Influences of Perinatal Environment on Impulsivity and Seizure Susceptibility
Differences in Seizure-Prone (Fast) and Seizure-Resistant (Slow) Rodents

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ABSTRACT

This study was designed to determine whether the Fast and Slow rat strains differed in the maternal care they elicit upon their litters. Through crossfostering we hoped to determine the influence environmental experiences may have on previously documented strain differences by comparing the strains during restraint, as response to stressful stimuli, and seizure susceptibility via kindling rates. From birth to PND 23 Fast pups, regardless of their rearing mother, outweighed Slow pups, suggesting that the distinct variations in pup weight of the strains at birth may give a possible link to variations in early neuronal development. The act of crossing pups resulted in decreased weight compared to control groups; specifically, crossing to Fast mothers might have been more detrimental to developing pups than crossing to Slow mothers. When examining nursing behaviour from PND1-PND10, Slow mothers performed Arched-Back Nursing less often than Fast mothers. Additionally, pups reared by Slow dams may have experienced a relative degree of maternal separation, since the frequency of No Contact was higher and the frequency of With Pups observations was lower. In the Restraint paradigm, Fast rats, regardless of rearing mother, were significantly more active than Slows – with no interaction with the crossfostering experience, suggesting that different rearing environments have little or no effect on the mechanisms that control the activity during restraint. Subsequent kindling of the left amygdala proceeded in a normal fashion, where Fast rats kindled much faster than Slow rats. Within the Slow groups (SC, SPSM, & SPFM), it seemed that

crossfostering had a greater effect on the pups' kindling rates than the strain of the rearing mother, suggesting that crossing can add to the speed of epileptogenesis; yet this effect was not seen in the Fast pups groups. The weight data suggested that while the act of crossing pups resulted in a decrease in weight compared to control groups, crossing to Fast mothers might have been more detrimental to developing pups than crossing to Slow mothers. This idea was further supported in the Kindling results, since the FPSM group had the same kindling rate as the Fast controls and both were significantly greater than the FPFM group. Thus, only the Fast pups were malleable by or vulnerable to the identity of the mother. This result suggests that the expression of genes is not equal given a similar experience, and that what may be beneficial for one genotype may not be for another.

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Introduction

Epilepsy defines a broad spectrum of disorders identified specifically by the onset of seizure activity (Shneker & Fountain, 2003). Symptomatic seizure disorders, such as temporal lobe epilepsy (TLE), are the most prevalent forms of epilepsy, affecting 1% of the general population (McNamara, 1994). It is also a disorder that has a high comorbidity with other disorders such as depression, anxiety disorders, ADHD and increased impulsivity, along with other mental illnesses (Hermann, 1982; Binnie & Marston, 1992; Alemayehu, Bergey, Krumholz, Wolf, Fleming & Frear, 1995; Kanner & Nieto, 1999; Ettinger Weisbrot, Nolan, Gadow, Vitale, Andiola, Lenn, Novak & Hermann, 1998).

Unfortunately, the origin and development of epilepsy has perplexed scientists for decades because it is unclear which factors contribute to its evolution. Seizures can develop in the aftermath of brain trauma or head injury, including stroke, infection and brain tumours (Benardo, 2003; Gottesman, Komotar & Hillis, 2003; van Zeijl, Mullaart & Galama, 2002). However, it is clear that people can also develop seizures in the absence of brain damage, which demonstrates that seizures can be triggered in the absence of any overt pathology (Elliot & Lowenstein, 2004). To complicate things further, some individuals who experience profound brain trauma do not develop seizures (Gottesman et al., 2003). Due to the varied nature and inconsistency of seizure participants, many of the underlying mechanisms and neural networks of epilepsy remain unclear.

To begin to comprehend epilepsy, scientists have asked the question of what makes one person more, or less, susceptible to seizures compared to another. This question begs the suggestion that epilepsy has a genetic basis. However, additional evidence also suggests that seizure susceptibility is not entirely dependent upon the genetic profile of an individual (Coulter, McIntyre & Löscher, 2002). In addition, recent research has suggested that perinatal environmental conditions can contribute substantially to brain development and behavioural response to adult experiences (Meaney, 2001), and could conceivably contribute to epileptogenesis. Since it is broadly agreed that critical phases in neuronal development occur during pregnancy, several researchers have proposed that “one or more” phenomena may occur during those critical periods that predispose an individual to seizure development. Later in development, often at birth, these predisposed individuals experience another event that triggers the onset of epilepsy. This hypothesis has been referred as the “Two-Hit Hypothesis” (Valesik and Moshe, 2003).

For decades, through the use of animal models of epilepsy, the search for a genetic basis of the disease has been ongoing (Coulter et al., 2002). Yet, it has remained unclear whether there is a changing role for environmental factors in the development and progression of the disorder. What is apparent at this point, however, is the need for an animal model that allows for the study of the interaction of both environmental and genetic contribution to epilepsy. The study of such interactive or epigenetic factors will permit scientists to better identify and understand possible influences and mechanisms more broadly associated with the development of seizure disorders.

Kindling Model of Epilepsy

Animal models constitute one of the most valuable tools in scientific research, allowing us to better understand the pathophysiology of neurological disorders such as TLE (Coulter et al., 2002). They provide scientists with the means to study a disease or disorder in a quasi-controlled environment. Therefore, through the manipulation of certain variables, scientists are able to learn about aspects of a disease/disorder that would otherwise be unavailable to them in human studies. Through the use of animal models, scientists hope to gain a better understanding of the mechanisms involved in the onset of seizures and aid in the development of therapies to improve treatment, ultimately leading to the discovery of a cure for epilepsy (Coulter et al., 2002).

The most popular animal model used in the study of TLE has been kindling. Kindling is defined as the progressive development of both electrographic and behavioural seizures following daily low-frequency stimulation of a specific brain region, such as the amygdala or hippocampus (Goddard, McIntyre & Leech, 1969). Kindling is termed progressive because it begins on the first day with a brief afterdischarge (AD), evoked using the minimum stimulus intensity, that outlasts the stimulus by two seconds or more, at the electrode tip (McIntyre, Kelly & Defresne, 1999a). It then evolves in duration and complexity over days of successive stimulation to recruit (i.e., stimulate and activate) other limbic structures, ultimately culminating in major convulsive behavioural seizures.

Racine (1972) identified five stages of behavioural/convulsive manifestations associated with amygdala kindling, beginning first with facial movements and eye blinking (stage 1) ipsilateral to the stimulation site, which is followed by head bobbing and drooling (stage 2). Stage 3, characterized by mild forelimb clonus, progresses to recruit more distal structures, resulting in forelimb clonus with rearing, where the animal is up on its hind limbs (stage 4). Ultimately, in stage 5, the stimulation triggers strong clonic-tonic-clonic seizures with rearing and falling. Pinel and Rovner (1978) identified additional seizure stages 6-8, whereby behavioural responses were more severe and characteristic of brainstem evoked seizures, such as barrel rolling, in which the animal experiences drooling and begins to flip onto its back and then its stomach in rapid succession. Eventually, after many evocations, in many of the rats spontaneous seizures appeared in the absence of the electrical stimulation trigger (Pinel & Rovner, 1978).

The development of spontaneous seizures has been considered a defining feature of human epilepsy. Interestingly, many patients with epilepsy experience spontaneous seizures with no apparent trigger. However, it is interesting that Pinel (1981) noted that not all the animals in his study developed spontaneous seizures, suggesting that even in rodents there is a genetic predisposition for seizure. As such, the use of selectively bred kindling-prone and -resistant rat strains developed by Racine and colleagues may provide insight into genetic influences on epileptogenesis (Racine, Steingart & McIntyre, 1999).

Seizure-Prone and Seizure-Resistant Kindling Rat Strains

Following the cross of two parent rat strains (Long Evans Hooded and Wistar rats), two new strains of rats were selectively bred based upon their rates of amygdala kindling (Racine et al., 1999). Animals that required larger numbers of stimulations (~45) to reach a secondarily generalized Stage 5 seizure were bred together to produce the kindling-resistant (Slow)-strain, while the animals that required less stimulation (~10) to generate the Stage 5 seizure formed the kindling-prone (Fast) strain. At the eleventh generation and thereafter, active selective breeding ceased, as the two strains were now stable but distinct populations. From that time on, only second cousin matings were employed in their continuance.

It is of interest to note that while the strains differed in their kindling rates, their afterdischarge (AD) *thresholds* in the amygdala were not different (Racine et al., 1999). Initially, the afterdischarge *durations* also did not differ dramatically between the strains, but in very few days the duration of the electrographic afterdischarge did become significantly longer in Fast than in Slow rats. In addition, when convulsive seizure first appeared, the behavioural profiles were similar between the strains, but the severity was greater in the Fast rats (Racine et al., 1999). The study of such differential responses to the same seizure-inducing stimulus between these two strains could provide many clues to genetically-based predisposing mechanisms that may underlie the development of epilepsy in humans.

In addition to the reported differences in kindling profiles between Fast and Slow rats, the strains exhibit several differences in brain neurochemistry and morphology. It has been shown that Fast and Slow rats differ in their response to GABAergic modulators. Slow rats require considerably lower doses of positive modulators, such as pentobarbital and diazepam, to elicit behaviours similar to drug injected Fast and Long Evans Hooded (LEH) rats (McIntyre & Anisman, 2000). In addition, the strains have differing GABA_A subunit expression in several temporal lobe structures (Poulter, Brown, Tynan, Willick, William & McIntyre, 1999). Specifically, via *in situ* hybridization, this study showed that the amygdala, perirhinal and piriform cortex of the Fast rats, had an overexpression of α_2 , α_3 , and α_5 (embryonic forms) subunits' mRNA compared to control (LEH) and Slow rats, while in Slow rats these subunits' mRNA are relatively underexpressed and the α_1 (adult form) subunit's mRNA is overexpressed compared to LEH and Fast rats.

Numerous other differences are beginning to emerge between the strains. Through the use of gene screening techniques (i.e., differential display, microarray analysis), a number of genes have been shown to be constitutively differentially expressed in limbic structures of naïve Fast and Slow rats (McIntyre, Poulter & Gilby, 2002; Laboratory communications). Moreover, recent work on neuroanatomy revealed that the overall volume of various limbic structures (hippocampal, temporal lobe) were significantly different in naïve Fast and Slow rats (Gilby, Hutcheon, Sauro, Malik, Sahota, Poulter & McIntyre, 2002). Specifically, the ventricles in the Fast rats are

significantly larger than in Slows, while the volumes of the dorsal hippocampus and the dentate gyrus are significantly smaller.

The McIntyre & Anisman laboratories have also observed striking differences between the Fast and Slow rats in their reaction toward neurogenic and psychogenic stressful stimuli (Anisman, Lu, Song, Kent, McIntyre & Merali, 1997; Mohapel & McIntyre, 1998). In various experiments that assessed habituation in the open field and inhibitory avoidance learning, Fast rats were more active and showed little habituation, while Slow rats tended to be more fearful (Mohapel & McIntyre, 1998). In contrast, Fast rats were more reactive when exposed to predator scent or to a conditioned startle reflex, while Slow rats performed better in most executive and spatial learning tasks (McIntyre & Anisman, 2000; Merali, Kent, Michaud, McIntyre & Anisman, 2001; Anisman & McIntyre, 2002). In a restraint paradigm, Slow rats showed a more passive behaviour, while Fast rats struggled vigorously to escape (McIntyre & Anisman, 2000).

Clearly, despite the fact that the Fast and Slow strains were developed to differ in their *amygdala* kindling rates, they also exhibited different personalities, represented as different behavioural responses to the same stimuli. These different behavioural profiles appear to be co-morbid with their predisposition for or against amygdala kindling. What is not apparent at this time is whether the differences between these strains occurred naturally via their genetic constitution or were a product of some early life experience(s) that differentially altered their gene expression and, as a result, their differential susceptibility to both seizure and responses to the different environmental stimuli described above.

Maternal Care

Studies using LEH rats have shown that naturally occurring variations in maternal behaviour altered the expression of developmentally regulated genes that control both, the endocrine response to stressors and brain and cognitive development (Liu, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky & Meaney, 1997; Weaver, La Plante, Weaver, Parent, Sharma, Diorio, Chapman, Seckl, Szyf & Meaney, 2001; Liu Diorio, Day, Francis & Meaney, 2002). In particular, female rats appeared to exhibit different nursing styles that were believed to improve or hinder pup development. There were three types of nursing styles observed, including Arched-Back Nursing (ABN), whereby the mother was positioned over all her pups, nursing, with her back arched and her hind limbs and forelimbs straightened. The other two other types of nursing styles were less proactive. In the Blanket Posture (BP), the mother laid completely on top of her pups while they nursed beneath her, and in the Passive Posture (PP), the mother laid on her side while the pups nursed. The mother often exhibited Licking and Grooming (LG) behaviour during nursing, and at other times as well, where she repeatedly licked and groomed her pups, most typically around the anogenital area.

Other maternal behaviours were recorded, which included simply spending time *with pups* (WP), while she did not perform any sort of overt mothering behaviour. During other observations, the mother may have been returning the pups to one nest or carrying them around the cage, termed pup Retrieval (RET). In addition, there were instances where the mother was away from the pups, termed No Contact (NC), and she

could have been observed sleeping, feeding, drinking, or grooming herself, nest building or doing nothing at all (Lui et al., 1997).

Interestingly, some of these behaviours have a tendency to be performed in tandem. For instance, studies have reported that licking of pups occurred most frequently while the mothers nursed in the arched-back position, and that the frequencies of the two behaviours were closely correlated ($r = +0.91$) across mothers (Lui et al., 1997). A mother who exhibited a high frequency of LG-ABN with her first litter continued to show this behaviour with her second and third litters. Subsequently, her female pups also showed a high frequency of LG-ABN behaviour with their own litters (Champagne Francis, Mar & Meaney, 2003). Caldji and colleagues reported that the offspring of high LG-ABN mothers showed a reduced response to stressful stimuli than rats that were raised by low LG-ABN mothers (Caldji, Tannenbaum, Sharma, Francis, Plotsky & Meaney, 1998). For instance, in a novel environment, rats of high LG-ABN mothers showed significantly more exploration time, shorter latency times to begin eating, and spent more time eating than the offspring of low LG-ABN mothers. In a startle paradigm, the high LG-ABN offspring exhibited a decreased startle response. As well, these pups, once matured, differed in their genetic expression for mechanisms controlling endocrine responses. Specifically, using autoradiography, it was discovered that corticotrophin-releasing hormone (CRH) receptor binding was significantly higher in the locus ceruleus and the nucleus tractus solitarius of the pups of low LG-ABN mothers compared with those of high LG-ABN dams (Caldji et al., 1998).

In addition to these behavioural and endocrine response differences found to be associated with the two differing maternal styles, there have also been distinctions found in the expression of certain mechanisms necessary for hippocampal development, synaptogenesis and apoptosis. Via western blot analysis of the hippocampus, BAX (Group-1 tumor necrosis factor family mediated cell death effector) -like immunoreactivity was significantly increased in the adult offspring of LEH rats that experience low LG-ABN maternal care. In addition, TUNEL (Terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick-end labelling) staining showed that the number of TUNEL-positive cells in both the dentate gyrus and the CA1 regions of the hippocampus was significantly increased in the offspring of low LG-ABN dams, suggesting that pups of low LG-ABN mothers may be more vulnerable to hippocampal cell loss through apoptosis (Weaver, Grant & Meaney, 2002).

Perhaps most noteworthy were the differences discovered in the GABA_A receptor levels of the basolateral and central nucleus of the amygdala of high and low LG/ABN adult offspring. Specifically, a study using *in situ* hybridization illustrated that the adult offspring of high LG-ABN mothers had an increased expression of α_1 subunit for the GABA_A receptor in the amygdaloid complex, while the adult pups of low LG-ABN dams overexpressed the α_3 and α_4 subunits (Caldji, Diorio & Meaney, 2000a; Caldji, Francis, Sharma, Plotsky & Meaney, 2000b; Weaver et al., 2001).

In addition to these findings were those of cross-fostering studies, in which pups from a High LG-ABN mother were switched to a Low LG-ABN mother and vice versa. There has been documented support of a nongenomic transmission of individual

differences in stress reactivity and maternal behaviour (Francis, Diorio, Liu & Meaney, 1999a). Interestingly, it was noted that the cross-fostered rats exhibited behaviours that were correlated with their rearing mothers and environment, rather than their genetic mother or siblings (Meaney, 2001). Specifically, biological offspring of low LG-ABN dams reared by high LG-ABN were significantly less fearful in novel paradigm than offspring reared by low LG-ABN mothers, including the biological offspring of high LG-ABN mothers (Francis et al., 1999a). Furthermore, the maternal behaviour followed the same pattern as the levels of fearfulness. The female adult offspring of low LG-ABN reared by high LG-ABN mothers did not differ from normal high LG-ABN offspring in the frequency of pup LG and ABN (Meaney, 2001).

The aforementioned studies have provided substantial evidence that variations in maternal care, specifically nursing styles, contributed significantly to changes in the behaviour and neuroanatomy of the offspring of female LEH rats. We then must question similarly whether the behavioural, brain physiology and excitability differences documented in our Fast and Slow strains could be associated with potential differences in maternal care of Fast and Slow mothers, rather than purely differential genetic predispositions. Thus, through the examination of early post-natal experiences in the two strains, we anticipate clarifying possible links between maternal care and seizure susceptibility.

It is our hypothesis that, similar to the cross fostering study conducted by Meaney and colleagues (2001), some of the behavioural and brain physiology differences documented between the strains may be altered when the rats are cross-fostered, which

will indicate that the strain differences were due to variations in early post-natal life experiences, specifically maternal care.

This study was two-fold in nature. First, we hoped to determine whether the Fast and Slow rat strains differed in their maternal behaviour, which in turn might impact neuronal development and behaviour. Second, we expected to ascertain the influence of putative differences in early environmental experiences on the previously documented strain differences. Specifically, we compared the strains during restraint, as a response to stressful stimuli, and seizure susceptibility via kindling rates of cross-fostered litters compared to appropriate strain controls. If maternal care has no effect on adult strain behaviour, including kindling rates, fear and impulsivity behaviours, the strains would be more firmly established as a genetic model for epilepsy unaffected by perinatal environmental alterations. However, should the strains demonstrate a reversal or normalization in their kindling profiles or restraint activity when cross-fostered, our research directives regarding the mechanism underlying seizure susceptibility would have to switch to more physiological and/or epigenetic factors that occur during early post-natal life that may subsequently be linked to these adults behavioural profiles.

Methods

Animals

Eighty female rats (40 Fast and 40 Slow) and 40 male rats (20 Fast and 20 Slow) were used as breeding pairs. Each male rat was housed with two females, of the same strain, in a clear plastic cage (dimensions 47 x 24 x 21 cm) with food and water available ad libitum. Rats were monitored daily until the female rats appeared pregnant (approximately 2-3 weeks). Females were then individually housed and checked daily for litter births. The time and date of birth for each litter was recorded.

Cross Fostering Procedure

Within twelve hours of birth, the entire litter was separated from the mother, sexed and weighed. Litters were then placed with a mother according to their assigned experimental groups, which were as follows: Slow Pups raised by Fast Mother (SPFM), Fast Pups raised by Slow Mother (FPSM), Slow Pups raised by different Slow Mother (SPSM) and Fast Pups raised by different Fast Mother (FPFM). Fast and Slow litters raised by their own mother served as control groups (FC and SC, respectively). Birth weights of all Fast and Slow pups were assessed prior to crossing the litters. Crossed litters were culled to ensure mothers received a litter containing no more pups than was in her original litter. Each group was made up of 10-12 mothers with litters of greater than five pups. After crossing, the number of pups allocated to different groups was follows: $N(FC) = 34$, $N(FPFM) = 40$, $N(FPSM) = 58$, $N(SC) = 73$, $N(SPSM) = 44$ and $N(SPFM) = 50$. Observation of maternal behaviours began on post-natal day 1 (PND1) and

continued throughout the pre-weaning period. On PND23, rats were weaned and males were selected and housed together in groups of two until PND50. At PND50, male rats were housed individually to prepare for surgery in anticipation of kindling.

Maternal Observations

Maternal observations were recorded from PND1-PND10. Observations were conducted six times per day for periods of one hour each at 08:00, 10:00, 12:00, 13:00, 15:00, and 17:00 hrs. Note that all observations were conducted during the light phase of the regular light cycle as nursing is reported to occur most frequently during that period (Liu et al., 1997). Individual maternal behaviours were recorded every 3 min during each one hr period, for a total of 21 observations/hr and 126 observations/day. Behaviours were classified into 7 categories: arched-back nursing (ABN), licking and grooming of any pup (LG), arched-backed nursing with licking and grooming (ABN/LG), blanket posture (BP), passive posture (PP), time with pups but not nursing (WP) and no contact with pups (NC). At the end of the day, pups were removed from the home cage and individual pup weights were recorded daily until PND10, and then every other day until PND20 (PND 12, 14, 16, 18, and 20).

Statistical Analysis of Maternal Behaviour

Statistical analysis that compared daily maternal behaviours across groups was conducted using repeated measures ANOVA. However, the sample size used in this experiment was not large enough to allow for accurate analysis of 60 repeated

measurements (6 measurements/day for 10 days). Thus, data collected from the 6 observations per day were summated, which resulted in 10 measures that were then analyzed using repeated measures ANOVA. Total frequency of behaviours experienced by the pups was also compared across the groups using ANOVA. Importantly, data collected on PND1-2 were not included in this analysis due to an effect observed from the initial cross. Post-hoc group comparisons were made using least squares difference (LSD). In addition, pup birth weight and successive weights from PND1-PND10 were analyzed using univariate ANOVA and repeated measures ANOVA, respectively. Again, LSD was used as a post-hoc test to analyze differences between individual groups.

Restraint Procedure

On PND50, male rats from each of the experimental groups (N=9/group) and from the Slow (N=14) and Fast (N=6) control groups were assessed in the restraint paradigm. Each rat was placed in the wide end of a triangular plastic bag with the narrow top end open to allow the nose to protrude. Once the rat had completely entered the bag the wide end was taped closed leaving the tail out. The bag snugly fit the rat to allow limited movement, and it was then placed on a table for a 10-minute observation period. Total percentage time spent active during that 10-minute period was recorded. The rat was then removed from the bag and returned to its home cage.

Statistical Analysis of Restraint Behaviour

ANOVA was used to compare the percentage time spent active in the restraint

paradigm between groups. In addition, the crossed groups (SPSM, SPFM and FPFM, FPSM) were collapsed together within strain and compared to respective control groups (SC and FC) in order to determine whether there was an effect of crossing on restraint behaviour.

Kindling Surgery

Male rats used in this testing paradigm were allowed to mature to 50 days of age and were then housed and handled individually to prepare for kindling surgery. Rats from each group (N=12), weighing 220-300g at the time of surgery, were anaesthetized using sodium pentobarbital (Somnotol, 60 mg/kg, i.p.). Bipolar stimulating/recording electrodes, consisting of two twisted strands of 0.127 mm diameter Diamel-insulated Nichrome wire attached to male Amphenol pins and cut approximately 9 mm, were implanted in both amygdalae. Co-ordinates for the implantation were 0.2 mm posterior to bregma, 4.5 mm lateral to the midline and 8.2 mm below the skull surface (Pellegrino, Pellegrino, & Cushman, 1979). Five stainless steel screws, embedded in the skull and secured with dental acrylic, were used to anchor the electrodes in place. The Amphenol pins were inserted in a plastic head cap and secured in place by dental acrylic according to standard procedures in our laboratory (Molino & McIntyre, 1972). Following surgery, rats were given acetaminophen rectal gel and a topical analgesic (Bupivacaine) and were placed in plastic cages under warming lamps to maintain normal body temperature until behaviourally active. Rats were then returned to the colony room and allowed a 2-week recovery period before the beginning of kindling.

Afterdischarge Threshold & Kindling Procedure

Afterdischarge thresholds (ADT) were determined in the right and left amygdala of each rat approximately 2 weeks after surgery. The ADT was defined as the minimum stimulus intensity required to provoke an afterdischarge that outlasted the stimulation by 2 seconds or more (McIntyre et al., 1999a). ADTs were determined using a 2 second 60 Hz sine wave stimulus at incremental intensities (15, 25, 35, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600 (A) until an AD was triggered. Successive stimulation for each amygdala during ADT determination was separated by a one-minute interval. Twenty-four hours following ADT determination, kindling began in the left amygdala of all rats unless the left electrode proved unusable. In that case, the right amygdala was used as the kindling site. Daily kindling stimulation of the chosen amygdala at the ADT occurred until six stage 5 generalized convulsive seizures were accumulated (Racine, 1972). Twenty-four hours following the final stage-5 seizure, ADT intensities for both the kindled amygdala and non-kindled amygdala were re-determined.

Histology

Two hours following the final ADT determination all rats, under deep anaesthesia, were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde and the brains were left *in situ*. Electrodes were extracted 24 hours later and the brains removed from the cranium and stored for at least 7 days in 4% paraformaldehyde before sectioning. To prepare for sectioning, brains were transferred

to a 30% sucrose cryoprotectant solution in phosphate buffer for 5 days. Brains were then sectioned (40 μm) on a microtome, and the sections containing the electrode tip were mounted on gelatin-coated slides and stained with cresyl violet to verify electrode placements.

Statistical Analysis

Differences in kindling data generated from the rat groups were analyzed using ANOVA. Kindling parameters of interest included those relevant to local excitability such as ADT intensities and durations, as well as those that pertain to the ease of network recruitment, including kindling rates and cumulative AD durations to the first Stage 5 seizure. Kindling rate is defined as the number of successive stimulations required to elicit the first generalized convulsive event (Stage 5). In addition, measures associated with recruitment of secondary generalized convulsions such as the latency to forelimb clonus and the AD duration of that clonic event, as well as the cumulative AD duration of the 6 stage-5 seizures were compared using ANOVA. Finally, post-kindling ADTs and AD durations were analyzed in both amygdalae using ANOVA in order to determine possible changes in local excitability after kindling.

Results

Strain Birth Weight

As can be seen in Figure 1, Fast rats (N=164) were significantly heavier at birth than Slow rats (N=150) $\{p < 2.15 \times 10^{-8}\}$. Specifically, mean birth weight ($\pm$ SE) of Fast rats was $6.3g \pm 0.05$ while Slow rats had a mean birth weight of $5.9g \pm 0.04$. Following assignment to crossfostering groups, analysis of birth weights for pups in the 6 groups was performed to ensure that random assignment of Fast or Slow pups to designated within-strain groups did not result in prejudiced distribution of birth weights to one or more groups. Indeed there were no significant differences in birth weights within the 3 Fast pup groups {FC, FPFM, & FPSM; $F(2, 110) = 0.86$, n.s.} or 3 Slow pup groups {SC, SPSM, SPFM; $F(2, 118) = 0.36$, n.s.; see Figure 2}.

Pre-weaning Weight Gain

Comparison of daily weights across all 6 groups (FC, FPFM, FPSM, SC, SPSM, SPFM) using repeated measures ANOVA revealed significant group differences in pup weight across days prior to weaning $\{F(5, 226) = 8.746$, $p < .001\}$. Perhaps most importantly, a basic *control* strain effect was revealed such that FC pups were significantly heavier than SC pups between PND1-PND20 $\{F(1,42) = 7.945$, $p < .01$; see Figure 3a}. In a more detailed analysis, differences in Fast and Slow control pup weight on PND1, PND10 and PND14-PND20 proved significant $\{F(1, 77) = 5.01$, $p < .05\}$. In the larger analysis, ANOVA of total weight gained prior to weaning between all 6 groups revealed that all Fast pup groups (FC, FPFM, and FPSM), regardless of rearing mother,

gained significantly more weight than all Slow pup groups (SC, SPSM, SPFM) $\{F(5,250) = 13.108, p < .0001$; See Figure 3b}. Thus, as expected, *post hoc* analysis showed SPFM pups weighed less than all other groups raised by Fast moms (FC, FPFM) $\{p_{\text{SPFMvsFC}} < .05, p_{\text{SPFMvsFPFM}} < .01\}$ and FPSM pups were significantly heavier than all other pup groups raised by Slow moms $\{p_{\text{FPSMvsSC}} < .005, p_{\text{FPSMvsSPSM}} < .01\}$.

In order to determine whether the crossing event within one strain alone had any effect on weight gained prior to weaning, data from the crossed groups (FPFM and SPFM) were combined and compared to FC pups. It was revealed that FC pups were consistently heavier than the combined crossed group raised by a Fast mother (FPFM & SPFM) between PND3-20, $\{F_{\text{Cross/Control}}(1, 101) = 4.04, p < .05\}$. Comparisons within the groups raised by a Fast mother showed that while FC pups were heavier than both crossed groups, FPFM and SPFM were similar in weight until PND8, and then FPFM began to gain weight at a faster pace than SPFM $\{p_{\text{FPFMvsSPFM}} < .005$; See Figure 3C}. A similar analysis of pups raised by Slow mothers (SC, SPSM, & FPSM) again showed differences in weight gained prior to weaning. While repeated measures ANOVA between groups, as well as between Combined Cross/Control groups, revealed that significant differences existed between pups raised by Slow mothers prior to weaning $\{F(2, 126) = 11.03, p < .0001, F_{\text{Cross/Control}}(1, 127) = 7.38, p < .01\}$, those differences did not appear until PND5, despite the fact that FPSM pups had been significantly heavier at birth than both the SC and SPSM groups. Thus, statistical analysis detected no significant weight differences between SC, SPSM and FPSM between PND1-5 $\{F(2, 135) = 2.14, \text{n.s.}\}$. However, significant differences were documented between the 3

groups between PND6-10 and PND12-20 $\{F_{\text{PND5-PND10}}(2, 165) = 6.13, p < .005, F_{\text{PND12-PND20}}(2, 165) = 8.96, p < .0001\}$. Specifically, between PND6-10 the weights of SC and FPSM pups did not differ $\{p_{\text{SCvsFPSM}} = 0.99\}$ and both groups were significantly heavier than SPSM pups $\{p_{\text{SPSMvsSC}} < .01, p_{\text{SPSMvsFPSM}} < .01\}$. Between PND12-20, SC and FPSM pups continued to exhibit comparable weight gain $\{p_{\text{SCvsFPSM}} = 0.209\}$ and remained significantly heavier than SPSM pups $\{p_{\text{SPSMvsSC}} < .05, p_{\text{SPSMvsFPSM}} < .001\}$. Interestingly, however, by PND16 all three groups differed significantly from one another such that FPSM pups were heaviest, followed by SC pups, while SPSM pups weighed the least $\{F_{\text{PND16}}(1, 121) = 22.91, p < .0001, F_{\text{PND18}}(1, 114) = 14.10, p < .0005, F_{\text{PND20}}(1, 118) = 19.26, p < .0001; \text{see Figure 3D}\}$.

The preweaning period was also segmented in order to assess weight differences between the groups across smaller intervals of time. Between PND1-5, it was shown that both groups crossed to the opposite strain (FPSM & SPFM) showed a reduced weight gain compared to the other 4 groups examined (SC, FC, SPSM, FPFM) $\{F_{\text{PND3}}(5, 269) = 5.65, p < .0001, F_{\text{PND4}}(5, 274) = 7.21, p < .0001, F_{\text{PND5}}(5, 287) = 11.21, p < .001\}$. However, by PND6 FC, SC and FPSM weighed significantly more than SPSM, SPFM and FPFM, and continued to do so until PND10 $\{F_{\text{PND6}}(5, 300) = 15.41, p < .0001, F_{\text{PND7}}(5, 290) = 14.74, p < .0001, F_{\text{PND8}}(5, 289) = 15.51, p < .0001, F_{\text{PND9}}(5, 300) = 14.77, p < .0001, F_{\text{PND10}}(5, 300) = 13.97, p < .0001\}$. By PND 12, FC was the heaviest group followed by FPSM and SC, then SPSM and FPSM, and finally the SPFM group $\{F_{\text{PND12}}(5, 287) = 11.61, p < .0001, F_{\text{PND14}}(5, 287) = 9.991, p < .0001\}$. Thus, crossing Fast pups to a Slow mother appeared to make the weight gained by the pups more similar

to control groups. This was not true when Slow pups were crossed to a Fast mother as those pups exhibited the least weight gain throughout the majority of the pre-weaning period. Importantly, by PND16, all Fast pup groups, regardless of rearing mother (FC, FPFM, and FPSM) weighed significantly more than every Slow pup group. Finally, comparison of within strain groups revealed a negative effect of the cross on weight gain as FC pups consistently weighed significantly more than both groups of crossed Fast pups (FPFM and FPSM) as did the SC pup group relative to the Slow crossed pups (SPSM and SPFM) $\{F_{\text{PND16}}(5, 291) = 11.92, p < 0.0001, F_{\text{PND18}}(5, 284) = 10.76, p < .0001, F_{\text{PND20}}(5, 287) = 13.06, p < .005\}$.

Figure 1: Birth Weight (Grams) of Fast and Slow Rats

(Mean $\pm$ Std. Error)

* $p < .001$

Figure 2: Birth Weight (Grams) According to Group

Mean $\pm$ Std. Error

Figure 3: (A) Weight of Control Groups From PND1-PND21 $\{^*p < .01\}$, (B) Total Weight Gain Prior to Weaning of Six Groups $\{^*p < .0001\}$, (C) Weight of Groups Raised by Fast Mothers $\{^*p < .05\}$, and (D) Groups Raised by Slow Mothers $\{^ap < .0001, ^*p < .01\}$ From PND1-PND21

Mean (grams) $\pm$ Std. Error

Maternal Behavior

Arched-Back Nursing (ABN)

A repeated measure ANOVA was used to analyze daily frequency of ABN behaviour between PND1-10 in all 6 groups. No significant difference was found between the groups $\{F(5, 56) = 1.31, \text{ n.s.}\}$. Univariate analysis further revealed that Fast mothers, regardless of their pup strain, did not differ significantly in their daily frequencies of ABN behaviour, $\{F(2, 28) = 1.77, \text{ n.s.}\}$ nor was there an effect of crossing the pups $\{F(1, 29) = 3.55, \text{ n.s.}\}$. Likewise, Slow mother groups did not differ from each other according to strain or crossing of the pups $\{F(2, 28) = 1.27, \text{ n.s.}; F(1, 29) = 1.38, \text{ n.s., respectively}\}$. When repeated measures analysis was used to compare the total frequency of ABN in all 6 groups between PND3-10 no significant differences between the groups were observed $\{F(5, 56) = 1.83, \text{ n.s.}\}$. However, subsequent planned comparisons analysis revealed that SC mothers performed ABN less frequently prior to PND10 than either FC $\{p < 0.01\}$ or FPSM $\{p < 0.05\}$ mothers. Thus, a strain effect was revealed between control groups. Specifically, FC mothers performed ABN more frequently than SC mothers between PND 3- PND10 $\{F(1, 18) = 7.81, p < 0.05; F_{\text{PND}3}(1, 18) = 7.81, p < 0.05, F_{\text{PND}4}(1, 18) = 5.09, p < 0.05, F_{\text{PND}5}(1, 18) = 11.85, p < 0.01, F_{\text{PND}8}(1, 18) = 17.21, p < 0.01, F_{\text{PND}9}(1, 18) = 6.39, p < 0.05, F_{\text{PND}10}(1, 18) = 5.94, p < 0.05; \text{ see Figure 4}\}$. Furthermore, for both control groups (FC & SC) ABN behaviour decreased as the pup aged from PND1 to PND10.

Blanket Posture (BP)

When a repeated measure ANOVA was used to analyze daily BP behaviour between PND1-10, differences were found between the 6 groups $\{F(5, 56) = 2.422, p < .05; \text{ see Figure 5}\}$. Importantly, univariate analysis revealed FC mothers performed BP nursing considerably less often than SC mothers from PND3 onward $\{F(1, 18) = 8.19, p < .05; < .001\}$. Thus, when the total frequency of BP nursing was analyzed between PND3-10 using ANOVA, a significant difference was observed between the 6 groups $\{F(5, 56) = 2.423, p < .05\}$. Subsequent *post-hoc* analysis again showed that FC mothers displayed BP less frequently than SC mothers $\{p < .05\}$. However, ANOVA within the Fast mother groups (FC, FPFM, SPFM) showed no significant differences in the frequency of BP nursing regardless of the strain of pups nursed or whether the litters were crossed $\{F(1, 28) = 0.98, \text{ n.s.}; F(1, 29) = 1.49, \text{ n.s., respectively}\}$. In contrast, within Slow mother groups (SC, SPSM, FPSM) there appeared to be a change in frequency of BP nursing that was dependent upon the strain of the pups. Specifically, the FPSM group performed BP nursing less often than the SPSM and SC $\{F(1,29) = 4.50, p < .05\}$.

With Pups Behaviour (WP)

Repeated measures analysis between PND1-10 between control groups revealed a strain effect such that FC mothers were observed more frequently with their pups than SC mothers $\{F(1, 18) = 5.25, p < .05\}$. However, when statistical analysis was conducted within each maternal strain, no differences in the frequency of mother/litter contact were

revealed regardless of whether the mothers were raising a crossed litter or their own $\{F_{\text{FastMom}}(2, 28) = 0.36, \text{n.s.}, F_{\text{SlowMom}}(2, 28) = 0.521, \text{n.s.}\}$. ANOVA comparison of the total frequency of WP behavior between PND3-10 revealed significant differences between the 6 groups $\{F(5, 56) = 4.13, p < .01\}$. Univariate analysis showed all Fast mothers (FC, FPFM, SPFM) were more frequently observed with their litters than were Slow mothers (SC, SPSM, FPSM) $\{F(1, 60) = 19.79, p < .05; \text{see Figure 6}\}$.

No Contact (NC)

While comparisons of the total frequency of NC behaviours between PND3-10 was not significant between the 6 groups $\{F(5, 56) = 2.175, p < .01\}$, subsequent planned comparisons revealed that SC mothers were more frequently away from their litters than FC mothers $\{p < .05\}$. Repeated measures analysis of the control groups (FC vs SC) confirmed this behavioural difference from PND7 onward $\{F_{\text{PND7}}(1, 18) = 5.259, p < .05\}$. Similarly, when repeated measures analysis of NC behaviours was analyzed within Fast maternal groups (FC, FPFM, and SPFM), SPFM mothers were found to be away from their pups more often than the two Fast pup groups raised by a Fast mother (FPFM, FC) $\{F(1, 29) = 4.23, p < .05\}$. In contrast, analysis within the Slow mother groups (SC, SPSM, FPSM) showed no differences between the three groups on any of the days $\{F(2, 28) = 1.38, \text{n.s.}; \text{see Figure 7}\}$.

Passive Posture (PP)

Overall analysis of all groups revealed that there were no significant differences between the six groups $\{F(5,56) = 1.54, \text{ n.s.}\}$. Repeated measures analysis of the control groups (FC and SC) between PND1-10 showed no difference between the control groups with respect to PP behaviour $\{F(1, 18) = 0.61, \text{ n.s.}\}$. In addition, analysis within maternal strains revealed no differences in the frequency of PP nursing regardless of the strain of litter or whether the mothers were raising a crossed litter or their own $\{F_{\text{FastMom}}(2, 28) = 1.01, \text{ n.s.}; F_{\text{SlowMom}}(2, 28) = 0.32, \text{ n.s.}\}$. However, analysis of the total frequency of PP behaviour between PND3-10 revealed that Fast mothers (FC, FPFM, SPFM), in general, differed significantly from Slow mothers (SC, SPSM, FPSM) $\{F_{\text{Strain}}(1, 60) = 4.72, p < .05\}$. Generally, pups raised by Fast moms experienced a significantly higher frequency of PP than pups raised by Slow moms (See Figure 8).

Licking Grooming (LG)

Overall group assessment of LG behaviour in the six groups showed using repeated measure ANOVA showed no significant differences $\{F(5, 56) = 1.16, \text{ n.s.}\}$. Furthermore, repeated measures analysis of the frequency of LG of pups between PND1-10 between the control groups (SC, FC) revealed no difference $\{F(1,18) = 1.62, \text{ n.s.}\}$. Subsequently, repeated measures analysis within the maternal strains (FC, FPFM, SPFM and SC, SPSM, FPSM) between PND1-10 revealed no differences in the frequency of LG regardless of litter strain or whether the mothers were raising crossed litters or their own $\{F_{\text{FastMom}}(2, 28) = 0.33, \text{ n.s.}; F_{\text{SlowMom}}(2, 28) = 1.73, \text{ n.s.}\}$. Similarly, ANOVA of the

total frequency of LG activity between PND3-10 revealed no significant differences between the 6 groups $\{F(5, 56) = 1.11, \text{n.s.}\}$.

Arched-Back Nursing & Licking Grooming

Overall frequency of ABN combined with LG of pups showed no differences among the six groups during PND 1-10 $\{F(5, 56) = 1.16, \text{n.s.}\}$. In addition, repeated measures analysis over PND1-10 revealed no difference between the control groups (SC, FC) $\{F(1, 18) = 1.67, \text{n.s.}\}$. Subsequently, further repeated measures analysis of this behaviour between PND1-10 within the maternal strains revealed no differences in the frequency of ABN-LG with respect to strain of litter or whether the mothers were raising a crossed litter or their own $\{F_{\text{FastMom}}(2, 28) = 0.94, \text{n.s.}; F_{\text{SlowMom}}(2, 28) = 0.74, \text{n.s.}\}$. Similarly, ANOVA of the total frequency of combined ABN-LG activity between PND3-10 revealed no significant differences between the 6 groups $\{F(5, 56) = 1.16, \text{n.s.}\}$.

Figure 4: Frequency of ABN for (A) Total Frequency $\{^{\text{Aa}}p < .05\}$ and over PND1 – PND10 for (B) Control Groups {FC and SC} $\{^*p < .05\}$, (C) Pups Raised by Fast Mothers {FC, FPFM, SPFM}, and (D) Pups Raised by Slow Mothers {SC, SPSM, FPSM}

Mean $\pm$ Std. Error

Figure 5: Frequency of BP for (A) Total Frequency $\{ *p < .05 \}$ and over PND1 – PND10 for (B) Control Groups {FC and SC} $\{ *p < .05 \}$, (C) Pups Raised by Fast Mothers {FC, FPFM, SPFM}, and (D) Pups Raised by Slow Mothers {SC, SPSM, FPSM}

Mean $\pm$ Std. Error

Figure 6: Frequency of WP for (A) Total Frequency (* $p < .01$) and over PND1 – PND10 for (B) Control Groups {FC and SC} {, (C) Pups Raised by Fast Mothers {FC, FPFM, SPFM}, and (D) Pups Raised by Slow Mothers {SC, SPSM, FPSM}

Mean $\pm$ Std. Error

Figure 7: Frequency of NC for (A) Total Frequency $\{^{\text{Aa}}p < .05\}$ and over PND1 – PND10 for (B) Control Groups {FC and SC} $\{^*p < .05\}$, (C) Pups Raised by Fast Mothers {FC, FPFM, SPFM}, and (D) Pups Raised by Slow Mothers {SC, SPSM, FPSM}

Mean $\pm$ Std. Error

Figure 8: Frequency of PP for (A) Total Frequency and over PND1 – PND10 for (B) Control Groups {FC and SC}, (C) Pups Raised by Fast Mothers {FC, FPFM, SPFM}, and (D) Pups Raised by Slow Mothers {SC, SPSM, FPSM}

Mean $\pm$ Std. Error

Experiment 2: Restraint Test

Analysis using ANOVA revealed that the percentage of total time (10 min) spent active in the restraint paradigm differed significantly between groups $\{F_{\text{Strain}}(1,54) = 20.58, p < .0001\}$. Specifically the mean percentage activity for all Fast pups, regardless of rearing environment, greatly exceeded that of all Slow pups. Importantly, as has been reported in our laboratory (Anisman et al., 1997), Fast Control rats were considerably more active than Slow Control rats ($p < .05$). Analysis within the Fast pup groups (FC, FPFM, FPSM) revealed no differences within the strain regardless of the rearing mother ($F(1, 22) = 3.33, \text{n.s.}$) or whether the rats experienced the stress of the cross ($F(1, 22) = 3.37, \text{n.s.}$). Similarly, Slow groups (SC, SPSM, SPFM) did not differ in their activity according to the rearing mother ($F(1, 30) = 0.93, \text{n.s.}$) or the stress of the cross ($F(1, 30) = 1.85, \text{n.s.}$; see Figure 9).

Figure 9: Percentage of Time Spent Active of (A) All Groups $\{ *p < .05 \}$ and between
Crossed and (B) Control Animals
(Mean $\pm$ Std. Error)

Experiment 3: Kindling Study

Animals

Of the 63 rats used in this study 61 completed the kindling experiment. Two rats were excluded due to loss of headcaps during the kindling procedure. Histological analysis then confirmed correct electrode placement in the basolateral complex of the amygdala in 52 of the 61 rats. The nine rats with incorrect placements had electrode tips located in the central nucleus of the amygdala, an area known to kindle far more rapidly than the basolateral amygdala, or the ventricle (which does not kindle at all) and were excluded from statistical analysis.

Afterdischarge Intensity and Duration

Pre-kindling ADT intensities of both amygdalae proved to be significantly different among all 6 groups $\{F_{\text{LeftAmygdala}}(5, 46) = 5.98, p < .001, F_{\text{RightAmygdala}}(5, 44) = 7.12, p < .001\}$. Specifically, in the left amygdala, the ADT intensity of the FC group was significantly higher than the SC group $\{F(1, 13) = 11.10, p < 0.01\}$. Analysis of the Fast pup groups revealed that the crossed groups (FPFM, FPSM) had a considerably higher ADT than the control group (FC) $\{F(1, 21) = 4.91, p < .05\}$. Similarly, analysis of the Slow groups revealed a comparable significant crossing effect where the ADT of the Slow control group (SC) was significantly less than the ADT of SPFM and SPSM groups $\{F(1, 27) = 16.34, p < .005\}$. Interestingly, however, the ADTs of all four Crossed groups (FPSM, FPFM, SPSM, SPFM) did not differ $\{F(3, 33) = 1.94, \text{n.s.}\}$.

Analysis of the right amygdala again revealed a significant effect of crossing the pups in both Fast and Slow pups. In particular, within the Fast pup groups (FC, FPFM, FPSM) the ADT of the FC group was significantly less than both crossed groups (FPFM, FPSM) $\{F(1, 23) = 14.88, p < .001\}$. Within the Slow groups, the ADT intensity of the SC group was considerably less than the SPSM and SPFM groups $\{F(1, 23) = 15.21, p < .005\}$. However, ADTs of the control groups (FC, SC) did not differ from each other $\{F(1, 13) = 2.69, n. s.\}$, nor did the intensities of the crossed groups (FPSM, FPFM, SPSM, SPFM) $\{F(3, 31) = 1.488, n.s.; \text{ see Figure 10}\}$.

Analysis of the ADT AD duration in the left and right amygdalae showed no significant differences between or within the two strains regardless of rearing environment $\{F_{\text{LeftAmygdala}}(5, 45) = 1.90, n.s., F_{\text{RightAmygdala}}(5, 45) = 1.93, n.s.\}$.

Cumulative AD Durations

Analysis of the cumulative AD duration over the repeated kindling trials in the stimulated amygdala prior to the first stage 5 seizure revealed that the Fast pup groups (FC, FPFM, FPSM) exhibited a significantly shorter cumulative afterdischarge duration score than Slow pup groups $\{F(5, 45) = 5.92, p < .001\}$. Further analysis within the Fast pup groups showed FPFM rats had considerably shorter cumulative AD durations compared to FC and FPSM $\{F(2, 21) = 7.73, p < .001\}$. Cumulative AD duration within the Slow pup groups (SC, SPSM, SPFM) was also significantly different $\{F(2, 24) = 4.33, p < .05\}$. In addition, Slow pups appeared to experience an effect from cross.

Specifically, Slow controls (SC) had significantly longer cumulative AD durations than the crossed Slow groups (SPSM, SPFM) $\{F(1, 25) = 8.365, p < .01; \text{ see Figure 11}\}$.

Similar statistical significance was observed in the non-stimulated amygdala as in the stimulated amygdala. In particular, FPFM exhibited significantly shorter cumulative ADD compared to FC and FPSM $\{F(2, 21) = 6.91, p < .005\}$. Although the cumulative AD duration did not appear to be significantly different within the Slow strain in overall analysis $\{F(2, 24) = 2.88, \text{ n.s.}\}$, planned comparisons revealed an effect of crossing the pups. Specifically, SC had considerably longer cumulative AD duration in the unstimulated amygdala than the two slow crossed groups (SPSM, SPFM) $\{F(1, 25) = 5.13, p < .05\}$.

Kindling Rate

The kindling rates of the Fast pup groups (FC, FPFM, FPSM) were significantly faster than the Slow pup groups regardless of rearing mother $\{F_{\text{Group}}(5, 47) = 16.12, p < .0001, \text{ see Figure 12}\}$. In addition, analysis of the rates within the Fast pup groups showed FPFM rats had considerably faster kindling rates compared to FC and FPSM $\{F(2, 21) = 5.52, p < .05\}$. Slow rats (SC, SPSM, SPFM) appeared to experience an effect of crossing with respect to their kindling rates. Specifically, Slow controls (SC) had significantly higher kindling rates than the crossed Slow groups (SPSM, SPFM) $\{F_{\text{CrossControl}}(1, 27) = 23.48, p < .0001\}$.

Seizure Latency & Duration

Consistent with the previously documented strain difference (McIntyre et al., 1999a), the Fast groups (FC, FPFM, FPSM) had considerably longer latencies to stage 5 convulsions over the 6 seizures than the Slow pup groups (SC, SPFM, SPSM) $\{F(5,43) = 5.81, p < .0005; \text{ see Figure 13}\}$. However, there were no significant differences within the two strains $\{F_{\text{Fastpups}}(2, 19) = 2.64, \text{ n.s.}, F_{\text{Slowpups}}(2, 24) = 0.55, \text{ n.s.}\}$.

Additionally, the Fast groups (FC, FPFM, FPSM) had considerably longer motor convulsion durations over the 6 seizures than the Slow pup groups (SC, SPFM, SPSM) $\{F(5, 43) = 15.66, p < .0001; \text{ See Figure 14}\}$. Furthermore, there were no significant differences within the two strains $\{F_{\text{Fastpups}}(2, 21) = 1.134, \text{ n.s.}, F_{\text{Slowpups}}(2, 24) = 1.37, \text{ n.s.}\}$.

Cumulative Seizure Duration

The duration of cumulative afterdischarges elicited during the 6 stage-5 seizures in both amygdalae were significantly different between the Fast pup groups (FC, FPFM, FPSM) and the Slow pup groups (SC, SPFM, SPSM). $\{F_{\text{Stimulated}}(5,43) = 16.89, p < .00001, F_{\text{Non-Stimulated}}(5,43) = 15.07, p < .0001\}$. Specifically, in both amygdalae, the Fast pup groups had significantly longer durations of cumulative ADs than the slow pup groups $\{\text{See Figure 15}\}$. However there were no significant differences in cumulative afterdischarge durations within strain in either the stimulated amygdala $\{F_{\text{Fastpups}}(2,19) = 0.92, \text{ n.s.}; F_{\text{Slowpups}}(2,24) = 1.08, \text{ n.s.}\}$ or non-stimulated amygdala $\{F_{\text{Fastpups}}(2,19) = 0.92, \text{ n.s.}; F_{\text{Slowpups}}(2,24) = 1.05, \text{ n.s.}\}$.

Post-Kindling ADT

Post-kindling ADT intensities of both amygdalae (kindled and non-kindled) proved significantly different between the groups $\{F_{\text{Stimulated}}(5, 43) = 3.54, p < .01, F_{\text{Non-Stimulated}}(5, 39) = .98, p < .01\}$. Specifically, there appeared to be a cross effect for both pup strains. In the stimulated or kindled amygdala, FC had a considerably lower ADT than the other Fast pup groups (FPFM, FPSM) $\{F(2, 19) = 5.37, p < .01\}$. Similarly, the ADT of the SC group was significantly less than the ADT of SPFM and SPSM groups $\{F(2, 24) = 5.37, p < .05\}$. Interestingly, the ADT intensities of the control groups (FC, SC) did not differ from each other $\{F(1, 11) = 0.02, n. s.\}$, nor did the intensities of the crossed groups (FPSM, FPFM, SPSM, SPFM) $\{F(3, 32) = 0.96, n. s., \text{ see Figure 16}\}$.

Similar to the ADT intensities of the stimulated amygdala, analysis of the non-stimulated amygdala revealed a significant effect of crossing the pups. In particular, within Fast pup groups (FC, FPFM, FPSM), the ADT of the FC group was significantly less than both crossed groups (FPFM, FPSM) $\{F(2, 19) = 10.98, p < .001\}$. As well, within the Slow pup groups, the ADT intensity of the SC group was considerably less than the SPSM and SPFM groups $\{F(2, 22) = 3.96, p < .05\}$. However, ADT intensities of the two Control groups (FC, SC) did not differ from each other $\{F(1, 10) = 2.20, n. s.\}$ nor did the intensities of the Crossed groups (FPSM, FPFM, SPSM, SPFM) $\{F(3, 29) = 1.98, n. s.\}$.

Post-Kindling AD durations

Analysis of AD durations in the stimulated amygdala during the post-kindling ADT determination showed the Fast control group exhibited a significantly longer AD duration than the crossed Fast pup groups (FPFM, FPSM) $\{F(2, 19) = 7.83, p < .005;$ see Figure 17}. The Slow groups (SC, SPSM, SPFM) did not differ from one another nor did they differ from the crossed Fast groups (FPFM, FPSM) $\{F(2, 24) = 0.16, p = 0.84, \text{n.s.}\}$. Analysis of the AD duration of the non-stimulated amygdala showed no significant group differences within the two strains $\{F_{\text{Fast}}(2,19) = 0.30, F_{\text{Slow}}(2,24) = 1.12,\}$. However, the two basic strains did differ from one another $(F(5, 39) = 6.99, p < .001)$. Specifically the Fast pup groups (FC, FPFM, FPSM) had a considerably longer AD duration than the Slow pup group (SC, SPSM, SPFM).

Figure 10: Pre-kindling ADT Intensity (μ Amps) of the (A) Left Amygdala $\{^a p < .01, ^A p < .005\}$ and (B) Right Amygdala $\{^* p < .005\}$

Mean $\pm$ Std. Error

Figure 11: Cumulative Afterdischarge Duration (seconds) of the (A) Stimulated Amygdala $\{^{AsBs}p < .001, ^{Aa}p < .001, ^{Bb}p < .005\}$ and (B) Non-Stimulated Amygdala $\{^{AsBs}p < .001, ^{Aa}p < .005, ^{Bb}p < .05\}$ until first Stage 5 Seizure

Mean $\pm$ Std. Error

Figure 12: Kindling Rate of Control (FC & SC) and Cross-Fostered (FPFM, FPSM, SPSM, & SPFM) Groups
 (Mean $\pm$ Std. Error)
 $\{^{AsBs}p < .001, ^{Aa}p < .05, ^{Bb}p < .001\}$

Figure 13: Latency of onset of Stage 5 seizure (sec) of Stimulated Amygdala

(Mean $\pm$ Std. Error)

*p < .0005

Figure 14: Duration (seconds) of Motor Convulsions of the Stage 5 Seizure

Mean $\pm$ Std. Error

*p < .0001

Figure 15: Cumulative Afterdischarge Duration (seconds) of Stage 5 Seizures in the (A) Stimulated Amygdala $\{ *p < .0001 \}$ and (B) Non-Stimulated Amygdala $\{ *p < .0001 \}$
Mean $\pm$ Std. Error

Figure 16: Post-kindling ADT Intensity (μ Amps) of the (A) Stimulated Amygdala $\{ *p < .01 \}$ and (B) Non-Stimulated Amygdala $\{ *p < .01 \}$

Mean $\pm$ Std. Error

Figure 17: Post-Kindling ADD (Seconds) of the (A) Kindled Amygdala $\{ *p < .005 \}$ and
(B) Non-Kindled Amygdala $\{ *p < .001 \}$
Mean $\pm$ Std. Error

Discussion

Experiment 1: Crossfostering

Numerous studies have begun to document the behavioural, physiological, and molecular differences that exist between the Fast and Slow rat strains. We now know that Fast rats consistently kindle faster than Slow rats regardless of stimulation site (Racine et al. 1999, McIntyre et al. 1999a). Kindling rates, of course, were the basis of the original selection for the Fast and Slow kindling strains. We also know that Slow rats require considerably lower doses of positive GABAergic modulators, like pentobarbital and diazepam, to elicit behaviours similar to drug-injected Fast and Long Evans Hooded (LEH) rats, the latter being one of the two original parent strains (McIntyre & Anisman, 2000). A relative overexpression of α_2 , α_3 and α_5 GABA_A subunit mRNAs, which are known to be highly expressed in embryonic forms of the GABA_A receptor, has been documented in the amygdala, perirhinal and piriform cortices of Fast rats compared to control rats (LEH rats), while a relative underexpression of these subunits has been observed in Slow rats (Poulter et al., 1999). Interestingly, in that same study, an overexpression of α_1 (adult form) GABA_A subunit mRNAs in Slow rats compared to LEH and Fast rats was also reported (Poulter et al., 1999). Examination of adult brain morphology has shown both sizeable and subtle differences between the strains. For instance, the lateral ventricles in Fast rats are considerably larger than in Slow rats while the volume of the dorsal hippocampus is significantly smaller (Gilby et al., 2002). Behaviourally, Fast rats have been shown to be hyperactive, impulsive and inferior in spatial learning tasks relative to Slow rats, which tend to be more fearful and excel in

most executive and spatial learning tasks (Mohapel and McIntyre, 1998; McIntyre & Anisman, 2000; Merali et al., 2001; Anisman & McIntyre, 2002). The origin of these and other differences between the strains, however, is currently unknown. The intent of this study was to examine whether maternal influence might play a critical role in determining relative seizure sensitivities between the strains and/or at least some of the differing behaviours evident in these strains as adults.

Pre- and postnatal environments can have profound influences on the development of animals (Stott, 1973; Cliver, Goldenburg, Cutter, Hoffman, Copper, Gotlieb & Davis, 1992; Takahashi, Turner & Kalin, 1992; Henry, Kabbj, Simon, le Moal & Maccari, 1994; Bronzino, Austin-LaFrance & Morgane, 1990). In humans, abnormal prenatal environments have been associated with adverse birth outcomes, such as preterm births and fetal growth retardations (Stott, 1973; Cliver et al., 1992). In rats, prenatal stress has been linked to vulnerability to anxiety and increased and prolonged corticosterone secretion in response to stress as adults (Takahashi et al., 1992; Henry et al., 1994). In light of that finding, it is interesting that Slow rats have been shown to exhibit an increased fear response compared to Fast rats as adults (Mohapel & McIntyre, 1998). It has also been reported in the broader literature that pups born to dams stressed during gestation have significant reductions in birth weights relative to non-stressed pups (Edwards, Dortok, Tam, Won & Burnham, 2002). Perhaps in a similar manner, our study found that Slow rats exhibited considerably reduced birth weights compared to Fast rats. Thus, Slow rats may experience a biological or environmental stressor during embryonic development that leads to both reduced birth weights and increased stress responsivity as

adults relative to Fast rats. Since the strains appear to be different at birth in at least one obvious characteristic (i.e., birth weight), it is conceivable that strain differences already existed in other aspects of development by this time as well. Identification of potential differences that occur prior to birth between the two strains, possibly involving neurodevelopment, could offer information pertinent to the differing brain morphologies and seizure susceptibility seen later in life. Because of this prospect, investigation into embryonic differences between the strains is an ongoing theme in our laboratory.

The difference in birth weight observed between Fast and Slow rats continued throughout pre-weaning days such that all Fast pups, regardless of rearing mother, ultimately gained more weight than Slow pups during that time. Even when pups were old enough to supplement nursing with adult rat chow, as a whole Slow pups did not reach the pre-weaning weights of Fast pups. However, significance in mean weights between the groups was not obtained until PND 8, which may have been due to 'rounding error' because of the relatively poor scale used to weigh the first litters. A more sensitive scale was used after PND9, where clear significant strain differences were obtained.

Analysis within the maternal strains showed that crossing litters had a significant effect on pup weight gain. Specifically, both Fast and Slow pups crossed to a Fast mother weighed less than Fast control pups. However, within Slow mother groups (SC, SPSM, & FPSM), only SPSM weighed significantly less than the other two groups. Clearly, crossing the pups (either Fast or Slow) may have created a stressful environment for both the pups and mothers, which impacted weight gain. As such, it is possible that the crossing event alone influenced the pups and/or moms in such a way that it a) stopped

pups from nursing or caused mothers b) to refuse attempts of pups to nurse or c) to experience a reduction in milk supply. Any one of these events could have resulted in the observed lack of weight gain in crossed pups compared to the control pups. However, since control groups were also removed from their mothers following birth, the interaction between the stress of the cross involving a new mother is likely the reason for the reduced weight gain rather than removal of the pups briefly from their natal mother or human interaction. Despite the apparent stressful nature of crossfostering, very little mortality occurred in the crossed groups.

Interestingly, crossing did not appear to affect the groups equally. Specifically, crossing Slow pups to Fast moms appeared to inhibit weight gain more than crossing Slow pups to Slow moms or crossing Fast pups to Slow moms. Furthermore, crossing Fast pups to Fast moms showed the same reduced weight gain as crossing Slows for the majority of the pre-weaning period. Thus, it would seem that crossing Fast pups to a Slow mom ameliorated at least some of the negative effects of crossing stress on weight gain.

One possible hypothesis to help explain the lack of weight gain during the early post-natal period in the FPFM and SPFM crossed groups compared to the SPSM and FPSM groups could potentially be a poorer quality of milk in the Fast strain moms. Conceivably, Slow moms may have a greater nutritional quality to their milk, which facilitated the cross of a Fast pup to a Slow mom over Fast pups crossed to Fast moms. In support of this hypothesis, it has been shown that nutrition early in life plays a pivotal role in the developing brain. Early malnutrition has been linked to brain damage and

mutated neuronal growth (van Gelder & Sherwin, 2003). Moreover, Bronzino and colleagues (1990) reported that early malnutrition influenced seizure susceptibility in Wistar rats regardless of nutrition in adulthood. Specifically, pups that were nursed by malnourished mothers had faster kindling rates than pups whose mother did not experience malnourishment (Bronzino et al., 1990). Importantly, however, the studies showing different kindling and behavioural profiles in Fast and Slow rat rats provided the same quality rat chow *ad libitum* to mothers and to pups once weaned (Mohapel & McIntyre, 1998; McIntyre et al, 1999a; Anisman et al., 2000). Thus, there was no nutritional difference provided to the Fast and Slow pups directly from the experimenter's food source. Furthermore, food consumption studies of the Fast and Slow strains have confirmed Slow rats, in fact, generally eat less than Fast (unpublished data). Therefore, maternal nutrition or pup nutrition post-weaning cannot explain the different kindling and behavioural profiles documented in the two strains, since available nutrition to the two strains did not vary. However, it is conceivable that embryos in well nourished mothers do not receive proper nutrition while *in utero* or during breast feeding in the pre-weaning period due to placental insufficiency or metabolic disturbances in the mother, respectively. Thus, ultimately, it is imperative that the Fast and Slow milk be analysed to determine if these rats indeed present significantly different qualities or quantities of milk to their suckling offspring (in progress).

On the other hand, it is unlikely that the quality of maternal milk is the sole explanation for the weight discrepancies, since pups from the Fast control group were consistently the heaviest pups and neither Slow pup/Slow mom group, crossed or control

(SPSM, SC) were heavier than the Fast pups raised by Fast Mom groups (FC, FPFM). Examination of the pup weight from PND10 onward revealed that all Fast pup groups were consistently heavier than their Slow counterparts even when both strains were given the same rat chow *ad libitum* in adulthood. Therefore, an alternate explanation for the weight difference could be that genetically determined metabolic rates of Slow rats are faster than Fast rats, which results in smaller animals. Supporting this idea is a theory proposed by Green and colleagues (2003) suggesting that many types of epilepsy with varied aetiologies ultimately involve disruptions of brain energy homeostasis, alternating between periods of hyper- and hypo-metabolism. Hyper-metabolism has been detected in brain epileptic foci upon seizure initiation, which spreads to other brain regions during seizure generalization, yet during the period between seizures, the epileptic foci are hypo-metabolic (Kulh, Engel, Phelps & Sellin, 1980; Chugani & Chugani, 1999). As would be expected, therefore, many forms of epilepsy can be potentially managed through specific diets including fasting, the ketogenic diet and caloric restriction, all of which encourage increased metabolism while the diet is maintained (Greene, Todorova & Seyfreid, 2003; Freeman, Freeman & Kelly, 2001; Bough, Valiyil, Han & Eagles, 1999). Speculatively, Slow rats may be born with a higher metabolic rate, which prevents them from experiencing low kindling rates as adults, whereas much heavier Fast rats may be in a relatively hypo-metabolic state. Any insult/provocation such as the electrical stimulation involved in kindling could put specific areas of the brain (the kindled site) into the hyper-metabolic state associated with seizure genesis but then return it to the lower metabolic state once the stimulation has ceased. As such, if Slow rats have a higher metabolism

than Fast rats, the kindling stimulation could cause a hyper-metabolic state at the kindling site, yet following stimulation, brain regions of Slow rats would not enter a hypo-metabolic state, and would not create the continuing flux between metabolic states that has been associated with the development of epilepsy, as proposed by Green et al. (2003).

Perhaps the most interesting finding with respect to weight differences between the strains was that at PND16, when rat pups were physically able to access the rat chow, the crossed fostered pup weights began to more closely resemble the control groups {See Figure 3(B) & (C)}. In fact, by PND20, Fast pup groups (FC, FPSM, & FPFM) were all significantly heavier than Slow pup groups (SC, SPSM, & SPFM). Thus, the strains may be predisposed to reach a predetermined weight range, where the Fast rats are destined to be heavier than Slow rats. It is interesting that while crossing litters reduced weight gain in the pups, crossing Slow pups to Fast moms resulted in even greater reduction in weight gain than other groups. This finding may suggest, as mentioned previously, that Fast maternal milk quality is inferior to that of Slow mothers.

Maternal Care

Mother – Pup Interaction

The first days of life for a rodent do not hold much stimulus diversity. Their environment is defined by contact with their mother and littermates (Meaney, 2001). Typically, the mother serves as the only direct link between developing pups and their environment, and contact with mothers gives pups the necessary stimulation to encourage neuronal growth, as well as some basic reflexive functions, such as the bladder reflex

(Meaney, 2001). In fact, tactile stimulation has been proven to increase neuronal development, while restricted contact with rat pups from mothers has been documented to dramatically increase stress hormones and their precursors in brain, such as corticotrophin releasing factor (CRF), mRNA levels in the amygdala, and increase CRF receptor in the locus ceruleus (Ladd et al., 1996, Meaney, 2001). Findings such as these suggest that maternal separation early in life can induce changes in the CRF system that regulates the monoamines, especially noradrenergic and serotonergic responses to stress. Not surprising then were similar findings that animals separated from their mothers during the early post-natal period were highly fearful in novel behavioural tests and acoustic startle responsiveness as adults (Caldji et al., 2000b). In the same way, Slow rats have consistently scored higher than Fast rats in fearfulness in similar behavioural testing paradigms (Mohapel & McIntyre, 1998; Anisman et al., 2000). Interestingly, the findings of this study suggest that Slow pups experienced a relative degree of maternal separation, with respect to the frequency of No Contact and With Pups observations, specific to the strain when compared to Fast pups, which could contribute to their higher response to stressful stimuli as adults, including the acoustic startle and novel paradigms (Anisman et al., 2000; McIntyre & Anisman, 2000).

Nursing Behaviour

Cross-fostering studies have shown that adult LEH rats that experience low ABN-LG rearing environments as pups exhibited an increased response to stressful stimuli (Weaver et al., 2001; Meaney, 2001). Similar to the low LG-ABN animals, Slow rats

have proven to exhibit increased responses to various stressful stimuli compared to Fast rats (Merali et al., 2001; Mohapel & McIntyre, 1998). For instance, Slow rats showed behaviours consistent with low LG-ABN nursing pups reported by both Caldji and Francis in open field paradigm, where Slow rats spent more time immobile than Fast rats (Caldji et al., 1998; Mohapel & McIntyre, 1998; Francis et al., 1999a; Merali et al., 2001). In contrast, Fast rats, like high LG-ABN nursing pups, were more active and seemingly curious when exposed to the open field. Similarly, when the elevated plus maze was used as a measure of anxiety, Slow rats tended to move out onto the open arms less often than Fast rats (Mohapel & McIntyre, 1998), reflecting higher anxiety. The results of the present study clearly showed significant differences in the nursing styles of the control strains in the first ten days of life. Specifically, when examining the control groups (FC & SC) clear differences in their frequency of ABN, a behaviour believed to critically influence the development of adult behaviours, were reported. Slow mothers performed ABN less often than Fast mothers over the first 10 days of the pups' lives. These findings, in conjunction with the findings reported by Caldji and Francis (Caldji et al., 1998; Francis et al., 1999a), suggest the lack of ABN exhibited by the Slow control mothers may contribute to the elevated anxiety response displayed by adult Slow rats in various stressor paradigms. The fact that significance of ABN was lost when the strains were crossed, both within and between strain (FPSM, FPFM, SPFM, & SPSM) suggests the crossing affected the mothers and/or pups in such a way as to alter their adult nursing behaviour patterns.

The three other nursing behaviours (Blanket Posture, Passive Posture and Licking/Grooming) were not significantly different between the strains suggesting that they are not critically involved in the differential development of the two strains.

The findings of this study support documented reports that certain maternal behaviours may result in altered behavioural responses of adult animals, and particularly the Fast and Slow rat strains, to various stressors (Caldji et al., 1998; Mohapel & McIntyre, 1998; Francis et al., 1999a; Meaney, 2001; Merali et al., 2001). However, we have yet to address whether the perinatal environment of Fast and Slow rats influences the documented differential expression of the GABA_A subunits as adults (Poulter et al., 1999). Weaver has reported that adult offspring of high LG-ABN mothers showed an increased expression of α_1 subunit for the GABA_A mRNA receptor in the amygdaloid complex, while the adult pups of low LG-ABN dams overexpressed the α_3 and α_4 subunits (Weaver et al., 2001). In apparent contrast to Weaver et al. (2001), our study showed that Fast rats predominantly experience high ABN during the perinatal period, but generally underexpress α_1 and overexpress α_3 subunits in the amygdala as adults. Slow rats experience less ABN than Fast rats but show an increased expression of the α_1 subunit (Poulter et al., 2002). Clearly, if one were to examine frequency of ABN in the strains based solely on GABA_A subunit expression, Slow rats would be expected to exhibit higher frequency of ABN than Fast rats, since Slows and High ABN-LG rats in Meaney's group have similar GABA_A subunit expression. This, however, was not the case. These findings suggest that differences in GABA_A subunit expression between the

strains, akin to those reported in pup weight, may not result from maternal influences on brain development, but some other perinatal or prenatal experience.

LG-ABN Correlation

Unlike studies that have reported high correlations between ABN and LG behaviours (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999a; Meaney, 2001; Weaver et al., 2001), neither Fast nor Slow mothers performed these two behaviours with a high correlation. In fact ABN-LG was the least recorded behaviour in both strains. Since the basis for this study stemmed from the aforementioned reports (Liu et al., 1997; Caldji et al., 1998; Francis et al., 1999a; Meaney, 2001; Weaver et al., 2001) on the High and Low LG-ABN behaviours in Norway and LEH rats, this lack of correlation between ABN and LG in the Fast and Slow strains needs to be addressed. Several possible explanations exist for the apparent low correlation between LG and ABN activities observed in our two strains. These explanations include the fact that in our experiment entire litters were crossed, where as Meaney and colleagues only crossed a small number of pups per litter (Liu et al., 1997; Meaney, 2001). As stated earlier, crossing of the pups may have acted as a stressor on the mom which, in turn, may have led her to modify her interaction and nursing style with the pups. The crossed groups (FPFP, FPSM, SPFM, & SPSM) still continued to arched-back nurse, but did not perform licking and grooming in tandem with ABN. However, it does not explain the low correlation between those two behaviours in the control Fast and Slow rats. Perhaps the answer lies in the prenatal environment. Weaver found that there were no differences in the weaning weights of

high and low LG-ABN pups (Weaver et al., 2001). However, Fast and Slow control rats have obvious weight differences at birth (and presumably prior), which continues prior to weaning. It has been suggested that prenatal stress results in significant decreases in birth weight of rats and can lead to negative behaviours later in life such as high chronic corticosterone levels (associated with CRF) and increased anxiety (Drago, De Leo & Giardina, 1999; Edwards et al., 2002). Maturation of the nervous system and consequent adult behaviours depends in a large part on prenatal nutritional and environmental factors (Kehoe, Mallinson, Bronzino & McCormick, 2001), which could be easily perturbed by pre- and perinatal exposures. Further investigation into the prenatal environment of Fast and Slow rat strains is critical at this point to further pinpoint when differences between the strains arise.

Experiment 2: Restraint Activity

Normally Fast rats struggle in restraint significantly more than Slow rats (Anisman et al., 1997). In the crossfostering paradigm, we again observed this same main effect -- that Fast rats, regardless of rearing mother, continued to struggle significantly more vigorously than Slows -- with no interaction with the crossfostering experience. This suggests that different rearing environments have little or no effect on the mechanisms that control the activity of the two strains during restraint, which possibly reflect naive strain differences in impulsivity. These findings are consistent with previously recorded findings using Fast and Slow rats (Anisman et al., 1997; McIntyre et al., 1999b). In those studies, Fast rats were shown to be consistently more active than

Slow rats. These findings implicate either a genetic control mechanism for the behaviour, or some other pre- or postnatal environmental condition, but clearly the behaviours are not due to differences in previous exposure to different maternal behaviours.

Experiment 3: Kindling

Pre-kindling Afterdischarge Threshold and Duration

Examination of the local excitability of the amygdala (including ADTs and durations) in the strains revealed several intriguing findings associated with the strains and crossfostering. Initially the ADTs in the left amygdala of the Fast rat controls were slightly but significantly higher than the Slow rat controls. Because this result has not been observed previously, nor was it evident in the right amygdala, we suspect it to be spurious. Yet, equally unexpected was the observation that the AD duration of that threshold value was similar in the Fast and Slow rats. This again is rarely seen, as Fast rats typically have significantly longer initial AD durations than do Slow rats. However, it must be noted in this experiment that all animals received vastly more handling prior to kindling than in any other studies involving our two rat strains, and we have observed that excessive handling before kindling has profound consequences (acceleration) for epileptogenesis. The vehicle for this facilitation is not known, but repeated/chronic stress is suspected.

In spite of the unusual results in the two basic controls during the ADT measurements, some very strong effects were observed in the crossed groups in both strains. Specifically, the significant increase in ADTs in the crossed groups (FPFM,

FPSM, SPSM, & SPFM) compared to the controls (FC& SC) in both amygdalae ADTs suggested that the early prenatal stressor of being crossfostered can interact perhaps with the chronic stress of handling and dramatically decrease local excitability in the amygdala (higher ADTs) in both strains and in both amygdalae. Of course, the amygdala is a well-known target for stressor-induced effects (Inoue, Li, Abekawa, Kitaichi, Izumi, Nakagawa & Koyama, 2004; Vyas & Chattarji, 2004; Anisman, Lacosta, Kent, McIntyre & Merali, 1998, Mohapel & McIntyre, 1998).

Kindling Rate

Subsequent kindling of the left amygdala proceeded in a normal fashion, where Fast rats kindled much faster than Slow rats. This speed was realized both as shorter kindling rates and shorter cumulative ADs in Fast compared to Slow groups. Interestingly, however, the 3 Fast groups were not equal in their speed of kindling. Indeed, the fastest group to kindle was the FPFM rats, which were significantly faster than the FC and FPSM groups. This suggests that the stress of crossing can add to the speed of epileptogenesis; yet this effect was not seen in the Fast pups crossed to a Slow mom (FPSM). In support, Edwards reported that prenatal stress significantly hastened the rate of kindling seizure development in outbred adult male rats (Edwards et al., 2002). We take from this result that the nature of the mother is an important one, in some manner, in influencing the expression of the seizure-prone genes in the Fast rats. This maternal influence could result from mother's milk quality, or nursing style, or differential stress or other unknown conditions. Indeed, Bronzino and colleagues (1990)

reported that early malnutrition influenced seizure susceptibility in Wistar rats regardless of nutrition in adulthood. Specifically, pups that were nursed by malnourished mothers had lower kindling rates than pups whose mothers did not experience malnourishment (Bronzino et al., 1990). In analysis of our weight data, it was suggested that while the act of crossing pups resulted in a decrease in weight compared to control groups, crossing to Fast mothers might have been more detrimental to developing pups than crossing to Slow mothers. This idea was further supported, particularly in the Fast pup groups (FC, FPFM, & FPSM), since the FPSM group had the same kindling rate as the Fast controls and both were significantly greater than the FPFM group. However in the case of the Slow groups (SC, SPSM, & SPFM), it seemed that the crossing the litters had a more influential effect on the pups' kindling rates than the strain of the rearing mother. Thus, only the Fast pups were malleable by or vulnerable to the identity of the mother. This result suggests that the expression of genes is not equal given a similar experience, and that what is beneficial for one genotype may not be for another.

Convulsion Profiles

As the kindled convulsions made their appearance, the latencies from stimulus onset to convulsion onset were typical of Fast and Slow rats, i.e., longer in the Fast than in Slow rats, with crossing contributing little to no influence. The longer latencies in the Fast rats might seem to be incongruous as a reflection of being seizure prone, but it must be remembered that animals that are seizure prone usually have longer periods of seizure recruited from an instigating stimulus before the convulsion appears. As a result, it takes

only a few exposures to develop epilepsy in such individuals. With repetitions of the triggering stimulus, however, these long latencies in Fast rats shorten dramatically to be equal to the Slow rats - who by definition take much longer to develop the neural networks subserving the epileptogenic process. The duration of the ADs on those convulsive seizures, and the convulsive seizures themselves, were longer in the Fast than the Slow rats, with no differences between the groups within a strain. Clearly the genetics of the strains have an important signature that is applied to the manifestation of the epileptogenic process. This strain specific signature seems to be invariant on the profile of the seizures.

Lastly, after developing the stage-5 convulsive seizures, reassessment of the ADTs in the two strains reconfirmed the differential excitability that was measured in those same foci before the kindling process was initiated. That is, the Fast controls had lower thresholds (greater sensitivity) than the crossed Fast groups, an outcome that was mirrored in the 3 Slow groups.

Further Direction

At the onset of this study, we hoped to answer two questions. One was to determine whether the Fast and Slow strains differed in their maternal behaviour. We now have evidence that maternal behaviours in the strains do differ in some distinct ways. The second question was - if there were differences in mothering, what influence would these variations have on the documented difference between Fast and Slow rats with respect to adult behaviours and seizure susceptibility? The answer to the second

question was not as easily answered, and perhaps has given us more questions than answers.

At the turn of the century Hughlings Jackson defined epilepsy as a disorder originating in a “morbid nutrition” of the neuron (van Gelder & Sherwin, 2003), and this may be the key to the strain differences. The distinct variations in pup weight of the Fast and Slow rat strains give a possible link to variations in early neuronal development. Supporting this, the ketogenic (high fat) diet has long been a treatment for children with many types of epilepsy, while malnutrition early in life has been documented to promote seizures. Both of these facts suggest that nutrition, specifically involving fatty acids and their metabolism, affect seizure development (Bough et al., 1999; Huang et al., 2003). Hence, it will be necessary to analyse the milk quality of the Fast and Slow strains. Other important assessments will be to determine if a) the differences in neurochemistry still exist in the crossed groups, b) the expression of GABA_A subunits has changed and/or their physiology, and c) the brain structure size differences previously documented remain. Moreover, we must look at the prenatal environmental influences on embryonic development. It is probable that the behavioural and brain morphological differences identified in naïve Fast and Slow strains originated during embryonic stages of development, some of which are under genetic control while others are dependent upon the changing *in utero* environment.

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