**Early environmental influences in the development of learning and activity
differences in seizure-prone and seizure-resistant rats**

By

Victoria Thorne, B.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment
of the requirements for the degree of Master of Science

Department of Psychology

Carleton University

Ottawa, Ontario,

Canada

April 2004

© Victoria Thorne 2004

National Library
of Canada

Bibliothèque nationale
du Canada

Acquisitions and
Bibliographic Services

Acquisitions et
services bibliographiques

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 0-612-93988-X

Our file Notre référence

ISBN: 0-612-93988-X

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.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this dissertation.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de ce manuscrit.

While these forms may be included in the document page count, their removal does not represent any loss of content from the dissertation.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Abstract

The FAST and SLOW kindling strains, selectively bred for differences in seizure susceptibility, also exhibit comorbid learning, activity and impulsivity differences. In the present investigation, we evaluated the effect of maternal care on the development of the FAST and SLOW phenotypes by fostering litters to a mother of the opposite strain, to a different mother of the same strain or returning them to their natural mother. We found that FAST mothers rearing their own pups had a higher frequency of arched-back nursing than SLOW mothers, and that the strain of pup influenced maternal care, so that SLOW mothers rearing FAST pups had a higher frequency of arched-back nursing than SLOW mothers rearing other SLOW pups or their own pups. As adults, strain differences in spatial learning and activity previously reported were maintained. Thus, the behavioural phenotype of the FAST and SLOW strains was not modified by a change in maternal care through crossfostering.

Acknowledgements

I would like to thank Dr. Dan McIntyre and Dr. Krista Gilby for their support throughout my graduate career. Completion of this thesis would not have been possible without their tireless effort, and working under their supervision has been a wonderful learning experience.

I must also thank my fellow labmates, Tina and Pete, who have always been ready to lend a helping hand. To Andrea, the only person who truly understands the reality of “living in a colony room”, thank you for making such a massive project so enjoyable. And to, Jim, thanks for all the laughs, I’ll sincerely miss my arch-nemesis!

On a personal note, I wish to thank Floyd, who provided the love and emotional support that allowed me to reach this point.

Table of Contents

	Page
Abstract	ii
Acknowledgements	iii
Table of Contents	iv
List of Figures	v
Introduction	1
Methods	13
Results	18
Discussion	80
References	90

List of Figures

	Page
Figure 1. Mean ($\pm$ SEM) of the total number of arched-back nursing (ABN) observations summed over all days for each condition.	19
Figure 2. Mean ($\pm$ SEM) number of ABN observations per hour (collapsed over time of day) for the first ten days post-natal (<i>top panel</i>). Mean ($\pm$ SEM) number of ABN observations for SLOW mothers (<i>lower left panel</i>) and FAST mothers (<i>lower right panel</i>).	22
Figure 3. Mean ($\pm$ SEM) of the total number of licking/grooming (LG) observations summed over all days for each condition.	24
Figure 4. Mean ($\pm$ SEM) of the total number of arched-back nursing with licking/grooming (ABN/LG) observations summed over all days for each condition.	26
Figure 5. Mean ($\pm$ SEM) of the total number of blanket posture (BP) observations summed over all days for each condition.	29
Figure 6. Mean ($\pm$ SEM) of the total number of passive posture (PP) observations summed over all days for each condition.	31
Figure 7. Mean ($\pm$ SEM) of the total number of with pup (WP) observations summed over all days for each condition.	34
Figure 8. Mean ($\pm$ SEM) of the total number of no contact (NC) observations summed over all days for each condition.	36
Figure 9. Mean ($\pm$ SEM) weight of FAST and SLOW pups (males and females) at birth.	39
Figure 10. Mean ($\pm$ SEM) weight of pups in each condition (collapsed over sex) for PND 0 through 20 (<i>top panel</i>). Mean ($\pm$ SEM) weight of SLOW pups (<i>lower left panel</i>) and FAST pups (<i>lower right panel</i>).	41
Figure 11. Mean ($\pm$ SEM) weight gain over the first and last ten days postnatal in all conditions.	44
Figure 12. Mean ($\pm$ SEM) latency to retrieve the first pup for all conditions in a retrieval task.	46

Figure 13. Mean ($\pm$ SEM) latency to retrieve all pups during a retrieval task with a 5 minute limit for all conditions.	49
Figure 14. Mean ($\pm$ SEM) water consumption (collapsed over day) for males and females in each condition.	51
Figure 15. Mean ($\pm$ SEM) latency to reach a submerged platform (collapsed over trials) in the Morris water maze for males in all conditions.	54
Figure 16. Mean ($\pm$ SEM) latency to reach a submerged platform (collapsed over trials) in the Morris water maze for females in all conditions.	56
Figure 17. Mean ($\pm$ SEM) time spent in the target quadrant (i.e., the quadrant where the platform was located during training) over 15 second periods for males in all conditions.	58
Figure 18. Mean ($\pm$ SEM) time spent in the target quadrant (i.e., the quadrant where the platform was located during training) over 15 second periods for females in all conditions.	60
Figure 19. Mean ($\pm$ SEM) latency to reach a raised platform in the Morris water maze on each trial for males in all conditions.	63
Figure 20. Mean ($\pm$ SEM) latency to reach a raised platform in the Morris water maze on each trial for females in all conditions.	65
Figure 21. Mean ($\pm$ SEM) number of line crosses per day in the open field for males of all conditions.	67
Figure 22. Mean ($\pm$ SEM) number of line crosses per day in the open field for females of all conditions.	69
Figure 23. Mean ($\pm$ SEM) number of rears per day in the open field for males in each condition.	71
Figure 24. Mean ($\pm$ SEM) number of rears per day in the open field for females in each condition.	73
Figure 25. Mean ($\pm$ SEM) weight of male brains for each condition.	76
Figure 26. Mean ($\pm$ SEM) length of male brains in each condition.	78

Introduction

The study of psychopathology has been greatly advanced by the use of genetic models, such as transgenic or knockout mice. Yet these models are limited to the assessment of the influence of one gene, and do not permit the evaluation of multiple gene effects or the interaction of genes with the environment. To address these questions, selective breeding of animals for the overexpression or underexpression of behavioural traits is a desirable method to employ. The FAST and SLOW kindling rat strains were selectively bred to create differences in seizure susceptibility, which interestingly also produced comorbid behavioural features in the strains. Currently, it is unknown whether environmental factors play a role in the development of the FAST and SLOW behavioural phenotypes. However, the fact that the maternal environment is of vital importance to proper development of the nervous system is well documented. Thus, the purpose of the present investigation was to examine the role of maternal care in the development of several of the behavioural traits known to be different between the FAST and SLOW strains as adults.

The FAST and SLOW Kindling Strains

Racine et al. (1999) developed two strains of rats, one that is seizure-prone (FAST kindling) and the other seizure-resistant (SLOW kindling), by selectively breeding rats with opposing rates of amygdala kindling. The parent population of the FAST and SLOW strains included both Long Evans Hooded and Wistar rats. Males and females with the fastest kindling rates were selected and bred together to create the FAST colony, and rats

with slower than normal kindling rates were chosen and bred together to create the SLOW colony. Such breeding selection for kindling rate continued for the first eleven generations, at which point there was no longer an overlap in kindling rates between the strains and selection was, therefore, relaxed. The FAST and SLOW strains are currently in the 56th generation, and there remains no overlap in their respective kindling rates.

The rate of kindling for the FAST strain is consistently more rapid than in the SLOW strain in all neuroanatomical structures with the amygdala kindling on average 4.8 times faster in the FAST strain than in the SLOW strain (McIntyre et al., 1999a). The FAST and SLOW kindling strains also differ with respect to local seizure excitability (i.e., the local afterdischarge threshold) in the hippocampus, piriform and perirhinal cortices, with the FAST strain exhibiting lower thresholds (McIntyre et al., 1999a). In contrast, afterdischarge thresholds do not differ in the amygdala, the structure used as the selection vehicle during breeding. Only the afterdischarge durations differ in the amygdala. Likely the differential speed of amygdala kindling between the strains is related to the rate of recruitment of the piriform and perirhinal cortices following locally triggered amygdala seizures, which then project to the motor networks to create the fully kindled, convulsive seizure.

Differences in strain seizure predisposition are complemented by numerous morphological, neurochemical and genetic differences. The ventricles and piriform cortex of the FAST strain are significantly larger than the SLOW strain, whereas the corpus collosum and dorsal hippocampus are significantly smaller (Gilby et al., 2002). Interestingly, preliminary data indicates that strain dependent hippocampal differences

are already evident at postnatal day 21 (Gilby, unpublished results). SLOW rats also have increased mossy fibre sprouting in the dentate gyrus, in comparison to FAST rats (Elmer et al, 1997). Further, adult constitutive gene expression differs between the strains, with approximately 35 genes found to be differentially displayed in the hippocampus thus far (Gilby et al., 2002). Perhaps even more importantly, strain differences are also apparent in gene regulation during embryogenesis; however, at this point only 3 strain-dependent expression differences have been identified across numerous embryonic timepoints, in comparison to the much larger number observed in adulthood (Gilby, unpublished results).

Pharmacological data shows that the FAST and SLOW kindling strains have remarkably different sensitivities to GABA modulators. Specifically, SLOW rats are more sensitive to positive modulators (e.g., pentobarbital), whereas FAST rats are more sensitive to negative modulators (e.g., picrotoxin) (McIntyre et al., 1999a; Racine et al., 2003). Furthermore, FAST rats develop convulsive seizures in response to negative modulators at doses significantly lower than SLOW rats, whereas SLOW rats experience sedation at lower concentrations of positive modulators than FAST rats. Underlying these differences in GABA functioning is a differential expression of GABA receptor subunits. In comparison to control rats (Long Evans hooded rats), FAST rats overexpress $\alpha 2$, 3 and 5 GABA_A subunits, but underexpress the $\alpha 1$ subunit in the amygdala, piriform and perirhinal cortices (Poulter et al., 1999). On the contrary, SLOW rats underexpress $\alpha 2$, 3 and 5 subunits, but overexpress the $\alpha 1$ receptor subunit, compared to control rats in those same structures. Interestingly, the $\alpha 1$ receptor subunit is normally the major subunit

expressed in the adult brain, whereas $\alpha 2$, 3 and 5 are highly expressed during development (Poulter et al., 1992). Thus, the FAST rats, as adults, exhibit immature or juvenile expression of the GABA_A α subunits. In addition, it is important to recognize that ‘normal’ immature rats are also more seizure-prone than adult rats (Szot et al., 2001). Thus, arrested GABAergic development in FAST rats may be one of their defining features.

Synaptic transmission at the GABA_A receptor also differs between the strains. FAST rats have smaller GABA miniature inhibitory postsynaptic currents (mIPSCs) that decay more slowly than control rats (McIntyre et al., 2002). On the contrary, GABA mIPSCs in SLOW rats are larger and decay more quickly than control or FAST rats. These physiological differences are known to promote greater synchrony in neural network behaviour and likely contribute to the epileptogenesis that characterizes the seizure-prone FAST rats.

Activity of other neurotransmitter systems has also been assessed in the FAST and SLOW kindling rats. There are no differences in basal noradrenaline, but FAST rats have higher levels of basal dopamine activity in the prefrontal cortex (McIntyre et al., 1999b; Anisman et al., 2000). In addition, there are no differences in NMDA and AMPA receptor binding in the temporal lobe or the number of cholinergic neurons in the basal forebrain (McIntyre, in progress). Differences in prefrontal dopaminergic activity are also seen in children with attention deficit hyperactivity disorder, and suggest a behavioural disposition that might be characteristic of FAST rats as well (Anisman and McIntyre, 2002).

Importantly, it has been shown that these two strains differ not only with respect to seizure susceptibility, but also in other behavioural measures. The strains exhibit markedly different responses to novel environments, stressors and learning paradigms. In the elevated plus maze, a test of anxiety, the FAST rats enter the open arms more frequently than the SLOW strain (Mohapel & McIntyre, 1998). Open-arm *avoidance* is usually associated with greater fear, implying SLOW rats are more fearful than FAST rats. Alternatively, exposure to a less anxiogenic task, such as the open field test, yields strikingly different results (Mohapel & McIntyre, 1998). The SLOW strain initially is more active than the FAST strain, yet displays a marked decrease in activity over days. This profile is consistent with the gradual decrease in exploration (i.e., habituation) seen in normal control rats. However, the FAST strain maintains consistently high levels of activity and no habituation throughout testing days. Thus, FAST rats demonstrate high levels of arousal and activity on each day.

Activity differences are also apparent with tests of physical restraint. When exposed to restraint stress, the SLOW strain immediately assumes an immobile posture whereas the FAST strain struggles vigorously throughout the trial (Ansman et al., 1997). FAST rats even periodically break free from the funnel-shaped plastic bag used for the restraint. Yet, if the FAST strain is reared on a diet supplemented with poly-unsaturated fatty acids (e.g., salmon oil), the FAST strain behaves identically to the SLOW strain in the restraint test (Gilby, unpublished results). In contrast, the behaviour of the SLOW strain is not affected by diet supplementation. Furthermore, the FAST strain consumes 30% more water daily than the SLOW strain, a common feature of essential fatty acid

deficiency (Gilby, unpublished results). Thus, FAST rats may have metabolic differences that differentiate them from the SLOW rats, which could be altered or 'normalized' by environmental manipulations such as diet.

Sexual reactivity also differs between the strains, with the FAST strain displaying an impulsive style compared to the SLOW strain. Normally, male rats will only attempt to mate with a female when given the appropriate cues indicating that the female is in estrous (McIntyre & Anisman, 2000). When introduced to a non-receptive female (i.e., a female not in estrous), FAST males attempt to mount immediately, whereas SLOW males never display this behaviour (Michaud et al., 1999). In fact, the latency to mounting for FAST males is identical regardless of the female's estrous state. SLOW males, on the other hand, take several minutes before mounting an estrous female. These differences might be related to dopamine function in that FAST males have increased DOPAC levels in the amygdala and nucleus accumbens in response to contact with a female (regardless of estrous state), unlike SLOW males who only have a modest increase in DOPAC levels in the nucleus accumbens (Shin et al., 2002). DOPAC is a metabolite of dopamine and reflects activity in that neurochemical pathway. Interestingly, despite the more aggressive sexual behaviour of FAST rats, their testosterone levels are significantly lower than SLOW rats.

Finally, the FAST strain exhibits learning deficits in several tasks in comparison to the SLOW strain (Mohapel & McIntyre, 1998; Anisman & McIntyre, 2002). Morris water-maze performance, which is a test of spatial learning, is consistently inferior for the FAST strain, regardless of the location of the hidden, submerged platform (e.g., fixed

versus variable over days). Furthermore, the FAST strain is more likely to be distracted by irrelevant cues during testing, whereas distracters have no effect on the SLOW strain, suggesting FAST rats are more distractible than SLOW rats. The FAST strain, however, does demonstrate improved performance when pre-training with a raised platform is performed a day prior to testing or when the location of the platform is marked by an overhanging cue. Thus, FAST rat behaviour can be improved by special training, much like humans with mild to moderate learning/performance disabilities.

Clearly, the FAST and SLOW strains show distinct differences in attention (e.g., Morris water maze performance), impulsivity (e.g., sexual responsiveness), and hyperactivity (e.g., open field test, restraint test), behaviours that are considered to be hallmark features of human attention deficit hyperactivity disorder (ADHD). As a result, it has been suggested that the FAST rat strain may serve as a model of attention deficit disorder with impulsivity. ADHD afflicts 3 - 5% of school-age children, yet, despite the prevalence, there are very little data concerning the epidemiology of the disorder (e.g., age, gender) (Paule et al., 2000). However, it is primarily considered to be a developmental disorder. The heritability of ADHD is estimated to be 0.75 to 0.91, thus the disorder is most likely the result of the interaction of several genes with environmental influences (Levy et al., 1997). Several environmental factors such as maternal psychopathology, family dysfunction and low social class have been associated with ADHD (Biederman et al., 2002; Faraone & Biederman, 1998). In fact, the risk of ADHD diagnosis increases with the number of Rutter's adversity indicators (psychosocial risk factors for childhood mental disorders) and higher Rutter's scores tend

to occur when ADHD is present with a comorbid psychopathology (Biederman et al., 1995). Comorbidities are very common in ADHD, and several disorders occur at a high frequency with ADHD, such as learning disabilities, anxiety and mood disorders. Epilepsy is also a common comorbidity of ADHD, as the prevalence rate of seizure disorders in patients with ADHD is about 20 times higher than expected from a normal population (Wolf & Forsythe, 1978). Barkley (1990) found that 20% to 30% of children with epilepsy have comorbid ADHD, while Hempel et al. (1995) have shown that 35% of children with intractable epilepsy were previously diagnosed with ADHD.

Maternal Care and Development

Parental care is believed to modulate neurological development. For instance, child abuse and neglect have long been known to negatively impact cognitive development of children (Mackner et al., 1997; Koenen et al., 2003). Moreover, in rodents, a strong link has been identified between the quality of maternal care and proper neural and cognitive development. As such, the maternal environment is considered to be one of the most important environments in the life of any mammal (Pryce & Feldon, 2003). However, the mechanisms underlying this influence are largely unknown.

In the rat, maternal behaviour consists of a variety of nursing postures with intermittent licking and grooming of the pups, yet there are naturally occurring variations in the proportion of time spent performing these behaviours. The tactile stimulation resulting from two important maternal behaviours, arched-back nursing (ABN) and licking/grooming (LG) are crucial for proper development. In contrast to other nursing

postures (e.g., blanket postures where the mother is flattened over the pups), ABN enables the mother to readily groom her pups while nursing, and allows the pups to move around beneath the mother, possibly switching nipples (Liu et al., 2000). Interestingly, the frequency of LG is associated with higher levels of oxytocin receptors in the medial preoptic area, amygdala, and hypothalamus (Champagne et al., 2001). Oxytocin is involved in the initiation of maternal behaviour, including milk ejection. Reduction in oxytocin release is associated with decreased milk ejection and slower body weight gain in pups (Ohta et al., 2002)

Maternal behavior has been shown to influence the types of behaviours exhibited by offspring. Adult offspring of mothers who had a high frequency of ABN and LG (high ABN/LG mothers) perform significantly better in the Morris water-maze than offspring raised by mothers with a low frequency of ABN and LG (low ABN/LG mothers) (Liu et al., 2000). This difference in spatial learning ability is similar to the superior learning ability of the SLOW strain versus the FAST strain. High ABN/LG offspring also are less fearful and have reduced adrenocorticotrophic hormone and corticosterone responses to stress (Liu et al., 1997). Interestingly, a reduced stress response is typical of the FAST strain, not the SLOW strain.

Various molecular and morphological differences underlie the behavioural phenotype of high and low ABN/LG offspring. Importantly, offspring of high ABN/LG mothers are reported to exhibit increased synaptogenesis and hippocampal NMDA receptor expression as pups and adults in comparison to offspring of low ABN/LG mothers. Hippocampal BAX (a pro-apoptotic protein) expression is also increased in

adult offspring of low ABN/LG mothers, suggesting an increased risk for neuronal loss through apoptosis (Weaver et al., 2002). Moreover, offspring of high ABN/LG mothers have superior hippocampal neuronal survival as evidenced by more BrdU labelled cells in the dentate gyrus at day 21 and 90 than offspring of low ABN/LG mothers (Bredy et al., 2003). Alterations in amygdala GABA_A receptor subunits also occur as a result of the maternal environment, with high ABN/LG offspring expressing significantly higher levels of $\alpha 1$ subunits (similar to SLOW rats), whereas low ABN/LG offspring have increased expression of $\alpha 3$ and 4 subunits (similar to FAST rats, or experimental treatments that increase seizure genesis) (Caldji et al., 2000).

Furthermore, crossfostering of pups from low ABN/LG mothers to high ABN/LG mothers has been shown to eliminate differences in fearfulness, Morris water-maze performance and hippocampal synaptogenesis (Francis et al., 1999; Liu et al., 2000). Low ABN/LG offspring reared by high ABN/LG mothers are less fearful in the open field than low ABN/LG offspring reared by their biological mothers (Francis et al., 1999). Moreover, the adult offspring of low ABN/LG mothers that were reared by high ABN/LG mothers perform as well as the normal offspring of high ABN/LG mothers in the Morris water maze (Liu et al., 2000). However, adult offspring of high ABN/LG mothers, reared by low ABN/LG mothers, do not suffer any effect of crossfostering. The performance of these rats on the Morris water-maze is indistinguishable from offspring born to and reared by high ABN/LG mothers. Thus, learning and fear responses are enhanced in offspring with a genetic predisposition for deficits (low ABN/LG offspring)

when the environment is improved, but unaffected in resilient offspring (high ABN/LG offspring) when the quality of the environment diminishes.

Similar effects of maternal care are seen in C57 and BALBc mice. BALBc mice have severe spatial learning impairments and hippocampal damage in comparison to C57 mice. BALBc mice have a lower frequency of ABN and LG behaviours in contrast to C57 mothers, and BALBc pups reared by C57 mothers have improved spatial task performance (Anisman et al., 1998). Furthermore, prenatal and postnatal fostering of C57 embryos to BALBc mothers produced adult offspring with behaviours characteristic of the birth mother (BALBc) not the genetic mother (C57) (Francis et al., 2003). Yet, if the C57 offspring are only fostered with BALBc mothers once, either prenatal or postnatal, their behaviour is identical to C57 offspring fostered to C57 mothers both prenatally and postnatally. Thus, the response of pups to the postnatal environment may be programmed *in utero*, independent of genetics (Francis et al., 2003).

Objective

To date, the influence that environmental factors (i.e., maternal care) may have on the creation of the behavioural differences that characterize the FAST and SLOW kindling strains has not been assessed. However, clear parallels exist between the offspring of high and low ABN/LG mothers and the FAST and SLOW kindling strains, which may suggest a possible role for differential maternal care in the development of these contrary behavioural phenotypes. For instance, SLOW rats have superior spatial learning ability and increased expression of *adult* GABA_A receptor subunits, similar to

high ABN/LG offspring. In contrast, FAST rats and low ABN/LG offspring have inferior spatial learning and increased expression of *embryonic* GABA_A receptor subunits. Thus, in the present experiment, we attempted to determine whether some of the documented behavioural differences between the strains could be attributed to differential early maternal influences, rather than to straight genetic mechanisms. In order to accomplish this, we crossfostered entire litters of each strain to mothers of the opposing strain on their birth date and evaluated the impact that crossfostering might have on their ultimate adult behavioural profiles, specifically the learning and hyperactivity differences known to exist between the two strains

Methods

Subjects

The subjects used in this study were 30 FAST mothers and 30 SLOW mothers and their respective litters. Mothers were housed individually with their litters in Plexiglas cages (32 cm x 22 cm x 20 cm) with food and water available *ad libitum*. On post-natal day (PND) 23, pups were weaned and 120 pups were selected (60 male, 60 female) for behavioural testing. Subsequently, adult rats were housed in pairs. The colony room was temperature-controlled (21 ± 2 ° C) and maintained on a twelve hour light-dark cycle with lights on at 8:00 a.m. All manipulations were conducted during the light phase. The Carleton University Animal Care Committee approved all experimental procedures under protocol P02-9.

Crossfostering Procedure

30 FAST and 30 SLOW mothers were selected for the study and assigned to one of the three crossfostering conditions per strain. Within twelve hours of birth, pups were removed from the mother, sexed and weighed. The whole litter was then returned either to a mother of the opposite strain, a different mother of their same strain or the birth mother. Thus, six groups were created: FAST pups to a SLOW mother (FPSM), SLOW pups to a FAST mother (SPFM), FAST pups to a different FAST mother (FPFM), SLOW pups to a different SLOW mother (SPSM), FAST control pups with birth mother (FC) and SLOW control pups with birth mother (SC). When pups were returned to a different mother (e.g., FPSM, SPFM, FPFM, SPSM), the litter size was culled so that a mother would not have a new litter larger than her original litter.

Maternal Behaviour Observations

Maternal behaviour observations began on PND 1 and continued daily until PND 10. Observations were conducted at 8am, 10am, 12pm, 1pm, 3pm and 5pm for one hour. All observations were conducted during the light phase since nursing occurs most frequently during that period (Liu et al., 1997). Each mother's behaviour was recorded every three minutes during the one-hour period, for a total of 21 observations per hour and 126 observations per day. Behaviours were classified into seven categories: arched-back nursing (ABN), licking and grooming any pup (LG), arched-backed nursing with licking and grooming (ABN/LG), blanket posture (BP), passive posture (PP), with pups not nursing (WP) and no contact with pups (NC). Blanket posture is defined as the mother lying over her pups to nurse and passive posture is classified as the mother nursing her pups while on her side or back.

Pup weights were recorded daily in the afternoon from PND 1 to PND 10, and then every other day until PND 20. In addition, pup retrieval was assessed every third day from PND 1 to PND 10. On the days of retrieval testing, litters were weighed and returned to the opposite end of the home cage from the nest. The latency for the mother to retrieve the first pup along with the total time to retrieve all pups and return them to the nest was measured.

Water Consumption

Water consumption was recorded over six consecutive days beginning at approximately 80 days of age. Each day water bottles were weighed (1 g = 1 ml) and consumption was calculated. Since animals were housed in pairs, an average

consumption for each animal was determined. Animal weight was again measured once daily over the six days.

Morris Water Maze

At approximately 90 days of age, animals began training/testing in the Morris water maze (a white polypropylene pool with a circumference of 158cm and a depth of 60cm). The pool was filled with water made opaque by the addition of 1000 c.c. of powdered skim milk. A clear Plexiglas platform, with a diameter of 14cm, was placed in the middle of the northeast quadrant of the maze, submerged two centimetres below the surface of the water, and therefore, concealed. Animals received four trials per day for six consecutive days and the latency to find the platform was recorded. Each trial began by placing the animal facing the edge of the pool in one of four possible starting positions (i.e., north, east, south, west). The order of starting positions was random, with the provision that all starting positions were used each day. Trials lasted until the animal located the platform and remained there for ten consecutive seconds with a maximum trial time of sixty seconds. If the rat did not find the platform, it was led to the platform and left there for ten seconds. Each trial was separated by a sixty-second rest period during which the rat was placed in a dry cage. On the seventh day, a probe trial was administered where the platform was absent. All rats started from the same quadrant and were allowed to explore the maze for sixty seconds. The time spent in each quadrant was recorded during fifteen-second intervals. This test was to determine how well the rat knew where the platform was located, and how persistent the rat was in locating it. Finally, on the last day of training the platform was raised out of the water so that it was

visible to the rat, however, in all other aspects the procedure was identical to previous training sessions (i.e., 4 sixty second trials). This test measured the rats' motoric abilities by measuring latency to a platform that could easily be seen. In all tests, the latency time for the rat to find the platform was recorded. All trials were videotaped using a camera positioned on the ceiling and analysed with Smart Tracking 1.5 software (San Diego Instruments, San Diego, CA).

Open Field

The "open field" used in this experiment was a white wooden box (69 x 69 inches) with a grid on the bottom dividing it into twenty-five identical quadrants. In a brightly lit room, each rat was placed into the top left corner of the open field, facing the corner, and allowed to explore for five minutes. Each rat received one five-minute trial per day for four consecutive days. The number of lines crossed by the rat, the tendency for thigmotaxia and number of rears were determined for each five-minute period. Thigmotaxic tendency was determined by calculating the time spent in the periphery of the box. Open field activity was videotaped by a camera positioned above the maze.

Histology

Male rats were deeply anaesthetized with SomnotolTM (Sodium pentobarbital, 65 mg/kg i.p.) and perfused through the heart with 60 c.c. of saline followed by 60 c.c. of 4% paraformaldehyde. Brains were removed and stored in a 4% paraformaldehyde solution at 4°C. Gross brain morphology was assessed by weighing the brain and measuring the length and width.

Statistical Analyses

Maternal behaviour, pup weight and Morris water maze data were analysed with a univariate repeated measures ANOVA. In the case that Mauchly's test of sphericity was violated, the results of multivariate tests were reported. A repeated measures MANOVA was used to analyse open field and retrieval data. Gross brain morphology was also analysed with MANOVA. A type I error rate of $p < .05$ was used in all analyses, and *post-hoc* multiple comparisons used the Bonferroni correction.

Results

Breeding Success

82.2% of SLOW females successfully mated and reared healthy litters during this experiment, whereas 6.7% of SLOW females had unsuccessful litters (i.e., pups died shortly after birth) and 11.1% of SLOW females did not become pregnant. Only 57.1% of FAST females successfully mated and reared litters, since 12.5% of FAST females that got pregnant produced unsuccessful litters and 30.4% of FAST females failed to become pregnant. Interestingly, the pups in unsuccessful litters appeared to have died due to starvation (no milk band was visible across their bellies), even though the mothers appeared to be nursing. The rate of unsuccessful litters was not related to the stress of crossfostering, as only one crossfostered litter was unsuccessful. There were no significant differences in litter size or nest quality as a result of strain or crossfostering.

Maternal Behaviour

Total arched-back nursing (*summed* over all observations and days) did not vary significantly by strain ($F[1,54]=0.84$, $p<.36$) or crossfostering ($F[2,54]=0.99$, $p<.38$) (Figure 1). However, the frequency of ABN over each day (i.e., the *daily* experience of the pups during development), varied as a function of day x mother's strain x crossfostering ($F[18,92]=2.54$, $p<.01$). Specifically, the simple main effect of strain was significant in control mothers (SC, FC) on PND 3, 4, 5, 8 and 10 with the FC mothers performing ABN significantly more often. The simple main effect of strain was only significant on PND 10 for litters reared by a mother of the opposite strain (FPSM performed ABN more frequently than SPFM), and the effect of strain was not significant

Figure 1. Mean ($\pm$ SEM) of the total number of arched-back nursing (ABN) observations (summed over all days) for each condition.

in mothers crossed with pups of the same strain (SPSM, FPFM). A significant simple main effect of crossfostering was found on PND 5 in the SLOW strain (FPSM mothers had the highest frequency of ABN) and on PND 8 and 10 in the FAST strain (FC performed ABN most often). Finally, the simple main effect of day was only significant in SPFM, SPSM, SC and FC. Figure 2 demonstrates that mothers rearing SLOW pups (SPFM, SPSM, SC) had a tendency to perform ABN more frequently early in the nursing period (PND1-4). However, the simple main effect of day in the FC group was the result of a lower than normal frequency of ABN on PND 6 only. The FPFM and FPSM groups had no significant effect of day on frequency of ABN. Finally, a significant main effect of time of day ($F[5,50]=4.22, p<.01$) was found, as mothers performed ABN more frequently in the early morning and evening compared to mid-day.

The total number of observations for licking and grooming (LG) or ABN with LG did not differ significantly between the groups as a result of strain or crossfostering (Figures 3 & 4, respectively). Similarly, the effect of strain and crossfostering on ABN/LG and LG over all time periods was not significant. There were significant main effects of day ($F[9,46]=3.82, p<.01$) and time ($F[5,50]=2.83, p<.03$) on ABN/LG. All mothers performed significantly more ABN with LG on PND 1 compared to later in the nursing period (PND 4 – 8) and ABN/LG occurred significantly more often in the morning. Similarly, there were significant main effects of day ($F[9,46]=3.96, p<.01$) and time ($F[5,50]=6.61, p<.01$) on LG. LG occurred most frequently in the morning (before 1pm) and evening (after 5pm), and was also more common on PND 1 and 2.

Figure 2. Mean ($\pm$ SEM) number of ABN observations per hour (collapsed over time of day) for the first ten days postnatal (*top panel*). Mean ($\pm$ SEM) number of ABN observations per hour for SLOW mothers (*lower left panel*) and FAST mothers (*lower right panel*) over the first ten days.

Figure 3. Mean ($\pm$ SEM) of the total number of licking/grooming (LG) observations (summed over all days) for each condition.

Figure 4. Mean ($\pm$ SEM) of the total number of arched-back nursing with licking/grooming (ABN/LG) observations (summed over all days) for each condition.

The total number of observations for blanket posture (BP) were significantly higher in the SLOW groups ($F[1,54]=4.15, p<.05$), but there was no significant effect of crossfostering (Figure 5). Throughout all observation periods, the frequency of BP observations varied as a function of day x time x strain x crossfostering interaction ($F[90,20]=2.36, p<.02$). The effect of strain was significant at 3pm on PND 4 in the mothers rearing pups of a different strain (FPSM, SPFM) and at various time points on PND 3, 7 and 10 in mothers rearing different pups of the same strain (SPSM, FPFM). In all cases, the SLOW mother (FPSM, SPSM) had a significantly higher frequency of BP. Likewise, the effect of strain was significant at various time points on PND 3 though PND10 in control groups (FC, SC), with the SC mothers showing BP more often than FC mothers. The simple main effect of crossfostering was significant during various time points on PND 1 – 5 and 8, with the FPFM performing BP more than FC and SPFM. Likewise, SPSM spent significantly more time BP than FPSM at various time points on PND 1, 5, 7 and 8. There were no consistent trends for either time or day, with respect to frequency of BP.

Total observations of passive posture (PP) were significantly greater in FAST mothers compared to SLOW mothers ($F[1,54]=4.93, p<.03$), yet the effect of crossfostering was not significant (Figure 6). Over all days, the frequency of PP varied as a function of day x strain x crossfostering ($F[18,92]=2.15, p<.01$) and day x time ($F[45,10]=3.10, p<.03$). The simple main effect of strain was only significant on PND 8 and 10 in mothers that reared litters of the opposite strain, and in both cases SPFM had a higher frequency of PP than FPSM. The simple main effect of crossfostering was

Figure 5. Mean ($\pm$ SEM) of the total number of blanket posture (BP) observations (summed over all days) for each condition.

Figure 6. Mean ($\pm$ SEM) of the total number of passive posture (PP) observations (summed over all days) for each condition.

significant only in the FAST strain on PND 8 and 10, with SPFM mothers performing PP more often than both FC and FPFM. The simple main effect of day was significant in all groups, except SPSM. In general, the frequency of PP increased over days in all groups, but PP was consistently the most infrequent nursing posture.

The total number of observations wherein mothers were with their pups yet were not nursing or licking/grooming (i.e., WP) was significantly greater in the FAST mothers ($F[1,54]=17.19$, $p<.01$) compared to SLOW mothers (Figure 7). Further, WP observations over all time periods varied as a function of time x strain ($F[5,50]=3.76$, $p<.01$) and day x time x crossfostering ($F[90, 20]=1.95$, $p<.05$). FAST mothers spent significantly more time WP early in the day than SLOW mothers, but both strains had an increased frequency of WP later in the day. There was no consistent trend in WP behaviour over days. Total observations of no contact (NC) between mothers and pups did not differ significantly as a result of strain or crossfostering (Figure 8). However, the frequency of NC varied as a function of day ($F[9,46]=7.01$, $p<.01$) and time x strain ($F[5,50]=3.56$, $p<.01$). FAST mothers spent more time away from the litter at 8am and 3pm compared to SLOW mothers, who had a higher frequency of NC at 10am.

Overall, when analyses were conducted on total behaviour of FAST and SLOW mothers with their own pups (i.e., the natural conditions, FC and SC), there were significant differences in total ABN and total BP (Figures 1 & 5, respectively). The FC mothers perform ABN significantly more often ($F[1,18]=7.89$, $p<.01$), while SC mothers perform BP significantly more often ($F[1,18]=8.18$, $p<.01$). However, there were no significant strain differences in total ABN/LG, LG and PP. In addition, there were no

Figure 7. Mean ($\pm$ SEM) of the total number of with pup (WP) observations (summed over all days) for each condition.

Figure 8. Mean ($\pm$ SEM) of the total number of no contact (NC) observations (summed over all days) for each condition.

significant differences in total observations of nursing behaviour (the total of all ABN, BP and PP observations) when control mothers are analysed separately. FC mothers also display more frequent WP behaviours ($F[1,18]=5.42$, $p<.03$), whereas SLOW mothers spend more time not in contact with pups (NC) ($F[1,18]=5.48$, $p<.03$).

Pup Weight

At birth, the weight of the FAST and SLOW strains differed significantly as a function of strain ($F[1,512]=105.66$, $p<.01$) and sex ($F[1,512]=17.12$, $p<.01$). The FAST pups were significantly heavier ($6.26 \pm 0.03\text{g}$) at birth than the SLOW pups ($5.80 \pm 0.03\text{g}$), and males were significantly larger ($6.12 \pm 0.03\text{g}$) than females ($5.94 \pm 0.03\text{g}$) (Figure 9). The interaction of strain and sex was nearly significant ($F[1,512]=3.64$, $p<0.06$), however, *post-hoc* analysis revealed that the simple main effect of sex was only significant in the SLOW strain. In addition, pup weight varied over days as a function of day x strain x crossfostering ($F[28,982]=4.91$, $p<.01$). There were no significant sex differences for pup weight ($F[1,504]=0.21$, $p<.88$). *Post-hoc* analysis revealed that simple main effects of strain were significant on all days for pups crossed to mothers of a different strain (i.e., SPFM and FPSM), with the FPSM pups being larger (Figure 10). Likewise, FC pups were heavier than SC pups on all days, although the simple main effect of strain did not reach significance on PND 4 through 8. FPFM pups were significantly heavier than SPSM on PND 1 and 12 through 20. Interestingly, the simple main effect of crossfostering was only significant on PND 3, and PND16 through 20 in the FAST strain, but in the SLOW strain the effect of crossfostering was significant on

Figure 9. Mean ($\pm$ SEM) weight of FAST and SLOW pups (males and females) at birth.

Figure 10. Mean ($\pm$ SEM) weight of pups in each condition (collapsed over sex) for PND 0 through 20 (*top panel*). Mean ($\pm$ SEM) weight of SLOW pups (*lower left panel*) and FAST pups (*lower right panel*).

PND 3 to 12. FPSM pups were significantly smaller than both FPFM and FC pups after PND 14, and SPFM pups were significantly smaller than SPSM and SC pups on PND 3 – 12.

Differences in weight gain over the first ten days postnatal (i.e., weight at PND 10 minus weight at PND1) versus the last ten days (i.e., weight at PND 20 minus weight at PND 10) varied as a function of time period x strain x crossfostering ($F[2,504]=31.17$, $p<.01$). *Post-hoc* tests indicated that a simple main effect of strain was significant in pups reared by a mother of a different strain (FPSM, SPFM) over the first ten days, but in pups reared by mothers of the same strain (FPFM, SPSM, FC, SC), the strain difference was only significant over the last ten days. Thus, FPSM pups gained more weight than SPFM pups initially, but FPFM and FC pups gained more weight than SPSM and SC pups after PND 10. A significant effect of crossfostering was found only after PND 10 in the FAST strain, but over all days in the SLOW strain. Specifically, FPSM pups gained less weight than FC and FPFM pups after PND 10, whereas, SPFM pups gained less weight than SPSM and SC pups before PND 10, but gained more weight after PND 10 (Figure 11).

Pup Retrieval

A repeated measures MANOVA revealed a significant effect of day ($F[6,41]=6.88$, $p<.01$), mother's strain ($F[2,45]=19.73$, $p<.01$) and crossfostering ($F[4,90]=4.33$, $p<.01$) on retrieval latencies. Univariate tests revealed that the effect of day was only significant for the latency to retrieve the first pup, and between-subjects analysis indicated that an effect of mother's strain was only found in the latency to retrieve the first pup (Figure 12). However, an effect of crossfostering was found in both

Figure 11. Mean ($\pm$ SEM) weight gain over the first and last ten days postnatal in all conditions.

Figure 12. Mean ($\pm$ SEM) latency to retrieve the first pup for all conditions in a retrieval task.

latency to retrieve the first and all pups. FAST mothers retrieved the first pup quicker than SLOW mothers, and all mothers retrieved the first pup faster on PND 1 compared to the remaining days (Figure 12). Bonferroni *post-hoc* tests indicated that control mothers (i.e., FC and SC) were quicker to retrieve the first pup in comparison to the other mothers (i.e., FPSM, SPFM, SPSM, FPFM). A similar tendency was apparent for latency to retrieve all pups, but it did not reach significance (Figure 13).

Water Consumption

A repeated measures MANCOVA, with mean weight as a covariate, revealed that consumption did not differ significantly over days ($F[4,103]=1.03$, $p=.39$), but varied as function of strain x crossfostering ($F[2,106]=5.77$, $p<.01$) and a strain x sex interaction ($F[1,106]=11.92$, $p<.01$). Interestingly, the strain x crossfostering x sex interaction did not reach significance. Bonferroni *post-hoc* comparisons indicated that the simple main effect of crossfostering was significant only for the FAST groups, but simple main effects for strain were significant in all crossfostering conditions. As illustrated in Figure 14, the FPSM group consumed significantly more water than both the FPFM and FC groups, and in all cases the FAST groups consumed more water than the SLOW groups. Furthermore, when controlling for weight, a significant simple main effect of sex was only found in the SLOW groups, with males consuming more water than females.

Morris Water Maze

Latency to find a submerged platform varied as function of day x trial x strain ($F[15,93]=3.73$, $p<.01$) and a trial x crossfostering x sex x strain interaction ($F[2,210]=2.55$, $p<.02$). Bonferroni *post-hoc* comparisons showed that a significant simple

Figure 13. Mean ($\pm$ SEM) latency to retrieve all pups during a retrieval task with a 5 minute (i.e., 300 sec) limit for all conditions.

Figure 14. Mean ($\pm$ SEM) water consumption (collapsed over day) for males and females in each condition.

main effect of strain was apparent on all testing days and trials, except the first trials on the initial day of testing. Overall, the performance of all the FAST conditions was inferior to the performance of all SLOW conditions, yet all groups had significant declines in latency over days (Figures 15 & 16). Interestingly, the female FPFM group performed significantly worse than FPSM females on trial 1 and FC females on trials 2 and 3.

In the probe trial, a repeated measures ANOVA revealed a significant blocks of time x strain interaction ($F[3,321]=13.14, p<.01$). Bonferonni *post-hoc* analysis indicated that there was a simple main effect of blocks of time in the SLOW conditions only. As shown in Figures 17 and 18, the SLOW conditions initially spent significantly more time in the goal quadrant than the FAST rats, but the FAST rats spent the lesser amount of time in the goal quadrant throughout all blocks of time. Furthermore, the simple main effect of strain was only significant during the first 15 seconds of the trial. Interestingly, there was also a significant strain x crossfostering interaction ($F[2,107]=5.00, p<.01$) and although significant main effects of strain were apparent for FPSM, FPFM, SPFM and SPSM, there was no significant difference between the FC and SC groups. The FC group spent significantly more time in the target quadrant than both the FPSM and FPFM groups. In addition, there was a significant time x sex interaction ($F[3,321]=2.90, p<.04$), as females spent significantly more time in the target quadrant during the first 30 sec.

Finally, performance with a raised platform varied as a function of trial x strain x crossfostering ($F[6,210]=3.00, p<.01$). All FAST groups were initially slower in reaching the raised platform, but performance was comparable to all SLOW conditions by the

Figure 15. Mean ($\pm$ SEM) latency to reach a submerged platform (collapsed over trials) in the Morris water maze for males in all conditions.

Figure 16. Mean ($\pm$ SEM) latency to reach a submerged platform (collapsed over trials) in the Morris water maze for females in all conditions.

Figure 17. Mean ($\pm$ SEM) time spent in the target quadrant (i.e., the quadrant where the platform was located during training) over 15 second periods for males in all conditions.

Figure 18. Mean ($\pm$ SEM) time spent in the target quadrant (i.e., the quadrant where the platform was located during training) over 15 second periods for females in all conditions.

fourth trial, despite a small difference between controls, as the FC group had a latency of 7.05 ± 0.88 s, in contrast to 4.35 ± 0.88 s for the SC group (Figures 19 & 20). Also, there was no significant influence of sex on performance ($F[1,107]=1.35$, $p<.25$).

Open Field

A repeated measures MANOVA indicated that open field behaviour (line crosses, rearing, thigmotaxia) varied as a function of day x strain ($F[9,99]=5.86$, $p<.01$) and day x sex ($F[9,99]=2.96$, $p<.01$). Univariate tests indicated that the day x strain interaction was significant for line crosses and rearing, whereas the day x sex interaction was significant for line crosses only. FAST rats had a significantly greater number of line crosses and reared more often than SLOW rats over each day of testing (Figures 21 - 24).

Furthermore, SLOW rats crossed more lines on day 1 compared to days 3 and 4, and spent significantly more time rearing on day 1 compared to all other days. In contrast, FAST rats crossed significantly more lines on days 2 and 4 versus day 1, but had a consistent frequency of rearing over all days. Interestingly, females were more active than males, crossing significantly more lines and rearing more frequently.

Gross Brain Morphology

A multivariate ANOVA revealed a significant effect of strain on gross brain morphology ($F[3,52]=5.15$, $p<.01$), but no significant interaction of strain and crossfostering ($F[6,104]=0.57$, $p<.76$). Between subjects tests indicated that there were significant strain differences in weight ($F[1,54]=7.73$, $p<.01$) and length ($F[1,54]=8.33$, $p<.01$), but not width. The mean brain weights for the FAST and SLOW groups were 2.25 ± 0.02 g and 2.17 ± 0.02 g, respectively, and the length of the brain was 20.60

Figure 19. Mean ($\pm$ SEM) latency to reach a raised platform in the Morris water maze on each trial for males in all conditions.

Figure 20. Mean ($\pm$ SEM) latency to reach a raised platform in the Morris water maze on each trial for females in all conditions.

Figure 21. Mean ($\pm$ SEM) number of line crosses per day in the open field for males of all conditions.

Figure 22. Mean ($\pm$ SEM) number of line crosses per day in the open field for females of all conditions.

Figure 23. Mean ($\pm$ SEM) number of rears per day in the open field for males in each condition.

Figure 24. Mean ($\pm$ SEM) number of rears per day in the open field for females in each condition.

$\pm 0.10\text{mm}$ for the FAST groups and $20.21 \pm 0.09\text{mm}$ for the SLOW groups. Thus, FAST rats had bigger (heavier and longer) brains than SLOW rats. Figures 25 and 26 illustrate these strain differences.

Figure 25. Mean ($\pm$ SEM) weight of male brains for each condition.

Figure 26. Mean ($\pm$ SEM) length of male brains in each condition.

Discussion

Maternal care and physiology are known to regulate pup growth and development (Pryce & Feldon, 2003). In the present investigation, we assessed the influence of maternal care on the FAST and SLOW adult phenotypes. Overall, adult strain differences in learning and hyperactivity, as measured in the Morris water maze and open field test respectively, were unaffected in the crossfostering paradigm. Interestingly, significant differences were observed in the style of maternal care provided by mothers in each strain and the way in which crossfostering influenced pup growth.

It is important to note that differences between FAST and SLOW rats were already apparent at birth, as FAST pups weighed significantly more than SLOW pups in both sexes. Birth weight variation can be regulated by genetics or by a number of factors in the perinatal environment, most significantly proper fetal nutrition. For instance, birth weight and development are dependent upon glucose and fatty acid supply through the placenta (Herrera, 2002). In our laboratory, adult FAST rats have been shown to have decreased blood concentrations of both glucose and free fatty acids compared to SLOW rats, suggesting that FAST rats may be relatively undernourished (Gilby, unpublished results). Thus, the fetal or prenatal nutritional environment appears to be different for the FAST and SLOW strains and one might, therefore, suspect that the FAST pups would be smaller than SLOW pups at birth. Yet, this was not the case, as SLOW pups were, in fact, smaller at birth. Nonetheless, in light of such obvious differences in birth weight, studies that link the *in utero* environment to adult behavioural patterns and physiology suggest that the perinatal environment may contribute to the determination of the ultimate strain phenotype. This influence could be determined by a prenatal crossfostering study

involving embryo transfers from FAST to SLOW females, and vice versa. This experiment is planned for the near future.

The observed pup weight differences were maintained throughout the nursing period for both control groups (FC, SC) and pups raised by mothers of the opposite strain (FPSM, SPFM), with the FAST pups being larger. This finding suggests that these differences may be simply genetic in nature or perhaps the result of the prenatal environment since FAST pups were larger than SLOW pups at birth. However, when considering absolute weight gain over the first ten days postnatal, FAST and SLOW pups reared by mothers of the same strain (FPSM, SPSM, FC, SC) all gained comparable amounts of weight. Yet, over the next ten days, these FAST pups had a growth spurt and gained significantly more weight than SLOW pups. Interestingly, crossfostering influenced weight gain such that SLOW pups raised by a FAST mother (SPFM) gained the least amount of weight at first, but then experienced a FAST-like growth spurt over the last ten days. On the contrary, FAST pups raised by a SLOW mother initially gained weight at a rate similar to other FAST pups, but did not experience the rapid growth over the last ten days. Clearly the mother's strain can impact the growth characteristics of the pup, which interacts with the genetics/epigenetics of the pup in interesting ways. The exact reason for the differential growth behaviour, however, will need further study. A possible explanation, however, may lie in the fact that postnatal development and growth are highly dependent on maternal milk quality. As such, an inferior nutritional value in the milk of FAST mothers may explain the decreased weight gain of SPFM pups over the first ten days postnatal. Long-chain fatty acids are an important aspect of milk quality,

and in humans, infants receiving breast milk with low levels of fatty acids exhibit impaired cognitive development (Farquharson et al., 1995). It is currently unknown whether differences in maternal milk quality exist between the strains, but it is reasonable to hypothesize that the nutritional content of milk will differ due to the aforementioned differences in plasma fatty acid concentration between the strains. Thus, analysis of milk from lactating FAST and SLOW mothers needs to be performed.

Furthermore, slower weight gain in pups is also associated with decreased growth hormone levels as a result of periods of maternal separation (Kuhn et al., 1978). Over the first ten days postnatal, SLOW control mothers spent more time not in contact with their litter (i.e., NC) than FAST control mothers, and it is conceivable that SLOW mothers increased the incidence of this behaviour substantially in the last 10 days prior to weaning, so that weight gain in SLOW pups was inferior to FAST pups. Thus, it is possible that the absence of a growth spurt in SLOW pups was the result of a reduced level of maternal contact during the pre-weaning period. Alternatively, it may be that maternal physiology, in particular milk availability and ejection, modulates weight gain in rat pups. Differences in pup growth are also seen in the high-avoidance (HAA) and low-avoidance (LAA) rat strains. LAA pups are smaller than HAA pups during nursing, yet crossfostering pups between strains eliminates differences in pup weight (Ohta et al., 1998). Furthermore, LAA mothers have decreased milk ejection compared to HAA mothers, a difference that is eliminated after oxytocin administration (Ohta et al., 2002). Therefore, it is possible that SLOW mothers have decreased milk availability in comparison to FAST mothers, yet these differences are only apparent when demand for

milk is increased (i.e., when pups are larger). Thus, decreased milk availability may explain why FPSM pups did not experience the growth spurt seen in other FAST pups.

Finally, it is interesting to note that differences in weight gain may ultimately be related to the point at which pups begin to feed on rat chow (around PND 18), in addition to the mother's milk (Thiels et al., 1990). Maternal behaviour may influence the beginning of self-feeding, so that litters reared by FAST mothers begin this activity earlier, allowing them to gain weight at an accelerated rate in comparison to pups reared by SLOW mothers during the pre-weaning period.

Differential pup growth was also associated with different nursing profiles in the FAST and SLOW strains. FAST control mothers exhibited a higher frequency of arched-back nursing (ABN) than SLOW control mothers during the first 10 days postnatal. However, there was no difference in total time spent nursing between the strains as SLOW mothers had an increased frequency of blanket posture (BP) compared to FAST mothers. Interestingly, it appears that pup-type influenced the expression of maternal behaviour late in the nursing period. All mothers rearing SLOW pups (SPFM, SPSM, SC) showed a significant decrease in ABN frequency over days. As seen in Figure 2, the frequency of ABN in the FPSM group is clearly more similar to FC than other SLOW mothers after PND 5. Initially, FPSM mothers exhibited an ABN frequency typical of a SLOW mother, but then begin to exhibit FAST-like behaviour (i.e., higher frequency of ABN). Likewise, SPFM mothers behave similarly to a FAST mother at first, but then exhibit a SLOW-like decline in ABN. Thus, it appears that the higher frequency of ABN in FAST rats, particularly later in the nursing period, may be a pup-driven behaviour, as

the behaviour of FPSM mothers is more similar to FC than SC mothers. Crossfostering of whole litters is known to influence maternal behaviour, an effect that can normally be avoided if only two pups are fostered into or from a litter (Maccari et al., 1995; Liu et al., 2000). Stern et al. (1989) reported that pups actually invoke the ABN posture in mothers, when she is hovered over them licking and grooming (LG), by gaining access to her ventrum and attaching to a nipple. Furthermore, older pups can stimulate ABN simply by burrowing under the mother's ventrum, and do not require the mother to engage in LG activities prior to nursing.

It is interesting to note that the maternal behaviour profile of the FAST and SLOW kindling strains is very similar to the hyperactive spontaneously hypertensive rat (SHR) and its normotensive control, Wistar Kyoto rat (WKY). SHR mothers spend a greater proportion of time showing ABN than WKY mothers, similar to our FAST mothers (Myers et al., 1989b). Interestingly, the SHR rats are also far more seizure prone than the Wistar controls (McIntyre, personal communication). Crossfostering of pups eliminates the differences in ABN and LG between the SHR and WKY mothers, and actually modified the behaviour of the WKY mothers so that they were more similar to SHR mothers (Myers et al., 1989a). Myers et al. (1989b) also noted that SHR pups were significantly more active than WKY pups when in the nest and in contact with their mother, which may increase the frequency of ABN. It is unknown whether an analogous pup activity difference is seen in the FAST and SLOW strains, however, as adults, FAST rats show significantly higher levels of activity (Mohapel & McIntyre, 1998). Thus, it is

plausible to suggest that the larger and more active FAST pups are more capable of stimulating ABN in mothers than the smaller, less active SLOW pups.

While pup behaviour has a strong influence on maternal behaviour, handling, (i.e., removing pups from the mother for 15 minute periods daily until weaning) also stimulates mothers to increase licking/grooming (LG) compared to mothers of non-handled rats (Liu et al., 1997). Our experimental procedure involved weighing pups daily in late afternoon and evening, thus we handled the pups for several minutes each day. Although, all groups received the same manipulation, it is possible that some groups may be more sensitive to the handling procedure. Our results indicated no significant effect of strain or crossfostering on the frequency of LG each day, and the frequency of LG at 8am was not significantly different from the frequency at 5pm. Thus, the handling procedure did not produce an immediate increase in LG frequency, yet it is impossible to determine whether handling increased LG differentially in any of our groups without a non-handled control for comparison.

Finally, in addition to a higher frequency of ABN, the FAST mothers also retrieved the first pup faster than SLOW mothers in a retrieval test. Interestingly, on PND 1, FPSM mothers retrieved the first pup just as quickly as the FAST mothers, but displayed latencies typical of SLOW mothers on subsequent days. Furthermore, there was no significant strain difference in latency to retrieve all pups. A similar difference in latency to retrieve pups is also seen in the SHR and WKY rats, with the hyperactive SHR mothers retrieving the first pup at a shorter latency than WKY mothers (Myers et al., 1989b). These authors noted that although the SHR strain retrieved the first pup faster,

they displayed more frantic behaviour following the retrieval, kicking cage shaving and repeatedly placing pups in and out of the nest. Similar behaviours were noted in this study, however, they were not quantified. Different pup retrieval latencies have also been reported in C57BL/6J and DBA/2J mice. Similar to our FAST strain and SHR rats, C57 mice, the more active strain, also exhibits shorter latencies to retrieve pups (Brown et al., 1999). Interestingly, pup retrieval is known to be highly dependent on dopaminergic function, and injection of dopamine antagonists increases retrieval latencies (Giordano et al., 1990). Recall, basal dopamine levels in the prefrontal cortex of the FAST strain are significantly higher than SLOW rats, and it has been suggested these dopamine neurons may respond to environmental stimuli in an abnormal fashion (Anisman et al., 2000). Thus, comorbid hyperactivity and impulsivity, coupled with aberrant increases in dopamine functioning in the FAST strain, may account for the decreased retrieval latencies in FAST rats compared to SLOW rats.

Despite differences in maternal behaviour and pup growth, these differences did not affect the behavioural phenotypes of the FAST and SLOW strains as adults. In the Morris water maze, normal strain differences were maintained in all groups, regardless of mothering experience. After six days of training, all FAST rats had significantly higher latencies to find the submerged platform than SLOW rats. Furthermore, in a probe trial where the platform was absent, SLOW rats spent more time searching in the target quadrant (where the platform should be) over the first 15 seconds of the trial than FAST rats. Further, differences in the acquisition of this spatial task were not related to differences in motor function, as FAST rats had latencies similar to SLOW rats after

training with a raised, visible platform. Similarly, in the open field, all FAST groups were significantly more active compared to all SLOW groups, in spite of the crossfostering condition. SLOW rats had a modest decline in rearing and line crosses over days, whereas, FAST rats actually had significant *increases* in line crosses over days. Interestingly, the offspring of high ABN/LG mothers are also more active in the open field, similar to our FAST rats (Francis et al., 1999).

Mohapel and McIntyre (1998) reported that SLOW rats show a dramatic decline in rearing and line crosses over days, whereas FAST rats maintain a consistent level of activity over all days. In particular, SLOW rats crossed an average of 10 lines by the fourth day of testing. Yet, SLOW rats in this study did not habituate to the same magnitude as SLOW rats in the earlier study, and this discrepancy may be the result of handling the strains as pups. Accordingly, Gilby et al., (unpublished results) have shown that increased handling (e.g., weighing every day, behavioural testing) actually diminishes the differences normally observed in activity levels between the strains. Simply put, the more handling the less habituation in the open field test. As would be expected, when FAST and SLOW rats are minimally handled during development, the habituation curve in SLOW rats again appears (Gilby et al., unpublished results). The exact reason for this effect is currently unknown, however, further work on the effect of handling the strains will be conducted in the near future. This work will be important as handling in the early stages of development appears to have made SLOW rats more FAST-like (little to no habituation over days) in the open field test.

Finally, the reliably consistent difference in water consumption between the strains did not change as a result of crossfostering, as all FAST rats continued to consume more water daily than SLOW rats, despite the maternal environment experienced. This greater need for water in FAST rats may reflect some basic metabolic difference between the strains, which certainly needs further study. Similarly, differences in gross brain morphology were not influenced by crossfostering, as brains of FAST rats were heavier and longer than SLOW rats in all conditions. It was not surprising that gross brain morphology differed between the strains in this experiment, as Gilby et al. (2002) demonstrated that FAST rats have larger ventricles and piriform cortex, but smaller hippocampi. The larger ventricles and smaller hippocampi are perfectly consistent with the spatial learning deficits in the FAST rats, as the hippocampus is a critically important structure for that behaviour. The larger ventricles, of course, are likely compensation for the loss of an adjacent hippocampal structure, which thus allows the ventricles to expand their territory.

The results of this study indicate that the early postnatal environment did not impact upon known behavioural differences between the FAST and SLOW strains as adults. However, the lack of behavioural and morphological changes as a result of postnatal crossfostering does not eliminate the possibility of environmental influences in the development of differential phenotypes. First, strain differences may be associated with prenatal *and* postnatal differences in maternal physiology. Recall that C57 mice had to be fostered both pre- and postnatally to BALBc mice in order to display the BALBc behavioural phenotype (Francis et al., 2003). This possibility is currently being addressed

in our laboratory. Secondly, many of our findings are supported by the literature. For instance, FAST mothers had a higher frequency of ABN than SLOW mothers and as adults, FAST rats are less fearful, similar to high ABN/LG offspring (Liu et al., 1997). However, SLOW rats, which experience less ABN, exhibit improved spatial learning ability, unlike low ABN/LG offspring. Thus, it is possible that fearfulness, in our strains, is related to the frequency of ABN, whereas learning is associated solely with genetic mechanisms. Accordingly, a high frequency of ABN in the FAST rats would account for their decreased fearfulness in comparison to SLOW rats. Unfortunately, we cannot determine how this would affect crossfostered animals, as fostering whole litters seems to have influenced the natural frequency of ABN in FAST and SLOW mothers. As a result, it will be important to assess the resultant behavioural phenotype of pups when only two pups are fostered to and from a mother.

In conclusion, modifying the postnatal environment of the FAST and SLOW strains did not influence learning or activity differences as adults. Thus, it appears that these characteristics may be predominantly genetic or prenatal in origin, and are influenced little by the postnatal environment.

References

- Anisman, H., Kelly, O., Hayley, S., Borowski, T., Merali, Z. & McIntyre, D.C. (2000) Acoustic startle and fear-potentiated startle in rats selectively bred for fast and slow kindling rates: relation to monoamine activity. *Euro. J. Neurosci.*, 12, 4405-4416.
- 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. & McIntyre, D.C. (2002) Conceptual, spatial and cue learning in the Morris water maze in fast and slow kindling rats: attention deficit comorbidity. *J. Neurosci.*, 22(17), 7809-7817.
- Anisman, H., Zaharia, M.D., Meaney, M.J. & Merali, Z. (1998). Do early life events permanently alter behavioural and hormonal responses to stressors? *Int. J. Dev. Neurosci.*, 16, 149-164.
- Barkley, R.A. (1990). *Attention Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment*, New York: Guilford Press.
- Biederman, J., Faraone, S.V. & Monuteaux, M.C. (2002) Differential effect of environmental adversity by gender: Rutter's index of adversity in a group of boys and girls with and without ADHD. *Am. J. Psychiatry*, 159(9), 1556-62.
- Biederman, J., Milberger, S., Faraone, S.V., Kiely, K., Guite, J., Mick, E., Ablon, S., Warburton, R. & Reed, E. (1995) Family-environment risk factors for attention-

- deficit hyperactivity disorder. *Arch. Gen. Psychiatry*, 52, 464-470.
- Bredy, T.W., Grant, R.J., Champagne, D.L., & Meaney, M.J. (2003) Maternal care influences neuronal survival in the hippocampus of the rat. *Eur. J. Neuro.* 18, 2903-2909.
- Brown, R.E., Mathieson, W.B., Stapleton, J., & Neumann, P.E. (1999) Maternal behaviour in female C57BL/6J and DBA/2J inbred mice. *Physiol. Behav.*, 67(4), 599-605.
- Caldji, C., Diorio, J. & Meaney, M.J. (2000) Variations in maternal care in infancy regulate the development of stress reactivity. *Biol. Psychiatry*, 48, 1164-1174.
- Castellanos, F.X. (2001) Neuroimaging studies of ADHD. In: Solanto, M.V., Arsten, A.F.T., Castellanos, F.X. Eds. *Stimulant drugs and ADHD: basic and clinical neuroscience*, New York: Oxford University Press, 243-258.
- Champagne, F., Diorio, J., Sharma, S. & Meaney, M.J. (2001) Naturally occurring variations in maternal behaviour in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *PNAS*, 98(22), 12736-12741.
- Elmer, E., Kokaia, Z., Kokaia, M., Lindvall, O. & McIntyre, D.C. (1997) Mossy fibre sprouting evidence against a facilitatory role in epileptogenesis. *Neuroreport*, 8, 1193-1196.
- Faraone, S.V. & Biederman, J. (1998) Neurobiology of attention-deficit hyperactivity disorder. *Biol. Psychiatry*, 44, 951-958.
- Farquharson, J., Jamieson, E.C., Abbasi, K.A., Patrick, W.J., Logan, R.W. & Cockburn, F. (1995) Effect of diet on the fatty acid composition of the major phospholipids

- of infant cerebral cortex. *Arch. Dis. Child*, 72(3), 198-203.
- Francis, D., Diorio, J., Liu, D. & Meaney, M.J. (1999) Nongenomic transmission across generations of maternal behaviour and stress responses in the rat. *Science*, 286, 1155-1158.
- Francis, D.D., Szegda, K., Campbell, G., Martin, W.D. & Insel, T.R. (2003). Epigenetic sources of behavioural differences in mice. *Nature Neurosci.*, 6(5), 445-446.
- Gilby, K., Hutcheon, B., Sauro, K., Malik, N., Sahota, S., Poulter, M.O., McIntyre, D.C. (2002). Differential gene expression correlated with reduced temporal lobe volume in seizure-prone versus seizure-resistant rats. American Epilepsy Society 56th Annual Meeting.
- Giordano, A.L., Johnson, A.E. & Rosenblatt, J.S. (1990) Haloperidol-induced disruption of retrieval behaviour and reversal with ampomorphine in lactating rats. *Physiol. Behav.*, 48(1), 211-214.
- Herrera, E. (2002) Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine*, 19(1), 43-55.
- Hempel, A.M., Frost, M.D., Ritter, F.J., & Farnham, S. (1995). Factors influencing the incidence of ADHD in pediatric epilepsy patients. *Epilepsia*, 36, 122.
- Koenen, K.C., Moffitt, T.E., Caspi, A., Taylor, A. & Purcell, S. (2003) Domestic violence is associated with environmental suppression of IQ in young children. *Dev. Psychopathol.*, 15(2), 297-311.
- Kuhn, C.M., Butler, S.R. & Schanberg, S.M. (1978) Selective depression of serum growth hormone during maternal deprivation in rat pups. *Science*, 201(4360),

1034-1036.

Levy F., Hay D.A., McStephen M., Wood C., & Waldman I. (1997). Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. *J. Am. Acad. Child Adolesc. Psychiatry*, 36(6), 737-744.

Liu, D., Diorio, J., Day, J.C., Francis, D.D. & Meaney, M.J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neurosci.*, 3(8), 799-806.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M. & Meaney, M.J. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, 277, 1659-1662.

Maccari, S., Piazza, P.V., Kabbaj, M., Barbazanges, A., Simon, H. & Le Moal, M. (1995) Adoption reverse the long-term impairment in glucocorticoid feedback induced by prenatal stress. *J. Neurosci.*, 15(1), 110-116.

Mackner, L.M., Starr, R.H. Jr., & Black, M.M. (1997) The cumulative effect of neglect and failure to thrive on cognitive functioning. *Child Abuse Negl.*, 21(7), 691-700.

McIntyre, D.C., Hutcheon, B., Schwabe, K. & Poulter, M.O. (2002) Divergent GABAA receptor-mediated synaptic transmission in seizure-prone and seizure-resistant rats. *J. Neurosci.*, 22(22), 922-9931.

McIntyre, D.C. & Anisman, H. (2000) Anxiety and impulse control in rats selectively bred for seizure susceptibility. In Mysblodsky & Weiner, Eds. *Contemporary issues in modeling psychopathology*, New York: Kluwer Academic, 29-45.

- McIntyre, D.C., Kelly, M.E. & Dufresne, C. (1999a) 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. (1999b) Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. *Brain Res.*, 840, 65-74.
- Michaud, D., McIntyre, D., Anisman, H. & Merali, Z. (1999) Rat strains with high vs. low sexual reactivity: behavioural & lateralized amygdaloid CRH responses in males. *Soc. Neurosci. Abstracts*.
- Mohapel, P. & McIntyre, D.C. (1998) Amygdala kindling-resistant (SLOW) or –prone (FAST) rat strains show differential fear responses. *Behav. Neurosci.*, 112(6), 1402-1413.
- Myers, M.M., Brunelli, S.A., Shair, H.N., Squire, J.M. & Hofer, M.A. (1989a) Relationships between maternal behaviour of SHR and WKY dams and adult blood pressures of cross-fostered F1 pups. *Dev. Psychobiol.*, 22(1), 66-67.
- Myers, M.M., Brunelli, S.A., Squire, J.M., Shindeldecker, R.D. & Hofer, M.A. (1989b) Maternal behaviour of SHR rats and its relationship to offspring blood pressures. *Dev. Psychobiol.*, 22(1), 29-53.
- Ohta, R., Matsumoto, A., Nagao, T. & Mizutani, M. (1998) Comparative study of behavioural development between high and low shuttlebox avoidance rats. *Physiol. Behav.*, 63(4), 545-551.
- Ohta, R., Shirota, M., Tohei, A. & Taya, K. (2002) Maternal behaviour, milk ejection and

plasma hormones in Hatano high- and low-avoidance rats. *Hormones Behav.*, 42, 116-125.

Paule, M.G., Rowland, A.S., Ferguson, S.A., Chelonis, J.J., Tannock, R., Swanson, J.M. & Castellano, F.X. (2000). Attention deficit/hyperactivity disorder: characteristics, interventions, and models. *Neurotoxicol. Teratol.*, 22(5), 631-651.

Poulter, M.O., Barker, J.L., O'Carroll, A.M., Lolait, S.J. & Mahan, L.C. (1992) Differential and transient expression of GABA_A receptor α -subunit mRNAs in the developing rat CNS. *J. Neurosci.*, 12, 2888-2900.

Poulter, M.O., Brown, L.A., Tynan, S., Willick, G., William, 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(11), 4654-4661.

Pryce, C.R. & Feldon, J. (2003) Long-term neurobehavioural impact of the post-natal environment in rats: manipulations, effects and mediating mechanisms. *Neurosci. Biobehav. Rev.*, 27, 57-71.

Racine, R.J., Steingart, M., Bureau, Y. & McIntyre, D.C. (2003) Differential sensitivity of genetically fast versus slow kindling rat strains to GABAergic convulsive agents. *Neuropharmacol.*, 54(7), 918-924.

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.

- Shin, R.S., Makinen, J., Anisman, H., Merali, Z. & McIntyre, D.C. (2002) Changes in monoamine levels in sexually impulsive (FAST) or quiescent (SLOW) rat strains. *Soc. Neurosci. Abstracts*.
- Stern, J.M. & Johnson, S.K. (1989) Perioral somatosensory determinants of nursing behaviour in Norway rats (*Rattus norvegicus*). *J. Comp. Psychol.*, 103(3), 269-280.
- Szot, P., White, S.S., McCarthy, E.B., Turella, A., Rejniak, S.X. & Schwartzkroin, P.A. (2001) Behavioural and metabolic features of repetitive seizures in immature and mature rats. *Epilepsy Res.* 46(3), 191-203.
- Thiels, E., Alberts, J.R. & Cramer, C.P. (1990) Weaning in rats: II. Pup behaviour patterns. *Dev. Psychobiol.*, 23(6), 495-510.
- Weaver, I.C.G., Grant, R.J. & Meaney, M.J. (2002) Maternal behaviour regulates long-term hippocampal expression of BAX and apoptosis in the offspring. *J. Neurochem.*, 82, 998-1002.
- Wolf, S.M. & Forsythe, A. (1978). Behavioural disturbance, phenobarbital, and febrile seizures. *Pediatrics*, 61, 728-731.