very marked (>400%) elevation of the startle response.

 Insert Figure 8 about here

In the second experiment an overall effect was apparent wherein the startle response was increased on CS trials relative to that evident on non-CS trials, $F(1, 8) = 8.77$, $p < 0.01$. However, as seen in Figure 9 a fear-potentiated startle response was not evident in saline treated Slow rats, but was in their Fast counterparts. Interestingly, in the Slow rats treated with diazepam, the fear-potentiated startle response was two-fold greater than that evident on non-CS trials. Interactions between the CS and non-CS trials with Rat line or Drug treatment did not reach significance, possibly owing to the limited sample size. Nevertheless, these data are consistent with the supposition that Slow rats are indeed more anxiety-prone than Fast rats, and under conditions where excessive arousal is diminished, in this instance by anxiolytic treatment, fear-potentiated startle is enhanced.

 Insert Figure 9 about here

Neurochemical changes elicited by cues associated with footshock

Plasma corticosterone levels were increased among rats that were exposed to the light cue, $F(1,71) = 32.15$, $p < 0.01$, irrespective of whether or not they had previously received the conditioning treatment (see Figure 10).

 Insert Figure 10 about here

Likewise, simply exposing rats to the light cue effectively increased NE utilization within the PVN, as reflected by increased MHPG accumulation. $F(1, 71) = 47.16$, $P < 0.01$. This was the case in both strains and was independent of prior conditioning procedures. The concentrations of 5-HIAA, in contrast, varied as a function of the Conditioning procedure x Test cue interaction. $F(1, 69) = 6.33$, $P = 0.01$. Subsequent comparisons confirmed that presentation of the test cues increased 5-HIAA among rats that had received the fear conditioning, relative to rats that had not been conditioned or had not been exposed to the conditioning cue on the test day (see Figure 11). The magnitude of the conditioning effect appeared to be greater in Fast than in Slow rats, but the interactions involving the Strain variable did not reach statistical significance.

Insert Figure 11 about here

The activity of NE within the locus coeruleus could readily be distinguished from that seen in the PVN. In particular, while the strains did not differ from one another with respect to MHPG or NE levels in the absence of treatment, MHPG accumulation varied as a function of the Strain x Test cue interaction. $F(1, 71) = 8.44$, $P < 0.01$. The multiple comparisons confirmed that exposing the rats to the light stimulus provoked a marked increase of MHPG in Fast rats, but not in the Slow strain. The increase was apparent irrespective of the conditioning procedure applied previously .

Insert Figure 12 about here

By contrast, hippocampal NE levels as well as the accumulation of MHPG were higher in the Slow than in the Fast rats. $F(1, 69) = 10.81, 7.52, p's < 0.01$. A modest rise of MHPG was elicited by the test cues, but this effect was not significant. Like NE, the levels of 5-HT within the hippocampus of Slow rats exceeded that of Fast rats. $F(1, 70) = 7.42, p < 0.01$, while 5-HIAA was comparable in the strains, irrespective of the treatment or test conditions.

Within the central amygdala the accumulation of MHPG varied as a function of the Strain x Conditioning treatment x Test cue interaction. $F(1, 67) = 7.17, p < 0.01$. As shown in Figure 13, and confirmed by the multiple comparisons, MHPG was elevated in Fast rats that had received conditioning and then reexposure to the test cues relative to similarly treated Slow rats or Fast rats that had not been reexposed to the conditioning cues. In contrast, neither levels of NE, DA, 5-HT, DOPAC, HVA nor 5-HIAA were affected by the conditioning or test procedures.

 Insert Figure 13 about here

The effects of the conditioning procedure seen in the central amygdala were not apparent within other amygdala nuclei. In the basolateral nucleus, 5-HIAA accumulation was increased upon exposure to the test cues. $F(1, 69) = 6.31, p < 0.01$, but this effect was independent of the initial conditioning treatment. The magnitudes of the effects were somewhat greater in the Fast than in the Slow rats, but the interaction with this variable did not reach statistical significance. Nevertheless, as *a priori* predictions had been made concerning differential reactivity between the strains, multiple comparisons were

conducted of the simple effects of the Strain x Conditioning x Test stimulus interaction. These comparisons indicated that the test stimulus significantly increased 5-HIAA in the Fast rats, irrespective of prior training, but had no effect in the Slow rats (see Figure 14). As in the case of 5-HIAA, the accumulation of MHPG was also increased by the test cue, but this effect was comparable between the strains and the Conditioning x Test cue interaction was not statistically significant. Finally, while DOPAC was not markedly affected by the treatments, HVA accumulation was appreciably increased in response to the test cue, $F(1, 68) = 8.84$, $P < 0.01$. The extent of the increase was relatively large (66%), but did not vary as a function of either the Rat strain or the Conditioning procedure (data not shown).

 Insert Figure 14 about here

Within the medial amygdala, the accumulation of 5-HIAA was increased by the test cue, $F(1, 69) = 6.66$, $P < 0.01$, but this effect did not interact with either the Strain or Conditioning variables. The accumulation of DOPAC, HVA and MHPG was modestly elevated by the test cue, but in no instance was the magnitude of the effects statistically significant.

NE and MHPG levels in the perirhinal cortex were similar in the two rat strains. Moreover, in both strains the presentation of the test cue, regardless of whether rats had received light-shock pairings, increased the accumulation of MHPG (see Figure 15). Neither perirhinal DA nor DOPAC varied between the strains, and were also unaffected by

the applied treatments. The accumulation of 5-HIAA within the perirhinal cortex likewise did not vary as a function of the Strain or the treatments applied.

The levels of NE and MHPG within the piriform cortex were also comparable in the two strains. However, MHPG accumulation was found to vary as a function of the Strain x Test cue interaction, $F(1, 71) = 3.29$, $P = 0.05$. As seen in Figure 15, among Fast rats the cue resulted in elevated MHPG accumulation, but not in Slow rats. Unlike MHPG, the accumulation of DOPAC was increased by the test cue in both Strains, $F(1, 71) = 7.04$, $P < 0.01$. In contrast, 5-HIAA accumulation was unaffected by the Strain or Treatment variables.

 Insert Figure 15 about here

The monoamine and metabolite levels within the PFC were quite different from those of other cortical regions. The concentration of NE was found to be higher in Slow than in Fast rats, $F(1, 70) = 11.63$, $P < 0.01$, while MHPG accumulation was comparable in the strains. Exposure to the test cue increased MHPG accumulation, irrespective of the strain, $F(1, 70) = 7.13$, $P < 0.01$ (see Figure 16). While the levels of DA were comparable in the two strains, the accumulation of DOPAC varied with the Strain x Test cue interaction, $F(1, 69) = 5.75$, $P < 0.01$. As seen in Figure 16, in the absence of cue presentation DOPAC accumulation was higher in Fast than in Slow rats. Presentation of the test cue resulted in an increase of DOPAC in the Slow rats, whereas a nonsignificant decline was evident in the Fast rats. Finally, 5-HIAA was elevated following exposure to the test cue, $F(1, 69) = 14.83$, $P < 0.01$. This effect was somewhat more pronounced in the

Fast rats, although the Strain x Test cue interaction was not statistically significant. Nevertheless, *post hoc* comparisons indicated that the increase of 5-HIAA was significant only in the Fast rats (Figure 16).

Insert Figure 16 about here

Discussion

It has been reported that Fast and Slow kindling rats differ with respect to several anxiety-related behaviors. Upon stressor exposure, Slow rats typically adopt an immobility posture, whereas Fast rats remain very active. Predictably, the immobility of the Slow rats favors proficient inhibitory avoidance performance, while disrupting acquisition of an active avoidance response (Mohapel & McIntyre, 1998). However, it was subsequently observed that the defensive styles of these rat strains were also dependent on the specific characteristics of the stressors. Upon being restrained, Fast rats display persistent struggling and emit vigorous efforts to escape, whereas Slow rats typically adopt an immobile posture. By contrast, when exposed to a ferret, the Fast rats displayed a greater degree of immobility than did the Slow rats (Anisman et al., 1997; McIntyre & Anisman, 2000).

In the present investigation, it was again observed that the behavioral responses of Slow and Fast rats were dependent upon the specific characteristics of the test situation. The Slow rats exhibited a more pronounced startle reflex in response to an acoustic stimulus. However, as the strains displayed a similar prepulse inhibitory effect, it is unlikely that the differential response to the startle stimulus (in the absence of the prepulse stimulus) was related to differences in attention to the acoustic stimulus (Grillon & Davis, 1997). Of course, this does not imply that attentional processes in the strains were similar in other test situations, particularly in tests involving visual stimuli or in the presence of distracter cues. Indeed, we have suggested that Fast rats may suffer from associative impairments, possibly secondary to impulsivity or disturbances of attention (McIntyre &

Anisman, 2000), and display signs of anxiety and/or impulsivity reminiscent of observations in epileptic patients (Gloor, 1992; Herman & Whitman, 1984).

In contrast to the acoustic startle response, the fear-potentiated startle response was greater in the Fast than in the Slow rats under conditions where a large number of relatively aversive conditioning trials were employed. It had been suggested that while the startle response to an acoustic stimulus is reflective of general anxiety, possibly involving the bed nucleus of the stria terminalis, fear-potentiated startle may be more closely aligned with a focused or stimulus-bound anxiety involving the central amygdala (Davis et al., 1997; Lee & Davis, 1997). Thus, it may well be that general anxiety levels are greater in Slow than in Fast rats, but to explicit fear evoking stimuli, a more pronounced reaction was seen in Fast rats. It is possible, of course, that the anxiety of the Slow rats was so great that it precluded potentiation effects. Indeed, it has been suggested by others that high levels of fear will mute potentiated startle (Walker et al., 1997). Yet, in exploring this issue further, using a smaller number of fear conditioning trials (5, 10 and 20), fear-potentiated startle was still not observed in the Slow rats. This does not rule out the possibility that potentiation effects could be similar or even greater in the Slow rats under other experimental conditions. The rigid appearance and tenacious grip of these rats on the grid bars in these experiments encourages the suggestion that the generalized anxiety provoked by the test situation itself in the Slow rats was sufficiently high to preclude potentiation of the startle response, independent of the number of conditioning trials.

Accordingly, in three further studies it was ascertained whether attenuation of both the shock and startle stimulus intensity, as well as administration of the anxiolytic, diazepam, would be successful in provoking fear-potentiated startle in the Slow-kindling

rats. Indeed, administration of a low dose of diazepam (0.5 mg/kg) was able to produce a small fear-potentiated startle effect in Slow-kindling rats that were trained and tested using established shock and startle stimulus parameters. In the second experiment, upon reducing both the number and strength of conditioning trials, as well as the startle stimulus intensity (to prevent ceiling effects from precluding further enhancement), modest fear-potentiation was apparent in the Slow rats, whereas no such increase was evident in the Fast line. Interestingly, once shock intensity was increased (but the conditioning trials maintained at the low level of the preceding study), the fear-potentiated startle response was not evident in saline treated Slow rats, but was again apparent in their Fast counterparts. However, in the Slow rats treated with diazepam the fear-potentiated startle response was twice that seen on non-CS trials. These data suggest that the generalized anxiety provoked by the test situation itself is indeed sufficient to attenuate potentiation of the startle response in the Slow rats. Of course, given the small number of subjects tested (owing to the limited availability of these selectively bred lines), firm conclusions must await further behavioral analyses.

One aim of the present investigation was to assess the effects of conditioned fear cues on endocrine and monoamine changes in the Fast and Slow rats. In this respect, it was of particular interest to examine the effects of the treatments under conditions that approximated those in which the fear-potentiated startle test was assessed. It is important to underscore that in measuring fear-potentiated startle the test situation itself, quite apart from the application of the conditioned fear cues or the startle stimulus, may have prominently influenced neurochemical activity. Moreover, presentation of the test stimulus, irrespective of whether that stimulus had previously been paired with footshock, may have influenced

neurochemical functioning. Ordinarily, in studies that assess the effect of stressors, such as restraint or immobilization, the procedure involves restriction of the animals movement, the extent of which is determined by the type of restraining device employed. In the present investigation, rats were not restrained in the commonly used sense; however, they were placed in a Plexiglas container that severely constrained their movement. When these rats were thus restricted, and were also exposed to a light stimulus (even if animals had not previously undergone fear conditioning), plasma corticosterone levels were elevated, suggesting that this procedure was perceived as being stressful - although the magnitude of the response was not as pronounced as those observed previously following predator exposure or immobilization (McIntyre et al., 1999).

It is curious that plasma corticosterone in response to the light cue was not greater in fear-conditioned animals relative to controls. The reason for the absence of such an effect is not immediately evident, as the fear conditioning procedure reliably increased startle amplitude, and also influenced monoamine activity in specific brain regions. Furthermore, given that Fast rats exhibited a greater fear-potentiated startle response, it was expected that the cues associated with the stressor would also induce a greater rise of corticosterone in that strain. However, blood was taken at only one time point following the stressor experience, and it is certainly possible that the corticosterone changes are induced more readily, and perhaps persist for a more extended period in the fear conditioned rats than in rats not previously exposed to cue-shock pairings. Indeed, in studies where *in vivo* arterial blood samples were collected before, during and after stressor exposure, the elevations of corticosterone in response to different types of stressors was greater in the Slow than in the Fast rats (Kent et al., 1996).

As previously observed in response to relatively intense challenges (e.g., footshock, restraint, predator exposure), the psychogenic stressor of simply being exposed to a series of light stimuli (after being placed in a novel environment, where movement was restricted) increased monoamine activity within brain regions often associated with stress and/or anxiety (Anisman et al., 1991). In the present investigation, this included increased NE activity within the locus coeruleus, PVN, and PFC, as well as increased DA activity within the PFC. In addition, the treatment provoked monoamine changes in several amygdaloid nuclei, and both the perirhinal and piriform cortices. Interestingly, some of these changes varied between the two rat strains where the Fast rats tended to show greater monoamine utilization in response to the stimulus relative to the Slow rats. This was the case with respect to MHPG elevations in the locus coeruleus, and in the piriform cortex, and to some extent with respect to 5-HIAA in the basolateral amygdala and PFC. These data are consistent with the effects previously observed in Fast and Slow rats exposed to ferret and restraint stressors (McIntyre et al., 1999), supporting the view that the light stimulus was acting as a stressor. The fact that these various stimuli all led to similar monoamine changes, yet provoked different behavioral response profiles (recall that restraint and ferret exposure induce strain-dependent effects) suggests that the monoamine and behavioral effects of these stressors may be independent of one another.

It has been reported frequently that cues that had been associated with a stressor may come to promote central neurochemical activity. To be sure, the effects of a conditioned stimulus are not nearly as widespread nor as profound as the primary stressor. It was demonstrated, for instance, that hypothalamic and locus coeruleus NE activity was subject to conditioning, as were variations of DA and 5-HT functioning in the PFC, and NE

activity within the central amygdala (Cassens et al., 1980; Coco et al., 1992; Herman et al., 1982; Kaneyuki et al., 1991; Wedzony et al., 1996). In the present investigation, as already mentioned, neurochemical functioning was increased in some brain regions by the light stimulus, irrespective of whether it had previously been paired with shock. However, in other instances, the neurochemical changes were particularly marked in response to the conditioned fear cues. For instance, 5-HIAA activity within the PVN of both rat strains was appreciably increased by the conditioned fear cues. Likewise, MHPG accumulation within the central amygdala was elevated by conditioned fear cues in the Fast rats, but not in the Slow rats. Importantly, it will be recalled that the Fast rats also showed a much greater fear-potentiated startle response than did the Slow rats. Although this result does not necessarily imply that an increase in NE activity in the central nucleus of the amygdala was causally related to the strain-dependent fear potentiation of the startle response, it is consistent with other data indicating the importance of the central amygdala for fear learning, in the presence of CRH and monoamines (e.g., Goldstein et al., 1996; Merali et al., 1998; Pacak et al., 1995), that critically and additionally involves input from the basolateral and lateral amygdala nuclei (e.g., Falls & Davis, 1995; LeDoux, 1995;).

It has frequently been reported that stressors markedly increase DA neuronal activity within the PFC (Anisman et al., 1991; Deutch & Roth, 1990), and that variations of DA activity can be induced in this region in response to cues that had been paired with a stressor (Herman et al., 1982). In the present investigation, the PFC was indeed found to be sensitive to stressors in that MHPG and 5-HIAA accumulation were increased by the test stimulus, but this occurred regardless of whether rats had previously been exposed to cue-shock pairings. Of particular interest was the finding that basal DOPAC levels in the PFC of Fast

rats considerably exceeded those of their Slow counterparts. Yet, following exposure to the test cue, DOPAC accumulation increased appreciably only in the Slow rats, and not in the Fast rats.

At this juncture, it would be prudent to note that stressor responses in these rat lines need to be evaluated using methods which more effectively control for divergent levels of basal anxiety. For example, as these lines may exhibit disparate levels of anxiety associated with the testing environment itself, it may be beneficial to examine their responses to stressors under conditions where animals are extensively pre-handled (to diminish this source of anxiety) and are housed and tested in a similar context. Indeed, the possibility exists that the Slow-kindling rats, in particular, may be exhibiting basal stressor responses secondary to those encountered in the course of testing (e.g. transport to the laboratory, handling).

It has been reported that in the presence of fear cues, the spontaneous unit activity of PFC neurons is reduced, and that this effect can be instigated by activation of the basolateral amygdala (Garcia et al., 1999). As fear cues normally increase DA activity in the PFC, it is not surprising that such an increase is antagonized by amygdala lesions (Davis et al., 1994). In accordance with these findings, as indicated earlier, increased DA activity in the PFC, measured by DOPAC accumulation, was provoked by the stressor situation, but only in the Slow rats. In addition, within the basolateral amygdala, a strain difference was also evident, but here 5-HIAA accumulation was increased in the Fast rats, but not in the Slow rats. Since increased 5-HT activity within the basolateral amygdala is associated with reduced amygdala excitability (e.g., Chen et al., 1998) and recruitment via the 5-HT_{1A} receptor (Wada et al., 1997), one might reasonably speculate that the stressor-provoked

increase of amygdala 5-HIAA activity in Fast rats reflects that outcome. In support of this view, it has been reported recently that mice with a knock-out for the 5-HT_{1A} receptor also show in the amygdala and cortex benzodiazepine-resistant anxiety and reduced expression of the major GABA_A subunit expressed in adulthood, the α_1 subunit (Sibillie et al., 2000). Perhaps in a parallel manner, our Fast rats naturally show in the cortex and amygdala reduced expression of the GABA_A α_1 subunit compared to Slow rats (Poulter et al., 1999) and, in an as yet unpublished report, we have also observed a relative benzodiazepine insensitivity in this strain. Thus, although it is premature to conclude that the differences between our strains in 5-HT activity within the basolateral amygdala is related to the observed differences in DA activity within the PFC, this possibility ought to be entertained.

It will be recalled that in addition to anxiety differences that exist between the strains, the Fast rats may also be suffering from an impulsivity-like problem (McIntyre & Anisman, 2000), as reflected in several learning situations, and by inappropriate approach responses to estrous and nonestrous female rats (McIntyre & Anisman, 2000; Michaud et al., 1999). The DA functioning in the PFC of the Fast rats is, indeed, consistent with the notion that impulsivity may be associated with excessive prefrontal DA functioning (Feldon & Weiner, 1992). Moreover, the possibility needs to be considered that DA neurons in the PFC of Fast rats do not react or interpret environmental stimuli in the same manner as in the Slow rats, giving rise to a variety of inappropriate behavior patterns (Grace, 2000).

Conclusion

Slow rats displayed a greater startle reflex to noise relative to Fast rats, implying differences with respect to generalized anxiety. Yet, these rat lines exhibited comparable rates of startle habituation and prepulse inhibition, suggesting that the differential startle reactivity was not directly to factors related to attentional processes (at least within this particular context). In contrast to the more pronounced basal startle response in the Slow line, a profound fear-potentiated startle effect was readily observed only in Fast rats. However, this outcome could be influenced by manipulating both the shock and startle stimulus intensity employed, as well as by treatment with diazepam. Together, these data affirm the notion that behavioral responses (e.g., fear-potentiated startle) may be largely dependent upon the specific characteristics of the test situation itself. These data also reinforce the view that analysis of anxiety and fear-potentiated startle requires considerable care as the relationship between anxiety and the startle response is not a linear one, and indeed likely is a quadratic function.

With respect to stressor-induced changes in neurochemistry, it was evident that when rats were constrained in a new environment, a novel stimulus (light) was able to increase NE, DA and/or 5-HT activity in typically stress-sensitive regions of the brain, such as the locus coeruleus and paraventricular hypothalamic nucleus. In general, these effects were more pronounced in the Fast rats, and NE utilization in the central amygdala was especially strong in response to a conditioned fear stimulus. Thus, while levels of generalized anxiety appeared to be greater in Slow rats, behavioral and central neurochemical reactivity to novel stimuli, as well as to transient fear-cues, were greater in Fast rats. Similarly, basal DA activity in the prefrontal cortex was augmented in Fast rats.

but DA utilization induced by a novel stimulus was more pronounced in Slow rats. This implies that relative to Slow rats, DA neurons in prefrontal cortex of Fast rats react abnormally to environmental stimuli, which could in turn, precipitate disturbances of attention or behavior reminiscent of impulsivity. Ultimately, these rat strains need to be evaluated using methods which more effectively control for disparate levels of basal anxiety, as the results observed may be related to basal stressor responses secondary to those encountered in the course of testing.

References

- Adler L.E., Hoffer L.D., Wiser A., & Freedman R. (1993). Normalization of auditory physiology by cigarette smoking in schizophrenic patients: In Dawson. M. E., Schell. A.M., & Bohmelt. A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133). United States of America: Cambridge University Press.
- Anisman. H., Hayley S., Kelly. O., Borowski. T. & Merali. Z. (2000) Psychogenic, neurogenic and systemic stressor effects on plasma corticosterone and behaviors: mouse strain-dependent outcomes. *Behav. Neurosci.* submitted
- Anisman. H., Lu. Z.W., Song, C., Kent, P., McIntyre. D.C. & Merali. Z. (1997) Influence of psychogenic and neurogenic stressors on endocrine and immune activity: differential effects in Fast and Slow seizing rat strains. *Brain Behav. Immun.*, 11, 63-74.
- Anisman, H., Zalcman, S., Shanks, N. & Zacharko. R.M. (1991) Multisystem regulation of performance deficits induced by stressors: An animal model of depression. In Boulton A. Baker G, Martin-Iverson M. (eds) *Neuromethods, vol. 19: Animal Models of Psychiatry, II*. Humana Press, New Jersey, pp. 1-59.
- Anthony, B.J. (1985). In the blink of an eye: Implications of reflex modification for information processing. In P.K Ackles, J.R. Jennings, & M. G. H. Coles (Eds.), *Advances in psychophysiology* (167-218) Greenwich, CT: JAI Press.

- Bitsios, P., Philpott, A., Langley, R.W., Bradshaw, C.M., & Szabadi, E. (1999). Comparison of the effects of diazepam on the fear-potentiated startle reflex and the fear-inhibited light reflex in man. *J Psychopharmacol*, 13(3), 226-234.
- Bubser M., & Kock M. (1994). Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133). United States of America: Cambridge University Press.
- Caine S.B., Geyer M.A., Swerdlow N.R. (1992). Hippocampal modulation of acoustic startle and prepulse inhibition in the rat. *Pharmacol Biochem Behav*, 43:4 1201-8.
- Campeau, S., Falls, W.A., Cullinan, W.E., Helmreich, D.L., Davis, M. & Watson, S.J. (1997) Elicitation and reduction of fear: Behavioral and neuroendocrine indices and brain induction of the immediate-early gene c-fos. *Neuroscience*, 78, 1087-1104.
- Campeau S., & Davis M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience*, 106, 569-574.

- Cassens, G., Roffman, M., Kuruc, A., Orsulak, P. J. & Schildkraut, J. J. (1980) Alterations in brain norepinephrine metabolism induced by environmental stimuli previously paired with inescapable shock. *Science*, 209, 1138 - 1140.
- Chalmers D., Lovenberg T.W., & DeSouza, E.B.(1995). Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 Receptor mRNA expression. *Journal of Neuroscience*, 15, 6340-6350.
- Chen, L.L., Wang, S.J. & Gean, P.W. (1998) Serotonin depresses excitatory synaptic transmission and depolarization-evoked Ca^{2+} influx in rat basolateral amygdala receptors. *Eur. J. Neurosci.*, 10, 2163-2172.
- Chiueh, C.C., Zukowska-Grojec, Z., Kirk, K.L. & Kopin, J.J. (1983) 6-fluorocatecholamine as a false adrenergic neurotransmitter. *J. Pharm. Exp. Therap.*, 225, 529-533.
- Coco, M.L., Kuhn, C.M., Ely, T.D. & Kilts, C.D. (1992) Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. *Brain Research*, 50, 39-47.
- Crabbe, J.C., Wahlsten, D. & Dudek, B.C. (1999) Genetics of mouse behavior: interactions with laboratory environment. *Science*, 284, 1670-1672.

- Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxon, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology*, 132, 107-124.
- Cullinen, W.E., Herman, J.P., Battaglia, D.F., Akil, H. & Watson, S.J. (1994) Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience*, 64, 477-505.
- Davis, M. & Shi, C. (1999) The extended amygdala: are the central nucleus of the amygdala and the nucleus of the stria terminalis differentially involved in fear versus anxiety? *Ann N Y Acad Sci.*, 877, 281-291.
- Davis, M. (1998). Anatomic and physiologic substrates of emotion in an animal model. *Journal of Clinical Neurophysiology Society*, 15 (5), 378-387.
- Davis, M., Walker, D.L. & Lee, Y. (1997) Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 352, 1675-1687
- Davis, M. (1994). The role of the amygdala in emotional learning. *International Review of Neurobiology*, 36, 225-266.

- Davis, M., Falls, W.A., Campeau, S., Munsoo, K. (1993). Fear-potentiated startle: a neural and pharmacological analysis. *Behavioural Brain Research*, 58, 175-198.
- Davis, M. (1986). Pharmacological and anatomical analysis of fear-conditioning using the fear-potentiated startle paradigm. *Behavioural Neuroscience*, 100 (6), 814-824.
- Davis, M. (1984). *The Mammalian Startle Response* : In Ed. Eaton, R.C. Neural Mechanisms of Startle Behaviour (chap. 10) New York: Pleaum Press.
- Davis, M., Gendelman, D.S., Tischler, M.D., & Gendelman, P.M. (1982). A primary acoustic startle circuit: lesions and stimulation studies. *Journal of Neuroscience*, 6, 791-805.
- Depaulis A, Keay KA, & Bandler R. (1992). Longitudinal neuronal organization of defensive reactions in the midbrain periaqueductal gray region of the rat: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) Startle modification: Implications for neuroscience, cognitive science, and clinical science (chapter 6 - 114-133). United States of America: Cambridge University Press.
- Deutch, A.Y. & Roth, R.H. (1990) The determinants of stress-induced activation of the prefrontal cortical dopamine system. In Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Comer, M.A. & Feenstra M.G.P. (Eds.), *Progress in Brain Research* Vol. 85, Elsevier, New York, pp. 367-403.

- Dudek, B.C. & Underwood, K.A. (1993) Selective breeding, congenic strains, and other classical genetic approaches to the analysis of alcohol-related polygenic pleiotropisms. *Behav Genet.* 23, 178-189.
- Elmér, E. Kokaia, M. Kokaia, Z. McIntyre, D.C. & Lindvall, O. (1998) Epileptogenesis induced by rapidly recurring seizures in genetically Fast but not slow kindling rats. *Brain Research.* 789, 111-117.
- Falls, W.A. & Davis M. (1995) Lesions of the central nucleus of the amygdala block conditioned excitation, but not conditioned inhibition of fear as measured with the fear-potentiated startle effect. *Behav. Neurosci.* 109, 379-387.
- Feldon, J. & Weiner, I. (1992) From an animal model of an attentional deficit towards new insights into the pathophysiology of schizophrenia. *J. Psychiat. Res.* 26, 345-366.
- Garcia R., Voulmba, R-M., Baudry, M., & Thompson, R.F. (1999) The amygdala modulates prefrontal cortex activity relative to conditioned fear. *Nature*, 402, 294-296.
- Gloor, P. (1992) Role of the amygdala in temporal lobe epilepsy. In Aggleton, J.P. (ed.), *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 505-538.

- Gloor, P., Olivier, A., Quesney, L.F., Anderman, F., & Horowitz, S. (1982). The role of the limbic system in experimental phenomena of temporal lobe epilepsy. *Annals of Neurology*, 12, 129-143.
- Goldstein, L.E., Rasmusson, A.M., Bunney, B.S., & Roth, R.H. (1996) Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J. Neurosci.*, 16, 4787-4798.
- Grace, A. (2000) Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res. Rev.*, 31, 330-431.
- Gray, T.S. (1991) Amygdala: Role in autonomic and neuroendocrine responses to stress. In: McCubbin, J.A Kaufmann, P.G., Nemeroff, C.B. (eds), *Stress, Neuropeptides and Systemic Disease*. Academic Press, New York, pp. 37-53.
- Grillon, C., Dierker, L., & Merikangas, R. (1998). Fear-potentiated startle in adolescent offspring of parents with anxiety disorders. *Society of Biological Psychiatry*, 44, 990-997.
- Grillon, C. & Davis, M. (1997) Effects of stress and shock anticipation on prepulse inhibition of the startle reflex. *Psychophysiology*, 34, 511-517.

- Grillon, C., & Davis, M., (1997). Fear-potentiated startle conditioning in humans: explicit and contextual cue conditioning following paired versus unpaired training. *Psychophysiology*, 34 (4), 451-458.
- Grillon, C., Ameli, R., Goddard, A., Woods, S.W., & Davis, M. (1994). Baseline and fear-potentiated startle in panic disorder patients. *Society of Biological Psychiatry*, 35, 431-439.
- Grillon, C., Falls, W. A., Ameli, R., & Davis, M. (1994). Safety signals and human anxiety: a fear-potentiated startle study. *Anxiety*, 1 (1), 13-21.
- Grillon, C., Ameli, R., Woods, S.W., Merikangas, K., & Davis, M. (1991). Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology*, 28 (5), 588-595.
- Hashimoto, S., Inoue, T. & Koyama, T. (1999) Effects of conditioned fear stress on serotonin neurotransmission and freezing behavior in rats. *Eur. J. Pharmacol.*, 378, 23-30.
- Hermann, B.P. & Whitman, S. (1984) Behavioral and personality correlates of epilepsy: A review, methodological critique, and conceptual model. *Psychol. Bull.*, 95, 451-497.

- Herman, J. P., Guillonneau, D., Dantzer, R., Scatton, B., Semerdjian-Rouquier, L. & LeMoal, M. (1982) Differential effects of inescapable footshock and stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. *Life Sci.*, 30, 2207-2214.
- Hitchcock, J. M. & Davis, M. (1991). The efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behavioural Neuroscience*, 105, 826-842.
- Hitchcock, J. M., & Davis, M. (1987). Fear-potentiated startle using an auditory conditioned stimulus: effect of lesions of the amygdala. *Physiological Behaviour*, 39, 403-408.
- Hoffman, H.S., & Ison, J. R. (1980). Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory output. *Psychological Review*, 87, 175-189.
- Kaneyuki, H., Yokoo, H., Tsuda, A., Yoshida, M., Mizuki, Y., Yamada, M. & Tanaka, M. (1991) Psychological stress increases dopamine turnover selectively in mesoprefrontal dopamine neurons of rats: reversal by diazepam. *Brain Research*, 557, 154-161.
- Kent, P., McIntyre, D., Anisman, H. & Merali, Z. (1996) Differential effects of psychological (ferret exposure) and physical stressors on neuropeptide and endocrine responses of "Slow" versus "Fast" seizure susceptible rats. *Soc. Neurosci. Abst.* 22, 461.

- Koch M., & Ebert U. (1998). Deficient sensorimotor gating following seizures in amygdala-kindled rats. *Biol Psychiatry* 44:4 290-7.
- Kodsi M.H., Swerdlow N.R. (1995) Prepulse inhibition in the rat is regulated by ventral and caudodorsal striato-pallidal circuitry.) *Behav Neurosci*. 109(5), 912-28.
- Kokaia. Z., Kelly. M.E., Elmér. E., Kokaia. M., McIntyre. D.C. & Lindvall. O. (1996) Seizure-induced differential expression of messenger RNAs for neurotrophins and their receptors in genetically Fast and slow kindling rats. *Neuroscience*, 75, 197-207.
- Kovacs. K.J. & Sawchenko. P.E.. (1996) Regulation of stress-induced transcriptional changes in the hypothalamic neurosecretory neurons. *J. Mol. Neurosci.* 7, 125-133.
- Lacosta. S., Merali, Z. & Anisman. H. (1998) Influence of interleukin-1 on exploratory behaviors, plasma ACTH and cortisol, and central biogenic amines in mice. *Psychopharmacology* 137, 351-361.
- LeDoux J. (1996). *The Emotional Brain - The Mysterious Underpinning of Emotional Life*. New York: Simon & Schuster.
- LeDoux. J.E. (1995) Emotion: Clues from the brain. *Ann. Rev. Psychol.* 46, 209-235.

- Lee, Y. & Davis, M. (1997) Role of the hippocampus, bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J. Neurosci.* 17, 6434-7446.
- Makino S., Gold P.W., Schulkin J. (1994a). Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Research.* 640, 105-112.
- Makino S., Gold P.W., Schulkin J (1994b) Effects of corticosterone on CRH mRNA and content in the bed nuclues of the stria terminalis: comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Research.* 657, 141-149.
- Mamalaki E., Kvetnansky R., Brady L.S., & Gold P.W. (1992). Repeated immobilization stress alters tyrosine hydroxylase, corticotropin-releasing hormone and corticosteroid receptor messenger ribonucleic acid levels in rat brain. *J.Neuroendocrinol.* 4, 689-699.
- Mansbach R.S. & Geyer M.A. (1989). Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) Startle modification: Implications for neuroscience, cognitive science, and clinical science (chapter 6 - 114-133). United States of America: Cambridge University Press

- Maric D., Maric I., Wen W., Fritschy, J.M., Sieghart, W., Barker J.L., & Serafini R. (1999) GABAA receptor subunit composition and functional properties of Cl⁻ Channels with differential sensitivity to zolpidem in embryonic rat hippocampal cells. *The Journal of Neuroscience*. 19(12), 4921-4937.
- McIntyre, D.C. & Anisman, H.. (2000) Anxiety and impulse control in rats selectively bred for seizure sensitivity. In Mysblodsky M. & Weiner, I. (eds), *Contemporary Issues in Modeling Psychopathology*. Kluwer Academic, New York
- McIntyre, D.C., Kelly, M.E. & Dufresne, C. (1999) FAST and SLOW amygdala kindling rat strains: Comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. *Epilepsy Res.* 35, 197-209.
- McIntyre, D.C., Kent, P., Hayley, S., Merali, Z. & Anisman, H. (1999) Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. *Brain Res.* 840, 65-74.
- Melia, K.R., & Davis, M. (1991) Effects of septal lesions on fear-potentiated startle, and on the anxiolytic effects of buspirone and diazepam. *Physiol Behav* 49:3 603-11
- Merali, Z., McIntosh, J., Kent, P., Michaud, D. & Anisman, H. (1998) Aversive as well as appetitive events evoke the release of corticotropin releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J. Neurosci.*, 18, 4758-4766.

- Michaud, D., McIntyre, D., Anisman, H. & Merali, Z. (1999). Rat strains with high vs. low sexual reactivity: Behavioral & lateralized amygdaloid CRH responses of males. *Soc. Neurosci. Abst.* 25, 346.
- Mogenson G.J., Nielson M. (1984). A study of the contribution of hippocampal-accumbens-subpallidal projections to locomotor activity. In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133), United States of America: Cambridge University Press.
- Mohapel, P. & McIntyre, D.C. (1998) Amygdala kindling-resistant (SLOW) or prone (FAST) rat strains show differential fear responses. *Behav. Neurosci.* 112: 1402-1413.
- Morgan, C.A., Grillon C., Southwick, S.M., Davis, M., & Charney, D.S. (1995). Fear-potentiated startle in posttraumatic stress disorder. *Society of Biological Psychiatry*, 38, 378-385.
- Pacak, K., Palkovits, M. & Kopin, I.J. & Goldstein, D.S. (1995) Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenal and sympathoadrenal activity: in vivo microdialysis studies. *Frontiers Neuroendocrinol.* 16, 89-150.
- Palkovits, M. & Brownstein, M.J. (1988) *Maps and guide to microdissection of the rat brain*. Elsevier Science, New York.

- Parmigiani, S., Palanza, P., Rogers, J. & Ferrari, P.F. (1999) Selection, evolution of behavior and animal models in behavioral neuroscience. *Neurosci. Biobehav. Rev.*, 23, 957-969.
- Patrick C.J., Berthot, B.D., Moorer, J.D. (1996) Diazepam blocks fear-potentiated startle in humans. *J. Abnorm Psychol.* 105 (1), 89-96.
- Paxinos, G. & Watson, C. (1998) *The rat brain in stereotaxic coordinates*. Academic Press, New York.
- Poulter, M.O., Brown, L.A., Tynan, S., Willick, G., Williams, R. & McIntyre, D.C. (1999) Differential expression of $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ GABA_A receptor subunits in seizure-prone and seizure-resistant rat models of temporal lobe epilepsy. *J. Neurosci.* 19, 4654-4661.
- Racine, R.J., Steingart, M. & McIntyre, D.C., (1999) Development of kindling-prone and kindling-resistant rats: Selective breeding and electrophysiological studies. *Epilepsy Res.*, 35, 183-195.
- Racine, R.J. (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalography and Clinical Neurophysiology*, 32, 281-294.

- Rigdon G.C. & Weatherspoon J.K. (1992) 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133). United States of America: Cambridge University Press.
- Rodgers, R.J.. (1997). Animal models of "anxiety": where next?. *Behavioural Pharmacology*, 8, 477-496.
- Rosen, J.B., Hamerman, E., Sitcoske, M., Glowa, J.R., & Schulkin, J. (1996). Hyperexcitability: exaggerated fear-potentiated startle produced by partial amygdala kindling. *Behavioural Neuroscience*, 110 (1), 43-50.
- Sananes, C.B., & Davis, M. (1992). N-Methyl-D-Aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behavioural Neuroscience*, 106, 72-80.
- Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.*, 21, 55-89.

- Seegal RF, Brosch KO, Bush G. (1986) High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. *J. Chromatog.* 377, 131-144.
- Shi, C., & Davis, M. (1999). Pain Pathways Involved in Fear Conditioning Measured with Fear-Potentiated Startle: Lesion Studies. *The Journal of Neuroscience*, 19 (1), 420-429.
- Sibillie, E., Pavlides, C., Benke, D. & Toth, M. (2000) Genetic inactivation of the serotonin_{1A} receptor in mice results in downregulation of major GABA_A receptor subunits, reduction of GABA_A receptor binding, and benzodiazepine-resistant anxiety. *J. Neurosci.*, 20, 2758-2765.
- Silverstein, L.D., Graham, F. K., & Calloway, J.M. (1980). Preconditioning and excitability of the human orbicularis oculi reflex as a function of state. *Electroencephalography and Clinical Neurophysiology*, 48, 406-417.
- Sipes T.A. & Geyer M.A. (1994) Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) Startle modification: Implications for neuroscience, cognitive science, and clinical science (chapter 6 - 114-133). United States of America: Cambridge University Press.

- Stanford, S.C. (1995) Central noradrenergic neurones and stress. *Pharmac. Ther.*, 68, 297-342.
- Steingart, M.O.. (1983). The selective breeding of seizure-prone vs.. seizure resistant rats based on amygdala kindling: Behavioural, electrophysiological and pharmacological measures. Unpublished doctoral dissertation. McMaster University, Hamilton, Ontario, Canada.
- Struass, E. (1989). Ictal and interictal manifestations of emotions in epilepsy. In F. Boller & J. Grafman (Eds.). *Handbook of neuropsychology: Vol. 3: Emotional behaviour and its disorder*. 315-344. New York: Elsevier.
- Swanson, L.W. & Petrovich, G.D. (1998) What is the amygdala? *Trends Neurosci.*, 21, 323-331.
- Swerdlow, N.R., Geyer, M.A. (1999). Neurophysiology and Neuropharmacology of Short Lead Interval Startle Modification: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science (chapter 6 - 114-133)*, United States of America: Cambridge University Press.
- Takahashi, J.S., Pinto, L.H. & Vitaterna, M.H. (1994) Forward and reverse genetic approaches to behavior in the mouse. *Science*, 264, 1724-1733.

- Wada, Y., Shiraishi, J., Nakamura, M. & Koshino, Y. (1997) Role of serotonin receptor subtypes in the development of amygdaloid kindling in rats. *Brain Res.* 747, 338-342.
- Wahlsten D. Single-gene influences on brain and behavior. (1999). *Ann. Rev. Psychol.* 50, 599-624.
- Walker, D.L., Cassella, J.V., Lee, Y., De Lima, T.C. & Davis, M. (1997) Opposing roles of the amygdala and dorsolateral periaqueductal gray in fear-potentiated startle.. *Neurosci. Biobehav. Rev.* 21, 743-753.
- Walker D.L. & Davis M. (1997). Involvement of the dorsal periaqueductal gray in the loss of fear-potentiated startle accompanying high footshock training: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133). United States of America: Cambridge University Press.
- Wan F.J., & Swerdlow N. R. (1996). Sensorimotor gating in rats is regulated by different dopamine-glutamate interactions in the nucleus accumbens core and shell subregions: In Dawson, M. E., Schell, A.M., & Bohmelt, A. D. (Eds.) *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (chapter 6 - 114-133). United States of America: Cambridge University Press.

Wedzony, K., Mackowiak, M., Fijał, K. & Golembiowska, K. (1996) Evidence that conditioned stress enhances outflow of dopamine in rat prefrontal cortex: A search for the influence of diazepam and 5-HT_{1A} agonists. *Synapse*, 24, 240-247.

Weiss, J.M. & Simson, P.E. (1989) Electrophysiology of the locus coeruleus: Implications for stress-induced depression. In Koob, G.F., Ehlers, C.L. & Kupfer, D.J. (eds.), *Animal Models of Depression*, Birkhauser, Boston, pp. 111-134

Figure 1: Schematic diagram (adapted from Paxinos & Watson, 1998) of the anterior (left) and posterior (right) faces of the brain slices taken from three coronal levels (A,B and C) showing the areas punched or transected for the **monoamine assays**.

Figure 2: Mean startle amplitude ($\pm$ SEM) in FAST and SLOW rats as a function of startle stimulus intensity. Asterisk (*) denotes a significantly higher mean startle amplitude in the SLOW rats in comparison to FAST rats.

.

Figure 3: Mean startle amplitude ($\pm$ SEM) in FAST and SLOW rats as a function of prepulse and startle stimulus intensity. Prepulse intensity of zero indicates that the rats received only the main startle stimulus.

Figure 4: Mean startle amplitude ($\pm$ SEM) over 5 consecutive days among Fast and Slow rats exposed to 115 dB noise. Rats received 60 trials on each day, and each point represents the average of 5 trials.

Figure 5: Mean startle amplitude ($\pm$ SEM) for startle alone trials and CS+ startle trials in FAST and SLOW. Double asterisks (**) denotes $P < 0.05$ relative to Slow kindling, while (*) denotes $P < 0.05$ relative to acoustic startle alone.

Figure 6: Mean startle amplitude ($\pm$ SEM) in Fast and Slow rats that had previously received 0, 5, 10 or 20 fear-conditioning trials. The startle amplitudes depicted are from the same set of animals, measured during noise alone (No CS trials; upper panel), or during trials where noise was preceded by the CS (middle panel). The lowest panel depicts the amplitude of startle responses during CS trials as a percentage of the No CS trials.

NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

65

This reproduction is the best copy available.

UMI®

Figure 7: Mean startle amplitude ($\pm$ SEM) for startle alone trials (startle) and CS + startle trials in Fast and Slow rats pretreated prior to testing with either vehicle or diazepam (0.5 mg/kg and 1.0 mg/kg).

Figure 8: Mean startle amplitude ($\pm$ SEM) in Fast and Slow rats that had previously received 10 conditioning trials (0.3 mA), and were then pretreated with either vehicle or diazepam (0.25 mg/kg) and tested using a startle stimulus intensity of 100 dB. The startle amplitudes depicted are from the same set of animals, measured during startle alone trials and CS + startle trials.

Figure 9: Mean startle amplitude ($\pm$ SEM) in Fast and Slow ($n = 3/\text{group}$) rats that had previously received 10 conditioning trials (0.6 mA), and were then administered either vehicle or diazepam (0.25 mg/kg) and tested using a 100 dB startle stimulus. Data are from the same set of animals, measured during noise alone (No CS trials), or during trials where noise was preceded by the CS.

Figure 10: Plasma corticosterone ($\pm$ SEM) as a function of strain, conditioning and test stimulus. Asteriks (*) denote $P < 0.05$ relative to rats not presented with the test stimulus.

...

Figure 11: Mean (+SEM) levels of MHPG (upper panel) and 5-HIAA (lower panel) in the PVN among Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 12: Mean ($\pm$ SEM) MHPG concentrations within the locus coeruleus of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 13: Mean (+SEM) levels of MHPG in the central amygdala of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test)

Figure 14: Mean ($\pm$ SEM) levels of 5-HIAA (lower panel) in the basolateral amygdala of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 15: Mean (+SEM) MHPG concentrations within the perihinal cortex (upper panel) and piriform cortex (lower panel) of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24 h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Figure 16: Mean (+SEM) concentrations of MHPG (upper panel), DOPAC (middle panel) and 5-HIAA (lower panel) within the medial prefrontal cortex of Fast and Slow rats that had either received fear-conditioning or no treatment, and then 24h later re-exposed to the CS that had been paired with foot-shock (Test stimulus) or were not given any subsequent treatment (No test).

Appendix A

Introduction:

With the increased focus on categorizing and defining the human genome, heightened attention has also been devoted to genetic and molecular genetic analyses among infrahumans, particularly mice. The most common techniques in this respect have been the use of knockout and transgenic mice that either lack or contain excessive numbers of genes for a particular phenotype. An alternative technique, often used in rats, is that of selectively breeding rats for (or against) a given phenotype, and then to assess the neurochemical concomitants of the selected characteristic. Experiments conducted by McIntyre and his associates (Elmer et al., 1998; Racine et al., 1999; McIntyre et al., 1999) have used this approach to evaluate the processes underlying the development of seizures induced by recurrent electrical stimulation of the amygdala (kindling). Interestingly, the seizure resistant rats also display other behavioral changes, such as increased anxiety, while the seizure prone rat displays problems with attention and/or impulsivity (Mohapel & McIntyre, 1998; Anisman et al., 1997; McIntyre & Anisman, in press). However, these strains have not been assessed across a sufficiently broad range of conditions that provide a clear view of the anxiogenic characteristics, nor have experiments been conducted to assess the effects of anxiolytic treatments in these rats. The present investigation represents an analysis of anxiety in the Fast (FAST) and Slow (SLOW) seizing rats using an acoustic startle paradigm, as well as that of fear-potentiated startle. As well, an index of attention in this paradigm was gained by examining a standard prepulse inhibition paradigm. Finally, the effects of the anxiolytic, diazepam, was assessed on startle responding in these rat lines.

Selective Breeding and Behavioral Genetic Analysis

Behavioral genetic analysis may provide a way to quantify the extent to which a particular gene or genotype may contribute to the development of a given pathology (Wahlsten, 1999). Through the development of transgenic or knockout mice (which either possess or lack a particular genetic substrate), as well as the selective breeding of animals for a given physiological or behavioral attribute, it has been possible to bring about strains of rodents which exhibit specific pathologies or particular behavioral profiles (Crawley, 1999). While these strains may serve as a useful instrument in determining physiological and behavioral correlates related to a given pathology, characteristics other than the target phenotype may have been selected in the course of strain development. These characteristics may be the result of pleiotropic effects, whereby a single gene may have the capacity to affect a number of different phenotypic characteristics or through linkage, whereby the genetic loci of interest may be placed closely to a number of other loci on a chromosome (Wahlsten, 1999). This gives these genes a greater probability of being inherited as a group. This, of course, complicates the assessment of the exact role of a particular gene or phenotype in the development of a pathology or specific pattern of behavior.

As it has been demonstrated that the amygdala mediates processes which lead to a central state of fear and anxiety, it may be useful to develop and examine rat strains with divergent amygdaloid characteristics. Given that changes in fear and anxiety can be induced in humans and animals through electrical stimulation of the amygdala (Gloor et al., 1982; Davis, 1994), and that repeated seizure activity in the amygdala brought about by electrical kindling can produce changes in emotionality (Hermann & Whiman, 1984;

Strauss, 1989). two new rat strains which have been selectively bred for their rate of amygdala kindling may provide a unique opportunity to clarify the relationship between emotionality and seizure susceptibility (Mohapel & McIntyre, 1998).

These two rat strains were originally developed at McMaster University in Hamilton, Ontario through the selective breeding of parental cross of Long-Evans and Wistar rats (Steingart, 1983). These rats were selectively bred without brother-sister matings for 11 generations (although there was no overlap in kindling rates after the sixth generation), based on the number of amygdala kindling stimulations required to elicit the first Stage 5 seizure (Racine, 1972). Those rats which required the fewest number of kindling sessions were bred together to produce a kindling-prone (FAST) strain, and those rats which required the greatest number of kindling sessions were bred together to produce a kindling-resistant (SLOW) strain (Mohapel & McIntyre, 1998). Following 11 generations of selective breeding, the two strains were transferred to the Carleton University Institute of Neuroscience in Ottawa, Ontario, where breeding has occurred non-selectively for over 40 generations within each respective strain (an exception being the disallowance of any brother- sister or first-cousin pairings). In addition to differential amygdala excitability, these strains have also demonstrated dissimilar kindling rates (although to a lesser extent) in the hippocampus, piriform and perirhinal cortex (McIntyre et al., 1999).

Fear and Anxiety

As already alluded to, kindling has been observed to produce changes in emotionality, particularly with respect to fear and anxiety (Hermann & Whiman, 1984; Strauss, 1989). Indeed, FAST and SLOW kindling rats have been observed to differ in

their performance on tasks traditionally used to assess levels of fear and anxiety in experimental animals. In an elevated plus-maze test, SLOW rats were observed to emit fewer open arm entries than FAST rats (Mohapel & McIntyre, 1998). Moreover, when placed in a novel environment after receiving a series of foot shocks, the SLOW rats exhibited a reduction in motor activity in comparison to the FAST rats (Mohapel & McIntyre, 1998). Likewise, it was noted that the SLOW rats demonstrated more freezing in response to foot shock and longer step-down latencies in a passive avoidance test (Mohapel & McIntyre, 1998). Interestingly, it has also been observed that these strains exhibit dissimilar patterns of habituation to stimuli over the course of successive days of testing. In an open-field test the SLOW rats initially showed a higher degree of motor activity than the FAST rats, but this pattern was reversed if testing continued over a number of successive days (Mohapel & McIntyre, 1998).

Finally, it would seem that these two divergent strains exhibit disparate responses to paradigms involving naturalistic stressors; both behaviorally and neurochemically. For instance, it was noted that when restrained in a triangular plastic "baggie" (restraint stress) the FAST rats struggled for almost half of the stressor period (15 minutes), whereas the SLOW rats remained virtually motionless. In contrast, when exposed to a ferret for a period of 15 minutes (predator stress), it was the FAST rats remained immobile approximately 90% of time, while the SLOW rats remained active, adopting a more active defensive posture (Anisman et al, 1997). Interestingly, it was observed that plasma ACTH and corticosterone levels corresponded with the defensive style adopted by each strain in each of the two stressor situations (i.e. in general, the more active the defensive posture adopted, the higher the levels of plasma ACTH and corticosterone) (Anisman et al, 1997).

Underlying these observed behavioral differences may be the fact that these strains differ in their GABA_A receptor system (Poulter et al., 1999). Through the determination of levels of mRNA and protein present in various brain regions, it has been revealed that the FAST and SLOW rats differentially express various α subunits of GABA_A receptor. Specifically, for the $\alpha 1$ subunit (found predominately in the mature brain) it was found that the FAST rats expressed 50% less mRNA than control animals, while the SLOW rats expressed 70% more mRNA (Poulter et al., 1999). In comparison, for α subunits typically found in the developing or embryonic brain ($\alpha 2$, $\alpha 3$, $\alpha 5$), FAST rats expressed 50-70% more mRNA than controls, while in the SLOW rats' levels of mRNA for these subunits did not reach detectable levels in many brain regions (Poulter et al., 1999). Subsequent tests employing immunocytochemistry to measure levels of $\alpha 1$ and $\alpha 5$ subunit protein expression yielded similar results (Poulter et al., 1999). Interestingly, it has been noted (Maric et al., 1999) that the specific combination of these various α subunits with other receptor components (e.g. β , γ subunits) may cause varying affinity for the benzodiazepine, diazepam.

Thus, it may appear that the differential expression of α subunits in the FAST and SLOW rats procures the behavior exhibited by these strains, such that the FAST rats seem to be hyperactive and impulsive (typically considered "immature" behavior), whereas the SLOW rats appear to overly fearful and apprehensive (Poulter et al., 1999).

From these data, it seems evident that the FAST and SLOW rats exhibit dissimilar defensive behavior, which seems to be contingent on the nature of the stressor (Anisman et al., 1997). Moreover, this behavior may be strongly tied to characteristics of their GABAergic systems (specifically the morphology of the GABA_A receptor) (Poulter et al.,

1999). While, in general, it appears that the SLOW rats may be more anxious and fearful than the FAST ones, it is important to note that some of the tests used to document measures of fear and anxiety in these strains (e.g. elevated-plus maze, open field) are highly susceptible to the natural variation in emotional reactivity in animals, and to variation in test parameters (Rodgers, 1997). In addition, many of these types of models may not be behaviorally equivalent to each other (Rodgers, 1997). Indeed, there appear to be different types of anxiety, including a generalized (non-specific) type of anxiety, as well as anxiety tied to specific environmental stimuli. Moreover, stimuli that provoke anxiety in one species or strain may be less likely to elicit a comparable anxiety response in a second strain. Accordingly, At this juncture it may be of value to examine these two rat strains using more definitive tests of fear and anxiety.

The Acoustic Startle Reflex

As an objective measure of fear and anxiety, the acoustic startle reflex enjoys a number of advantages (Davis, 1984). As the startle reflex is under stimulus control, it implies that inhibitory and excitatory effects can be measured. Moreover, in that the startle reflex is sensitive to a large number of drugs and demonstrates plasticity through habituation, sensitization and prepulse inhibition, the context into which it can be employed is fairly extensive. Additionally, the startle response in rodents is very similar to that observed in humans, which gives the startle paradigm high predictive and face validity (Davis, 1984). Finally, as the reflex itself has a remarkably short latency (e.g. 8 msec), the neural pathway governing this reflex must be simple, thereby creating an opportunity to reveal which anatomical substrates constitute the startle pathway, and the structures and treatments which may modulate it (Davis, 1984).

In a typical startle experiment, a rat is placed on a floor plate (within a sound-proof startle chamber) which is able to transduce and translate the magnitude of the pressure resulting from the downward motion of the startle reflex into an arbitrary value. Following a period of acclimation, a stimulus is delivered to the rat, which usually consists of an intense, highly-graded acoustic stimulus (e.g. white noise) which reaches a certain intensity within a minimum amount of time. It has been demonstrated (Davis, 1984) that the minimum intensity and duration of the stimulus needed to elicit startle are 90dB and 12 milliseconds, respectively. In response, the rat should then demonstrate a slight extension of the forepaws and hindpaws, which causes an overall shortening of the body and consequently, a hunched position. This extension of the rat's limbs causes an overall downward movement of the cage which can be used to quantify startle magnitude, and by extension, infer the amount of fear or anxiety that is experienced by the animal (Davis, 1984). This process is repeated over a number of trials, using a given inter-stimulus interval (e.g. 30 seconds) to obtain an index of habituation.

Initially, it was thought (Davis et al., 1982) that the acoustic startle pathway was mediated at four synapses within four distinct brain nuclei; the ventral cochlear nucleus, the area medial and ventral to the ventral nucleus of the lateral lemniscus, the nucleus reticularis pontis caudalis and the motor neurons of the spinal cord. It now appears that the acoustic startle pathway may be simpler, being comprised of only three synapses at three nuclei—auditory nerve fibers synapse onto cochlear root neurons present in the auditory nerve, cochlear root neurons then project through the ventral acoustic stria and send projections to the nucleus reticularis pontis caudalis, and cells in the nucleus reticularis pontis caudalis project to form the reticulospinal tract, which makes monosynaptic and

polysynaptic synapses in the spinal cord (Davis et al., 1998). Neurochemically, the excitatory amino acid, glutamate, may mediate and modulate startle at each of the three central synapses, with NMDA and non-NMDA receptors involved at the spinal cord and nucleus reticularis pontis (Davis et al., 1998).

At this juncture, it seems prudent to make distinctions between fear and anxiety, as it will be suggested that different startle paradigms seem to increase either fear *or* anxiety based on those brain structures which are sensitive to the treatment employed. In this context, the construct of fear is defined as a natural, adaptive change elicited by a potentially threatening stimulus which prepares an animal to either escape or cope with provocation (Lee & Davis, 1997). Conversely, anxiety is operationally defined as change in state, with signs and symptoms similar to fear, but not clearly associated with a single eliciting stimulus and may last for long periods of time once activated (Lee & Davis, 1997).

Fear-Potentiated Startle

Fear-potentiated startle, a variant of the startle paradigm, is a procedure wherein the amplitude of the startle reflex can be modified by the induction of a transient state of fear (Davis, 1998). In this paradigm a conditioned fear response is first established (by means of pairing a stimulus, such as light, with shock). Then during subsequent testing, the startle response is elicited by another stimulus (such as a burst of noise) either in the presence or absence of the CS (usually a light). Typically, the magnitude of the startle reflex is greater when elicited in the presence of the CS than on its own (Davis, 1986)

Fear-potentiated startle has a number of inherent features that address and rectify problems previously associated with models of fear and anxiety in animals (Davis, 1986).

First, as fear-potentiated startle is a measure of within subjects difference in startle magnitude in the absence or presence of a visual CS, it is more effective in reducing the problem of between subjects variability in startle and reactivity (Davis, 1986). Second, it allows the evaluation of specific vs. nonspecific effects so that qualitative *and* quantitative drug profiles can be compared (Davis, 1986). Third, because different intensities of the auditory stimulus can be used with the same test session to elicit startle on the noise-alone or light/noise trials, potentiated startle can be measured at comparable points on the same scale. This reduces problems of interpretation associated with different measurement scales (Davis, 1986). Fourth, due to the fact that shock is not applied during testing, drug effects that are observed upon subsequent testing cannot be explained in terms of sensitivity to shock (Davis, 1986). Fifth, because there is a lag time between training and testing sessions, it is possible to evaluate a drug alters original learning, and likewise tests for state-dependent learning can be easily evaluated (Davis, 1986). Finally, because fear-potentiated startle does not involve an operant and is based upon a reflex, the animal is not required to make or withhold an operant response, which would be subject to sedative actions of drugs, to indicate the presence or absence of anxiety (Davis, 1986).

As fear-potentiated startle can also be demonstrated in humans, this paradigm offers high face and predictive validity (Davis, 1986). Indeed, elevated fear-potentiated startle has been demonstrated in patients with panic disorder (Grillon et al., 1994), PTSD (Morgan et al., 1995), as a marker of vulnerability for the development of anxiety disorders in the children of parents with the same disorder (Grillon et al., 1998) and in the normal population (Grillon et al, 1991; Grillon et al., 1994, Grillon et al., 1997). Benzodiazepines

also reduce startle amplitudes in humans using the fear-potentiated startle paradigm (Patrick et al., 1996) as is the case in the animal model (Davis, 1993).

Role of the Amygdala in Fear-Potentiated Startle

It has been established that lesions to the amygdaloid region have a number of effects on expression and acquisition of fear-potentiated startle (Davis, 1998). For instance, lesions of the central amygdala block the expression of fear-potentiated startle in rats, using visual or auditory-conditioned stimuli (Hitchcock & Davis, 1987; Campeau & Davis, 1995). Furthermore, it appears (Hitchcock & Davis, 1991) that the central nucleus of the amygdala projects directly to the nucleus reticularis pontis caudalis (a nucleus in the startle pathway) and that lesions at any of several points along this pathway block the expression of fear-potentiated startle. When lesions of cell bodies (by way of local infusion of neurotoxic doses of NMDA) are performed in the lateral and basolateral nuclei of the amygdala (which receive sensory input and project to the central nucleus of the amygdala) there is a complete elimination of fear-potentiated startle if these lesions are made either before or after training. This effect is observable using either an audio or visual CS (Sananes and Davis, 1992). Interestingly, in a study which explored the effects of kindling on fear-potentiated startle (Rosen et al., 1996), it was shown that partial kindling of the amygdala (brief electrical stimulation over the course of two days) caused rats to exhibit augmented startle amplitude (using a fear-potentiated startle paradigm) in comparison to sham-kindled rats.

The fear response associated with the amygdala is characterized by a variety of symptoms usually associated anxiety. In this respect, efferent projections from the amygdala synapse on centers which have effects known to be related to the fear response

(Davis, 1994). Among these targets are the lateral hypothalamus which controls sympathetic activation (increased heart rate, increased blood pressure, galvanic skin response, and pupil dilation), the dorsal motor nucleus of the vagus which controls parasympathetic function (urination, defecation, bradycardia), the pontine reticular formation which controls reflexes (increased startle amplitude), the central grey which is involved in certain behaviors and analgesia (freezing, CER, conditioned analgesia), the ventral tegmental area, locus coeruleus and the dorsal lateral tegmental area which control dopamine, norepinephrine and acetylcholine release, respectively and may be involved in arousal/vigilance, and the paraventricular nucleus of the hypothalamus (PVN), a main component of the hypothalamic-pituitary-adrenal circuitry, as the PVN releases corticotropin releasing hormone which stimulates release of pituitary adrenocorticotrophic hormones (ACTH) (Davis, 1994).

In parallel, afferent projections to the amygdala arise from various brain regions which are concerned with functions that are highly cognitive in nature (LeDoux, 1996). These brain regions include the sensory thalamus (stimulus features), the sensory cortex (objects), the rhinal cortex (memories), the hippocampus (memories and contextual information) and the medial prefrontal cortex (extinction of the fear-potentiated startle effect) (LeDoux, 1996). In terms of a specific pathway, it has been suggested (Davis, 1993), that the amygdala receives highly processed sensory information from all modalities (e.g. nociceptive) through the lateral and basolateral nuclei. It is thought that visual and auditory inputs from modality-specific areas of the thalamus and cortex synapse specifically on the lateral amygdaloid nucleus, which may be the site of plasticity for CS and US associations. This assumption is based on the fact that only the lateral amygdaloid

nucleus receives CS and US input (Shi & Davis, 1999). In turn, these nuclei project to the central amygdaloid nucleus, the ventral caudate putamen and the nucleus accumbens. The central amygdala then projects to a variety of hypothalamic (e.g. paraventricular nucleus and lateral hypothalamus) and brainstem (e.g. parabrachial nucleus, pontine reticular formation) nuclei which are known to be active in behaviors related to a state of fear (Davis, 1993). Thus, it appears that conditioned fear may result when a formerly neutral stimulus comes to activate the amygdala through pairing of the neutral stimuli with aversive stimuli (Davis, 1993). Moreover, fear-potentiated startle may arise because the amygdala is involved in the assessment and retention of emotionally significant stimuli (Davis, 1993).

Curiously, it has been noted that when trained with higher than normal shock intensities (e.g. 0.9 mA), rats tend to demonstrate poor fear-potentiated startle (Davis et al., 1999). As the dorsal periaqueductal gray (PAG) has been associated with active behavior in response to aversive stimuli (Depaulis et al., 1992), it has been suggested that high intensity footshock may stimulate the PAG, causing the rat to adopt more active defensive behaviors, which may serve to attenuate fear-potentiated startle. In concordance, axon-sparing NMDA lesions of the dorsal PAG reversed the inhibition typically seen at higher shock intensities (Walker et al., 1997), while chemical activation of the dorsal PAG interfered with fear-potentiated startle, even at low shock intensities (Walker et al., 1997). Thus, the apparent deficit in conditioning seen with the use of high shock intensities may be the result of poor performance as opposed to poor learning (startle (Davis et al., 1999)

Role of the Amygdala and the Bed Nucleus Stria Terminalis in Fear and Anxiety

Recently (Lee & Davis, 1997), the Bed Nucleus of the Stria Terminalis (BNST), a structure of the extended amygdala which receives inputs from stress sensitive centers such as the dorsal hippocampus (Cullinan et al., 1993) and amygdala (Lee & Davis, 1999), has been implicated in processes which promote a long-lasting, unconditioned increase in the startle reflex. For instance, it has been reported (Lee & Davis, 1997) that there is a double dissociation between the amygdala and the BNST with respect to the long-term increase in the startle observed after an intracerebroventricular infusion of CRH (CRH-enhanced startle). While lesions of the BNST were able to completely block CRH-enhanced startle, lesions of the central and basolateral amygdala had no effect. In contrast, lesions of the BNST did not block fear-potentiated startle, whereas lesions to the central and basolateral amygdala eliminated the effect completely (Lee & Davis, 1997). Similarly, in experiments involving CRH antagonists (e.g. α -h CRH), it was revealed that the infusion of a CRH antagonist in the BNST (but not the central amygdala) attenuated CRH-enhanced startle (Lee & Davis, 1997). This is consistent with the notion that only CRH receptors in the BNST are involved with CRH enhanced startle (Lee & Davis, 1997).

In addition to CRH-enhanced startle, the BNST has been implicated in other paradigms which produce an unconditioned, long-lasting potentiation of startle amplitude. For example, it has been observed (Davis et al., 1999) that the blockade of glutamate receptors in the BNST (but not the central amygdala) blocked the tonic, unconditioned increase of startle amplitude normally observed when a rat is exposed to a bright light for a period of 20 minutes (light-enhanced startle). In addition, lesions of the BNST of rats employed in a fear-potentiated startle experiment blocked long-term sensitization to startle,

but had no effect on fear-potentiated startle itself (Davis et al., 1999). It has also been noted that the rise of plasma ACTH and corticosterone ordinarily induced when rats are reintroduced to an environment associated with a stressor, are attenuated by lesions of the BNST (Davis et al., 1999). Taken together, it appears that the BNST is a system which responds to signals more akin to anxiety, while the amygdala seems sensitive to those cues related to fear.

It has been suggested that stress or anxiety is associated with the release of CRH in the basolateral amygdala which, in turn, causes a release of CRH in the BNST (Lee & Davis, 1999). Activation of the BNST by CRH would then activate various brainstem target areas known to be involved in stress and anxiety (e.g. locus coeruleus, reticular formation). Thus, it may be that phasic activation of the basolateral amygdala by certain stressors could lead to a long term activation of the BNST via CRH (Lee & Davis, 1999). Specifically, it has been observed (Davis & Shi, 1999) that some cells in the basolateral nucleus of the amygdala project exclusively to the ~~central amygdala~~, while other cells project solely to the BNST. It may be that those cells in the basolateral amygdala that project to the central amygdala receive afferent pain information (i.e. conditioned fear), whereas cells in the basolateral amygdala projecting to the BNST may receive more diffuse sensory information (general anxiety) (Davis & Shi, 1999). Thus, it is conceivable that the distinct pathways between neurons in the basolateral amygdala, the central amygdala and BNST constitute the neural substrate responsible for the differential roles of the central amygdala and BNST in fear versus anxiety (Davis & Shi, 1999).

Prepulse Inhibition

Prepulse inhibition (PPI) (another variant of the basic startle paradigm), refers to the ability of a weak lead stimulus (usually a sub-threshold startle stimulus) presented at appropriate time intervals (<250ms), to inhibit a reflex response (i.e. the acoustic startle reflex) in response to a powerful sensory stimulus (e.g. startle stimulus) (Grillon & Davis, 1997). PPI is independent of the characteristics of the startle-eliciting stimulus (Hoffman & Ison, 1980), and does not require cognitive awareness of the prepulse (Sliverstein et al., 1980). However, PPI can be increased by manipulations that alter the effectiveness of the prepulse stimulus (i.e. increasing the intensity of the prepulse) (Anthony, 1985), or through the introduction of stressors (Grillon & Davis, 1997). Consequently, it has been suggested (Grillon & Davis, 1997) that PPI is an especially sensitive measure of sensory functioning.

It appears that in PPI, the first acoustic stimulus activates the ventral cochlear nucleus and pontis caudalis. One of these regions may then directly or indirectly activate cells in the pontine tegmentum, in the area of the pedunculopontine nucleus. These neurons then directly innervate and possibly inhibit the nucleus reticularis pontis caudalis, such that, its response to a subsequent startle stimulus is reduced (Swerdlow & Geyer, 1999).

Neurochemically, PPI is regulated by a variety of neurotransmitters (Swerdlow & Geyer, 1999). For instance, PPI is reduced by drugs which facilitate dopamine (DA) activity, including apomorphine, amphetamine and cocaine (Swerdlow & Geyer, 1999). Likewise, PPI is reduced by non-competitive NMDA antagonists (e.g. PCP), which impede the function of glutamate; the major neurotransmitter of the cortico-striatal efferents (Mansbach & Geyer, 1989). It has also been observed that PPI can be reduced through

administration of direct serotonin agonists (Sipes & Geyer, 1994; Rigdon & Weatherspoon, 1992), as well as through increased stimulation of nicotinic cholinergic receptors via systemic administration of nicotine (Adler et al., 1993).

Physiologically, PPI appears to be influenced by areas of the descending forebrain, specifically the limbic cortex and striatum. For example, in the hippocampus, cholinergic activation may impair PPI by stimulating glutamate release (Caine et al., 1992). In the medial prefrontal cortex, it has been discovered (Bubser & Koch, 1994) that interventions which decrease medial prefrontal cortex DA (e.g. 6-OHDA and D1 and D2 agonists) reduce PPI, possibly through the disinhibition of descending glutamate fibers, which in turn results in elevated DA levels in the nucleus accumbens. Interestingly, small cell-specific lesions of the basolateral amygdala significantly reduce PPI, as well as fear-potentiated startle, but not basal startle magnitude (Wan & Swerdlow, 1996). Additionally, there is also evidence to suggest that anomalous DA function in the basal ganglia (i.e. striatum and pallidum) may impact upon PPI (Mogenson & Nielson, 1984). Finally, it has been noted that lesions of the pedunculopontine nucleus reduce PPI (Swerdlow & Geyer, 1993; Kodsi et al., 1995). As the pedunculopontine nucleus innervates the nucleus reticularis pontis caudalis (which of course, is ultimately responsible for the startle response), it may regulate PPI via effects on ascending cholinergic projections to the thalamus (Swerdlow & Geyer, 1999).