Starving for attention, eating for excitement: Assessing the ability of caloric restriction to alter kindled seizure and behavioural profiles in seizure-prone (Fast) versus seizure-resistant (Slow) rats

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by

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ABSTRACT

Caloric-restriction (CR), primarily known for extending lifespan, has proven anti-convulsant in several seizure models and anti-epileptogenic in a strain of inherently seizure susceptible mice (EL). Since our animal model includes an innately seizure-prone, ADHD-like (Fast) strain versus a seizure-resistant (Slow) strain, we evaluated CR's effect on the typical seizure sensitivities and behavioural profiles of each strain. In this study, Fast and Slow rats were fed *ad libitum* or calorically restricted to 80% of free feeding body weight. Subjects were then tested in the open field, Morris water maze, and restraint paradigms and finally kindled from the amygdala. Ultimately, CR abolished signs of abnormal hyperactivity, retarded the kindling rate, and paradoxically increased local amygdala excitability in Fast rats. Importantly, CR did not seem to significantly affect Slow rats. The results of this study clearly endorse further investigation into the potential benefits of CR for epilepsy and ADHD.

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INTRODUCTION

Ketogenic diet- a fasting mimetic

For millennia, fasting has been recognized as an effective treatment for seizure control. This anecdotal observation was recorded periodically throughout history by the Babylonians, Greeks, Romans, Chinese and others. Whether it was believed that epilepsy was demonic possession or an accumulation of toxins, treatment often involved strict diet regimens (Eadie and Bladin, 2001). By the 1920s, based on the initial observation of an osteopathic practitioner that fasting prevents epileptic seizures, researchers had examined fasting-induced seizure control in a wide variety of seizure disorders (Geyelin, 1921; Wilder, 1921; Lennox & Cobb, 1928). This seizure control was attributed, at the time, to an increase in blood ketone levels and reduction in blood glucose (Lennox & Cobb, 1928). Geyelin (1921) also observed that breaking the fast would eliminate this reduction in seizure frequency, which Wilder ascribed to the reduction in ketone bodies upon consumption of carbohydrates (Wilder, 1921; Greene et al., 2003). Considering that anorexia is a prescription of limited usefulness to those afflicted with intractable epilepsy, Wilder developed a dietary manipulation designed to maintain the elevation of ketone bodies induced by fasting. He found that a diet high in fat, low in protein and free of carbohydrates, if administered after an initial fast, could maintain the patient's relative state of ketonemia (Wilder, 1921). This aptly named ketogenic diet (KD) was found to be impressively effective, particularly in children (Lennox & Cobb, 1928), and has since been adjusted to include very low levels of carbohydrates (Sinha & Kossoff, 2005). These days, the "classic" KD is comprised of long-chain saturated triglycerides with a fat to carbohydrate plus protein ratio of 4:1 by weight (Schwartz et al., 1989; Stafstrom & Bough, 2008; Livingston &

Berman, 1972). The KD's efficacy hinges on compliance, with the greatest reduction in seizure frequency seen in patients who maintain the diet for a year or more (Stafstrom & Bough, 2008; Livingston & Berman, 1972; Freeman et al., 2000; Hartman & Vining, 2007). Unfortunately, in its classical form, it is generally considered to be unpalatable and labour intensive, making it difficult to prepare and administer consistently, particularly since the majority of patients on the KD are children (Freeman et al., 2000; Stafstrom & Bough, 2008). Thus, with the discovery of phenytoin in the 1930s, and other effective anticonvulsants over subsequent decades, the KD diminished in common practice (reviewed in Swink et al., 1997).

The KD's unpalatability was addressed by Huttenlocher, who developed an alternative diet that induced ketonemia by employing medium chain triglycerides (MCT) as the majority of dietary fat (Huttenlocher, 1976). The MCT diet proved to be remarkably ketogenic, which enabled more flexibility for food choices; however, it was associated with painful cramping and diarrhea, which in turn proved to be more problematic for compliance (Greene et al., 2003; Sinha & Kossoff, 2005; Schwartz et al., 1989). The classical KD resurfaced in the 1990s, due in part to Hollywood director Jim Abrahams. Upon effective treatment of his son with the KD, Abrahams involved the mainstream media and helped push the KD into the forefront of public awareness (Charlie Foundation, 2002). The diet quickly regained popularity, and due to its remarkable efficacy in treating a significant portion of childhood refractory epilepsy disorders (Stafstrom & Bough, 2003; Sinha & Kossoff, 2005), was embraced as an alternative treatment strategy. This sparked a resurgence of scientific interest and funding that still persists today. However, despite the attention in recent decades,

and its use for nearly a century, the mechanisms by which the KD proffers this seizure resistance are poorly understood.

Fats and manipulations of cerebral metabolism

The majority of hypotheses with respect to the efficacy of the KD diet implicate a shift in the energy metabolism of the brain, particularly the process of fat metabolism and utilization. Although glucose and glucose derivatives are the brain's preferred energy substrate during normal physiological conditions, the brain is able to metabolize fat-derived ketone bodies for energy when circulating glucose levels decrease (Sokoloff, 1973), as seen in therapeutic fasting (Geyelin, 1921). The ketone bodies acetoacetate (ACA) and beta-hydroxybutyrate (BHB) are generated in liver mitochondria by the beta oxidation of fatty acids (Greene et al., 2001; Bhagavan, 2002) and cross the blood brain barrier via facilitated diffusion mediated by the monocarboxylic transport system (Pierre & Pellerin, 2005; Nordli et al, 1997), with the expression of these transporters partially regulated by circulating ketone and glucose levels (Leino et al., 2001). Unlike glucose, ketone bodies bypass glycolysis and enter the mitochondrial krebs cycle upon oxidation into acetyl-CoA. With repeated observations that glucose uptake and metabolism increase more during seizures than most or all other brain activities (Cornford et al., 2002), it has been proposed that ketones, while able to provide sufficient energy for regular cerebral functions, may not be able to deliver energy quickly enough to initiate or sustain seizure activity (Greene et al., 2001). Elevated ketone levels may further suppress seizure activity indirectly, based on observations that ketones elevate GABA content in synaptosomes by promoting glutamate decarboxylase (Yudkoff et al., 2001). Hence, the ketogenic diet may confer its seizure suppression by increasing

inhibitory neurotransmission and shifting cerebral metabolism to an energy state ill-suited for seizure activity.

Historically, a great deal of our knowledge of neurological processing has been derived from the investigation of pharmacological treatments already in use; consequently, refining our understanding as to what aspects of the KD influence its efficacy is critical. The KD is a broad stroke treatment accompanied by a multitude of physiological changes, and among these changes lay the foundation of its remarkably efficacious seizure reduction. Considering that administration of polyunsaturated fatty acids can ameliorate seizure profiles (Yehuda et al., 1994, Schlanger et al., 2002), that certain epilepsies are associated with differences in fatty acid metabolism (Sedel et al., 2007), and that the ketogenic diet itself is a lipid based treatment (Wilder, 1921), it seems that cerebral metabolism is at the heart of the matter and that the mechanisms involved are amenable to dietary treatment. Teasing apart which underlying systems are related to the KD's conferred seizure resistance will inform the development of new dietary treatments to manage these systems, and in turn contribute to a deeper understanding of epilepsy itself. Recently, several researchers have begun to do just that, by investigating the contribution of caloric restriction (which often accompanies the KD in effective clinical practice) to the KD's seizure reduction properties.

From ketosis to caloric restriction

The metabolic changes most often implicated in the KD's seizure suppression are the reduction of blood glucose, which shifts cerebral metabolism to utilize ketone bodies for energy production (Greene et al., 2003; Stafstrom & Bough, 2003; Greene et al., 2001), the direct or indirect action of these elevated ketone bodies (Likhodii et al., 2003; Rho et al., 2002; Ma et al., 2007), and the increase in certain circulating free fatty acids (Fraser et al.,

2003; Eagles et al., 2003). Interestingly, these changes also occur during periods of caloric restriction (Mahoney et al., 2006; Hammer et al., 2008; Greene et al., 2003; Keenan et al., 1999; Weindruch et al., 2001), which may explain the dramatic improvement to the KD's efficacy when administered during a state of reduced caloric intake (Freeman et al., 2000; Mantis et al., 2004; Greene et al., 2003; Harney et al., 2002; Bough et al., 1999). Furthermore, caloric restriction without implementation of the ketogenic diet has demonstrated seizure suppression in several animal models (Bough et al., 1999; Harney et al., 2002; Bough et al., 2003; Eagles et al., 2003; Greene et al., 2001; Mantis et al., 2004).

Recent evidence has begun to question if a state of ketosis is sufficient or even necessary for the seizure reduction provided by the KD (Likhodii et al., 2000; Stafstrom & Bough, 2003; Likhodii & Burnham, 2002; Eagles et al., 2003; Mantis et al., 2004). Originally thought to be anticonvulsant, several studies in humans and animals have failed to demonstrate any direct connection between seizure resistance and levels of ketone bodies (Appleton & DeVivo., 1974; Bough et al., 1999; Todorova et al., 2000). Yet, rodent models have shown that an association between elevated blood ketone levels and reduced seizure susceptibility is consistently observed if glucose levels and/or body weights are also reduced (Todorova et al., 2000; Greene et al., 2001). These findings lend themselves to an alternate proposal: that an elevation in ketone bodies is not sufficient to shift cerebral metabolism from carbohydrate to fat, but requires a significant reduction in glucose levels. In an environment of reduced glucose, the organism may promote the utilization of alternate catabolic pathways, namely the breakdown of fat into ketone bodies and their mobilization into the brain for use as an alternate fuel. Without reduced glucose levels to signal a compensatory switch to ketone body utilization, the brain would continue to metabolize glucose as its preferred fuel,

which is well-suited as an energy source to initiate and sustain the high energy-demand of seizure activity.

This potential for glucose levels to modulate the efficacy of the KD agrees with findings 1) that seizure protection seems greatest in patients that experience a reduction in body weight (Livingston., 1972; Schwarzkroin, 1999; Sinha & Kossoff, 2005), 2) a calorically restricted ketogenic diet demonstrates greater increases in Pentylenetetrazol seizure threshold than an ad libitum ketogenic diet (Bough et al., 2000), 3) that ketone levels are significantly higher in response to calorically restricted ketogenic diets than unrestricted ketogenic diets (Zhou et al., 2007) and 4) that those patients who cheat by consuming carbohydrates experience rapid breakthrough seizures (Lennox & Cobb, 1928; Livingston, 1972; Freeman et al., 2000). Since the KD is typically administered in the clinic with a 10-25% restriction of calories (Bough et al., 2003; Hartman & Vining, 2007), and may even be ineffective if feeding is unrestricted (Mantis et al., 2004), it is highly likely that a restriction in caloric intake modulates its effects, perhaps by assisting in the maintenance of reduced blood glucose levels.

Given that the KD is highly unpalatable and has weight loss-inducing side-effects such as diarrhea and vomiting, reductions in food intake and glucose levels will often accompany the KD even if unrestricted in practice (Greene et al., 2003). Thus, an examination of caloric restriction on its own, as a metabolic manipulation for seizure control, could elucidate systems worth targeting in order to adjust pathological states of brain energy homeostasis that may underlie many forms of epilepsy.

Caloric restriction (CR) is defined as a reduction of total energy intake without causing nutrient deficiencies or pathological metabolic extremes, such as hypoglycemia or

ketoacidosis. Though some similar metabolic changes occur in both the KD and CR, the way in which they are actualized seem to differ. Whereas the elevation in ketone bodies from the KD is elicited by a near complete deprivation of carbohydrates and concomitant overload of dietary fat (Wilder, 1921; Sinha & Kossoff, 2005), CR naturally elevates ketone levels via a physiological response to reduced glucose levels that are still sufficient for normal functioning (Weindruch et al., 2001; Greene et al., 2003; Mahoney et al., 2006). Furthermore, CR has been observed to elevate glucocorticoid circulation, which would inhibit glycolysis and operate in tandem with reduced blood glucose to limit cerebral glucose utilization (Birt et al., 1999; Kadekaro et al., 1988). Hence, a moderate restriction in caloric intake may create an environment of reduced glucose that would shift cerebral metabolism from glucose to ketone body utilization, as well as stimulate fatty acid metabolism in the liver to offer up more ketone bodies as a fuel source (Birt et al., 1999; Sokoloff, 1999). It has also been observed that the increased ketone body metabolism punctuated by food restriction can increase sodium and potassium ATPase activity, which may serve to increase membrane potential and thereby decrease neuronal excitability (Srinivasarao et al., 1997; Schwartzkroin, 1999; Ma et al., 2007; Eiam-Ong & Sabatini, 1999; DeVivo et al., 1975). In essence, CR is a sustainable approach to induce some of the metabolic changes that accompany therapeutic fasting, while circumventing the pathology of starvation and the pitfalls of a predominantly fat diet.

Clinical relevance of caloric restriction

Caloric restriction, beyond its potential to elucidate the cerebral metabolics of seizure resistance, stands to be a treatment of significant clinical value. CR has been shown to delay the onset of many age-related diseases (Weindruch et al., 2001), stimulate neurogenesis (Lee

et al., 2002; Lee et al., 2000) decelerate age-related cognitive changes (Means et al., 1993), resist protein oxidative damage (Dubey et al, 1996), and extend maximum life span in rodents, non-human primates, and possibly humans (Keenan et al., 1999; Weindruch et al., 2001; Mattson et al., 2003; Mamczarz et al., 2005; Willcox et al., 2007; Everitt & LeCouteur, 2007). In contrast, the KD's severe protein and carbohydrate restriction, high saturated fat content, and constant state of ketonemia are known to cause kidney stones, high cholesterol, dehydration, constipation, diarrhea, vomiting, slowed growth, bone thinning, and possibly even impeded neurodevelopment (Furth et al., 2000; Wheless, 2001; Stafstrom & Bough, 2003; Zhao et al., 2004). Should some form of CR be developed as a clinical treatment for epilepsy, its multitude of associated health benefits will set it in stark contrast to the array of antiepileptic drugs riddled with cognitive side effects, and the ketogenic diet's associated health problems. Thus, the clinical application of caloric restriction for seizure reduction is an exciting alternative; a longer maximum lifespan is one side effect patients can live with.

Support for CR as an anti-convulsant and the kindling model

The utility of CR as a seizure suppressant has been bolstered by recent animal studies. CR has conferred seizure resistance in rats using the pentylenetetrazole (Harney et al., 2001; Bough et al., 1998; Eagles et al., 2003) and maximal dentate activation models (Bough et al., 2003), as well as demonstrated remarkable suppression of spontaneous seizures in EL mice (Mantis et al., 2004; Greene et al., 2001). In some cases, the increase in seizure resistance was similar (Mantis et al., 2004) or even equal to (Eagles et al., 2003) that conferred by the KD. Altogether, CR appears to be a simply implemented manipulation whose multifactorial effects exert some degree of seizure resistance. Given the neoteric

research supporting its anticonvulsant effects, CR is a fitting vehicle with which to delve into the metabolic control of seizures.

At present, we are unaware of any studies examining CR using the kindling model of epilepsy. This is remarkable, as kindling is one of the most widely used seizure models for temporal lobe epilepsy (reviewed in Bertram, 2007). A benefit of using a non-chemical seizure-induction model like kindling resides in the fact that CR is a metabolic manipulation, and as such there may be interactions between the metabolic changes induced and systemic drug absorption. Driving the seizures via direct electrical stimulation of the brain may circumvent these potential interactions.

While CR has not been addressed in the kindling model as of yet, the KD has demonstrated some significant effects in this paradigm, suggesting that kindling-induced epileptogenesis may be amenable to dietary manipulation. While only a few kindling-based KD studies have been performed, it has been reported that the KD transiently increased afterdischarge and stage 5 seizure thresholds (Hori et al., 1997). Thus, CR may also show efficacy in the kindling model, as it induces certain metabolic changes reminiscent of some seen in the KD. However, it should be recalled that CR is a powerfully effective treatment in several domains, and is itself distinct from the KD, therefore it may provide seizure resistance via mechanisms largely unaffected by the KD.

When investigating a treatment as multifactorial as CR for an affliction as multifactorial as epilepsy the use of an appropriate animal model is imperative. In this vein, there exists an animal model of rodent strains that naturally differ in seizure susceptibility and demonstrate substantial differences in energy utilization strategies. As such, these strains

seem particularly well suited to an exploration into the ability of CR (a metabolic manipulation) to confer seizure resistance.

Advantages of using Fast and Slow kindling rat strains

The natural characteristics of the Fast (seizure-prone) and Slow (seizure-resistant) kindling rat strains are of particular relevance to the metabolic exploration of epilepsy. Bred for their amygdala kindling rates, these strains model a dichotomous genetic disposition for seizure susceptibility (Racine et al., 1999; McIntyre et al., 1999). Interestingly, amygdala kindling rate was the only selection factor in generating these strains, and yet they exhibit remarkably different behavioural, molecular, neuroanatomical, and physiological profiles (McIntyre & Gilby, 2007; Gilby et al., 2007[a]; Gilby et al., 2007[b]). The consistency of these characteristic profiles over 64 generations suggests a genetic link between these expressed traits and susceptibility or resistance to seizures. Considering the potential role of cerebral metabolism and lipids in seizure resistance, it is unsurprising that the strains demonstrate very different metabolic profiles. Repeated anecdotal observations and unpublished data suggest that these strains differ in average body size and food/water consumption. Additionally, the seizure-prone Fast strain may differ from the seizure-resistant Slow strain in lipid handling, particularly since the constitutive concentration of serum free fatty acids in the Slow rats is nearly twice that of the seizure prone Fasts (Gilby et al., in press; Gilby et al., 2007[a]), despite maintenance on the same diet. This striking difference in free fatty acid concentration between two strains with very different susceptibilities to seizure further suggests that lipid handling may be involved in epilepsy. Recalling that the ketogenic diet, polyunsaturated fatty acid administration, and fasting are all anti-convulsant treatments associated with an increase in free fatty acid levels (Fraser et al., 2003; Eagles et al., 2003;

Goldrick & Hirsch, 1964; Yehuda et al., 1994; Geyelin, 1921), utilization of these strains may help explore whether the effects of a CR-induced increase (or the increase itself) in free fatty acids is tempered by their differing metabolic profiles.

Metabolic undercurrents in comorbidities to epilepsy

The remarkable difference in behavioural profiles observed between these two strains presents an additional opportunity. Specifically, the relative impulsivity, hyperactivity, inferior learning, and polydipsia routinely observed in Fast rats have established them as a natural model of attention deficit hyperactivity disorder (ADHD) (Anisman & McIntyre, 2002; McIntyre & Gilby, 2007; Gilby et al., 2007[b]). Considering the significantly greater prevalence of ADHD in patients with epilepsy than in the general population (Aldenkamp et al., 2006), the demonstrated ability of polyunsaturated fatty acid administration to ameliorate ADHD symptoms in humans (Sinn & Bryan, 2007; Richardson, 2004), the KD's use in animal models to reduce activity levels (Murphy & Burnham, 2006; Murphy et al., 2005), and the observation that ADHD symptoms decrease in adults and children on the KD (Pulsifer et al., 2001; MacCracken & Scalisi, 1999; Kinsman et al., 1992), it seems possible that caloric restriction may affect both the kindling and behavioural profiles of the Fast strain. If CR does impact both seizure disposition and ADHD-like behaviours, it might imply that the comorbidity between ADHD and epilepsy in humans has a shared metabolic undercurrent. Specific metabolics of the comorbidity could then be explored by comparing CR with other metabolic treatments/manipulations to discern any common threads. Furthermore, putative metabolic "culprits" could be easily assessed in the strains, as they dichotomously model both ADHD-like behavior and epilepsy, and thus likely differ with respect to any potentially relevant aspects of metabolism.

PURPOSE

Chronic caloric restriction

We set out to assess the effect of chronic caloric restriction in Fast and Slow rats by studying their behaviour in open field, performance in morris water maze, behaviour during restraint, and their amygdala kindling profiles. These paradigms provide measures thought to index levels of activity/hyperactivity, learning capacity, attention, impulsivity, and seizure susceptibility. These strains consistently and reliably differ from one another on these measures and naturally demonstrate marked metabolic differences; therefore it may be informative to determine whether a metabolic manipulation such as caloric restriction affects the strains differently. Given that the typical behavioural and kindling profiles of the Fast strain are suggestive of ADHD-like behaviour and seizure susceptibility, we are primarily interested in their response to caloric restriction; however, the response of our seizure-resistant Slow rats is also of considerable interest in light of recent experimental findings within the laboratory showing that another dietary treatment (omega-3 supplementation) affected each strain very differently (Gilby et al., in press).

METHODS

Animals

The effects of caloric restriction on the relative seizure sensitivity and behavioral profiles of adult Fast versus Slow rats was observed using a behavioural test battery (open field exploration, morris water maze, restraint; in that order) and basolateral amygdala kindling. The amount of water consumed by each animal was assessed for three days prior to the initiation of CR, at which point their free feeding body weight (FFBW) was also recorded. Chronic caloric restriction of Fast and Slow rats involved a gradual reduction of

each animal's allotment of Purina rat chow (1.5% saturated fat, 8.5% unsaturated fat, 23.4% protein, 5.3% fibre, 6.9 % ash, 49% carbohydrate), with the intention of stabilizing them at 80% of FFBW (+/-5%). Upon reaching target weights, water consumption was reassessed in order to determine whether the relative polydipsia normally associated with Fast rats was affected by caloric restriction. A pilot study was performed initially; it consisted of 7 calorically restricted Slow rats and 6 calorically restricted Fast rats. These pilot animals were run through the behavioural test battery, but not kindled. Subsequently, the main study was initiated and utilized 16 males of each strain (spread across two cohorts), which in turn were split into restricted and *ad libitum* fed groups.

Animals were assigned randomly to the calorically restricted or control group; however, the animals were to be reassigned if a significant difference in FFBW between the control and restricted groups of either strain was found. Ultimately, the random assignment did not result in imbalanced groups with respect to FFBW. The pilot study data was included in the analysis of the behavioural test battery, thus each behavioural test represented data from 8 Slow controls, 8 Fast controls, 15 calorically restricted Slow rats, and 14 calorically restricted Fast rats. The kindling paradigm excluded any rats found (via histology) to have been stimulated with an electrode placed outside the basolateral amygdala, prompting use of a third cohort (3 rats representing each group) to ensure a sufficient number of subjects in each group being kindled. Consequently, the kindling data represents 7 Fast controls, 7 calorically restricted Fast rats, 9 Slow controls, and 7 calorically restricted Slow rats. All animals were 4-5 months old upon the initiation of the study, and 6-8 months old upon the onset of behavioural testing and/or kindling.

Behavioural test battery: (open field, Morris water maze, restraint)

Open Field (Hyperactivity):

Cohorts 1 and 2 were first assessed in the open field paradigm. The apparatus consisted of a 69x69 inch white plexiglass floor divided into a grid of 25 identical squares enclosed by 4 interlocking white wooden walls. In a brightly lit room, each rat was placed in the centre square, and allowed to explore for ten minutes. Each rat received one ten minute trial per day for four consecutive days. These sessions were videotaped from overhead to allow rescoring, and the apparatus was cleaned with 50% ethanol between trials. Measures of interest included the latency to leave the centre square, defecation, urination, incidences of grooming, and the number of lines crossed.

This test is commonly used as an index of activity for these strains. Typically Slow rats habituate (reduce activity across trials) to this environment whereas Fast rats maintain a high level of activity during each trial (Mohapel & McIntyre, 1998; McIntyre & Gilby, 2007; Gilby et al., 2007[b]), which is thought to imply abnormal hyperactivity.

Morris Water Maze (Learning and Attention)

Cohorts 1 and 2 were next assessed in the Morris water maze paradigm two days after the completion of open field. The water maze apparatus consisted of a white polypropylene pool with a depth of 80 cm and circumference of 158 cm. The pool was filled to 55 cm with water at a temperature of 21-22 degrees Celsius and made opaque by the addition of white powder paint. A clear Plexiglass platform with 14 cm diameter was placed in the middle of one quadrant of the maze and submerged 2 cm below waterline. Each trial began by placing the rat in the pool, facing the wall, in one of 4 starting position quadrants, and each trial was separated by 60 s of rest in a dry cage. Each rat received 4 trials per day, and starting positions are randomly chosen with the provision that all were used each day and that this

order was consistent for all animals on that day. Animals that did not locate the platform within 60s were guided to and placed on the platform for 10s. The 5th and final day of testing involved a visible platform trial (swim test) wherein the platform was raised 1 cm above the water line with a dark towel wrapped around it for greater visibility and to facilitate ascension. Trials were videotaped from an overhead fixture and rescored by a confederate blind to the study.

Previous studies have demonstrated that Fast rats consistently display a significantly inferior performance on this task in cued and uncued variants, which implies that this is not due to a spatial deficit. Their delayed acquisition of the task relative to the Slow strain, and their ease of distraction by irrelevant cues, has suggested that this task measures an attentional disturbance and memory or learning deficits (Anisman & McIntyre, 2002) As such, Morris water maze testing is included to assess whether CR treatment has an effect on these processes.

Restraint (Impulsivity):

Cohorts 1 and 2 were assessed in the restraint paradigm two days after the completion of Morris water maze. Each rat was placed in the wide end of a triangular plastic bag with a hole in the narrow end allowing the rat's nose to protrude. Once securely in the bag, the wide end was taped closed with the rat's tail protruding. The bag fit snugly enough that only limited movement was possible, and the restrained rat was placed on a table for 10 minutes of observation. The percentage of time spent active during the 10 minutes was recorded, as well as the # of vocalizations, incidences of defecation, presence/absence of urine, and the presence/absence of porphyrin secretion from the nose. Only one animal was in the testing room at a time, the tester was blind to the strain or treatment group of the animal, and the

animal was removed from the bag and returned to their home cage immediately after the 10 minutes.

This test is included in the battery as a measure of impulsivity, as it has been consistently observed that Fast rats struggle to a much greater degree throughout the test than do the Slow rats (Gilby et al., 2007[b]; McIntyre & Gilby, 2007).

Kindling surgeries:

All rats were anaesthetized with sodium pentobarbital (Somnotol, 60mg/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, were implanted in both amygdalae in the basolateral region. Coordinates for the implantation were 2.8 mm posterior to bregma, 4.8 mm lateral to the midline, and 8.5 mm below the skull surface (Paxinos & Watson., 1998). Five stainless steel screws, embedded in the skull and secured with dental acrylic, anchored the electrodes. A Diamel-insulated ground wire was soldered to a stainless steel screw, attached to a male Amphenol pin, and embedded into the skull. The Amphenol pins were inserted into a plastic head cap and secured with dental acrylic (Molino & McIntyre, 1972). Following surgery, rats received acetaminophen rectal gel and Bupivicaine, a topical analgesic, and were transferred to cages placed atop heating pads to maintain normal body temperature until they resumed behavioural activity. Rats were then returned to the colony room and allowed 2-4 weeks recovery prior to after discharge threshold (ADT) determination.

Afterdischarge Threshold (ADT) Determination & Kindling procedure:

Afterdischarge thresholds were determined in the left and right amygdala of each rat 2 weeks following surgery. The ADT is defined as the minimum stimulus intensity required

to elicit an afterdischarge (AD). An AD is defined as a spike and wave discharge that outlasts the stimulation by 2 or more seconds (McIntyre et al., 1999). The stimulus used was a 2 s, 60 Hz sine wave, presented in incremental intensities (25, 35, 50, 75, 100, 150, 200, 300, 400, 500 microamperes) until an AD was elicited. Successive stimulations within the same amygdala were separated by a one-minute interval. Threshold tests between the two amygdalae were separated by 24 hours.

Daily kindling began twenty-four hours after ADT determinations. For consistency, the left amygdala was kindled if both ADT determination sessions yielded reasonably low thresholds and AD traces that suggest correct placement of the electrode. In the case of a suspect left electrode, the rat's right amygdala was kindled. Daily stimulation of the amygdala proceeded at the predetermined ADT intensity until 6 Racine stage-5 generalized convulsive seizures (Racine, 1972) were recorded.

On the day following an animal's 6^{th} stage-5 seizure, the ADT was re-determined for the kindled amygdala. Twenty-four hours later, the ADT was re-determined for the contralateral amygdala.

The ADT intensity and associated AD duration were parameters of interest as a reflection of local excitability. The number of daily stimulations required to elicit the first stage-5 seizure, defined as the kindling rate, was also assessed for each animal, as well as the cumulative AD duration required to elicit the first stage 5-seizure. These parameters are relevant to the ease of network recruitment, and are well established in the seizure prone and resistant rat strains. The behaviour of the animals during kindling trials, including the Racine stage of their seizure activity (Racine, 1972), will be recorded daily. In order to assess the severity and characteristics of generalized seizures in fully kindled rats, the latency to

forelimb clonus, duration of tonic/clonic seizure activity, and AD duration will be recorded for each of the 6 stage 5 seizure trials.

Histology:

All fully kindled rats, under deep anesthesia via sodium pentobarbital, were perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. The electrodes were then extracted and the brains removed and stored in 4% paraformaldehyde for at least 7 days before sectioning. Brains were sectioned (40 μm) on a microtome following immersion in a 30% sucrose solution to circumvent freezing damage. Sections containing the electrode tip were mounted on gelatin-coated slides and stained with cresyl violet to confirm electrode placement within the basolateral amygdala.

Data Analysis:

ANOVA and repeated measures ANOVA were used to analyze kindling data and behavioural test performance. Where appropriate, post-hoc oneway ANOVA was used to compare specific groups, particularly since our primary planned comparison was between calorically restricted Fast rats and Fast *ad libitum* fed controls.

RESULTS

Animal weights and weight loss

	Initial weight (g)	Weight at onset of testing (g)	Time to 80% FFBW (days)
Fast control	404.3 +/- 8.1	494.5 +/- 22.8	n/a
Fast restricted	419.8 +/- 13.4	335.9 +/- 10.7	28.4 +/- 0.9
Slow control	407.4 +/- 10.4	474.8 +/- 8.0	n/a
Slow restricted	416.9 +/- 9.9	333.5 +/- 7.9	35.7 +/- 2.4

Table 1: No significant differences between groups were seen in initial body weight, but restricted Fast rats achieved 80% FFBW significantly sooner than restricted Slow rats ($F(1, 34)=8.269$, $p=0.0069$).

Behavioural results

Open Field Test

The open field test was employed as a gauge of relative activity levels between the groups; hence the number of lines crossed by each animal over trials was the primary measure of interest. In this paradigm, repeated measures ANOVA demonstrated a robust main effect of strain on the number of lines crossed over the four day testing period [$F(1, 41) = 18.959$, $p < 0.001$]. Consistent with previous studies, Fast rats scored significantly more line crosses over trials than Slow rats. In addition to the strain effect, repeated measures ANOVA detected a significant interaction between strain and treatment, a significant within factor effect of trial, and a significant strain by treatment by trial effect.

The significant trial main effect [$F(3, 123)= 29.671$, $p<0.001$] observed was expected, as most rats demonstrate habituation to the open field environment by reducing line crossing over trials (Weijers & Weyers, 1998; Herman et al., 1986; Pedrazza et al., 2007). In fact, a lack of this habituation is often a characteristic of animal models of ADHD (Laming et al., 1989; Sagvolden et al., 1992). Previous studies using these strains have consistently demonstrated that while Slow rats reduce line crosses over successive trials, similar to most outbred animals, Fast rats exhibit poor to no habituation. This effect was again observed in this study when Fast and Slow control rats were compared ($F(3, 42)=4.903$, $p=0.0052$). Interestingly, this tendency in Fast rats is one of the characteristics bringing them forward as model of inherited hyperactivity.

The significant interaction between strain and treatment factors [$F(1, 41) = 5.543$, $p=0.0234$] on total line crossing activity suggested a differential effect of caloric restriction on each strain, which necessitated further analysis.

Upon closer analysis, calorically restricted Fast rats were found to cross significantly fewer lines (figure 1) than the Fast control group [$F(1, 20)=5.247$, $p=0.0330$ as analyzed post hoc by a oneway repeated measures ANOVA. Indeed, calorically restricted Fast rats showed habituation in the open field (figure 2) that was quite similar to that observed in both Slow groups (day x group vs slow controls $p=0.8670$, day x group vs slow restricted $p=0.9525$). Despite showing no significant difference from Fast control rats on the first day of the open field, indicating both groups of Fast rats had similarly high levels of activity in the novel open field environment, calorically restricted Fast rats crossed significantly fewer lines than Fast controls on day two [$F (1, 20)=4.689$, $p=0.0426$], three [$F (1,20)=6.097$, $p=0.0227$], and 4 [$F (1, 20)=6.423$, $p=0.0197$] as determined by post hoc oneway ANOVA. A similar analysis of the Slow groups found no significant difference in total line crosses between the calorically restricted and *ad libitum* fed rats ($p=0.2818$). Thus, caloric restriction appears to have lessened the Fast rat tendency for hyperactive behavior whilst having no demonstrable effect on the corresponding behavior of Slow rats.

Restraint

Two way ANOVA revealed a robust main effect of strain on the percentage of total time each rat spent actively struggling in the restraint trial [$F(1, 28)=26.174$, $p<0.0001$]. Specifically, Fast rats actively struggled for a significantly greater percentage of the ten minute trial than did Slow rats. Unlike the open field data, however, the ANOVA did not suggest a significant effect of treatment or a strain by treatment interaction. Hence, both control and calorically restricted Fast rats demonstrated significantly higher activity levels during the restraint trial than either Slow group (figure 3). Despite the non-significant result within strains, there was a trend for calorically restricted Fast rats to reduce activity levels

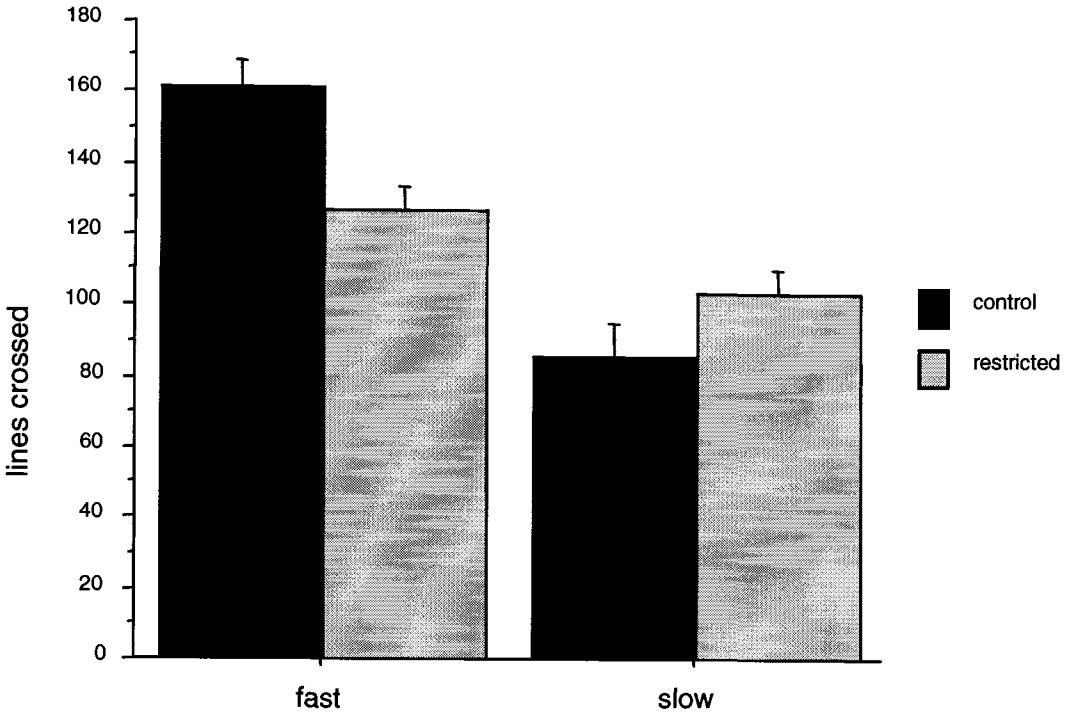

Figure 1: Overall activity in open field (+/-1 st.err). Calorically restricted Fast rats are more active overall than *ad libitum* fed Fast controls.

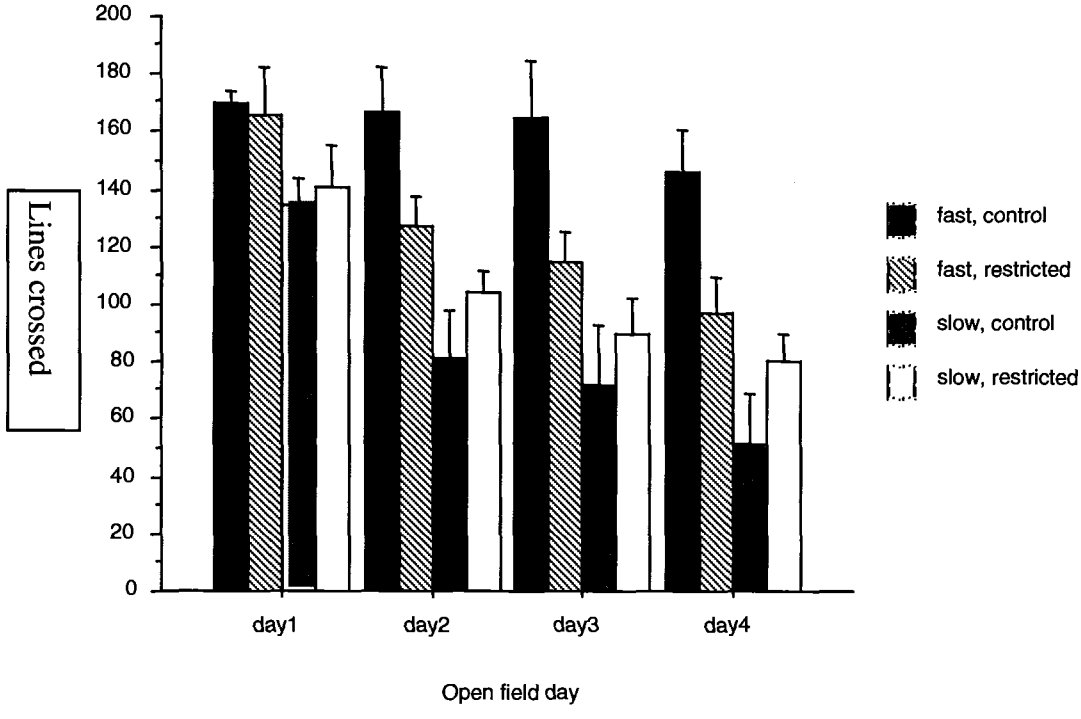

Figure 2: Activity across all 4 consecutive open field trials (+/- 1 st.err). Calorically restricted Fast rats habituate to open field while Fast controls do not.

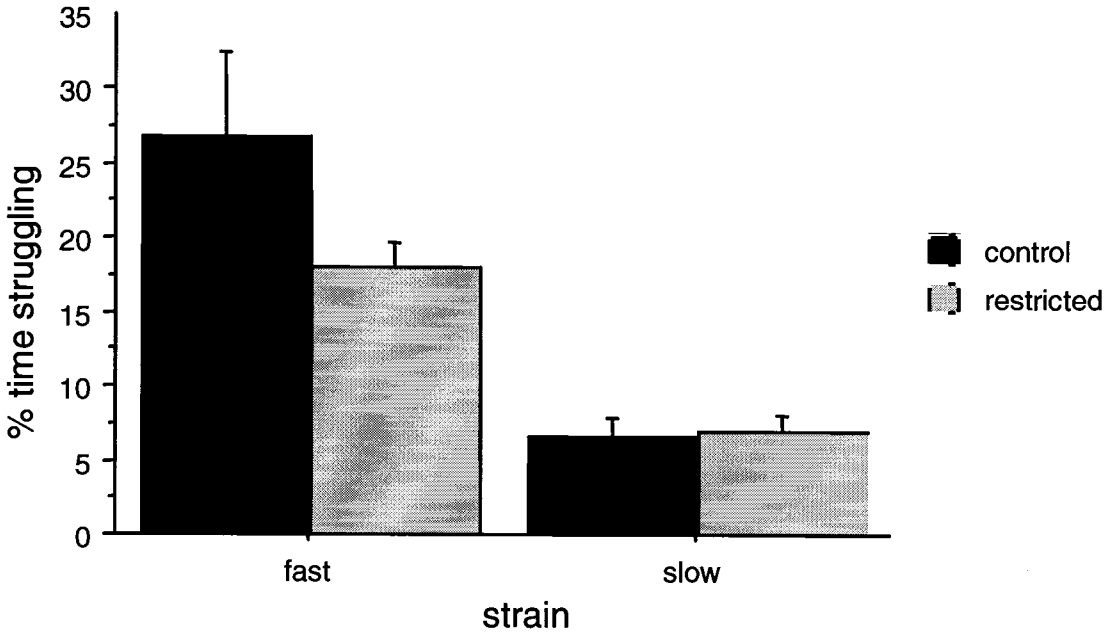

Figure 3: Active struggling during restraint trial (+/- 1 st. err).

relative to Fast control rats ($p = 0.10$) which, considering the variability within the Fast control group, may be of note. This tendency of the Fast strain to struggle significantly more in the restraint paradigm than Slow rats has been consistently reported by previous studies (Anisman et al., 1997; Merali et al, 2001; Gilby et al., 2007), and is thought by some to represent relative impulsivity. Thus, these findings suggest that caloric restriction did not significantly affect the typical behavioral patterns of either strain, and thereby did not impact the perceived impulsive nature of the Fast strain.

Another measure routinely taken during our restraint procedure is the number of vocalizations emitted by each animal during restraint trials. Fast rats have been routinely observed to produce a significantly greater number of vocalizations under restraint than Slow rats (unpublished data). In this study, two way ANOVA of the collected data revealed that not only does the Fast strain demonstrate significantly more vocalizations than the Slow strain [$F(1,28)=20.469$, $p=0.0001$], but that caloric restriction was associated with significantly fewer vocalizations as a main effect of treatment [$F(1,28)=5.737$, $p=0.0235$]. There was no statistically apparent strain by treatment interaction, although it did approach significance [$F(1,28)=3.809$, $p=0.0610$].

Upon more detailed analysis, post hoc testing of the Fast rats via oneway ANOVA suggested that caloric restriction significantly reduced the number of vocalizations during restraint compared to *ad libitum* controls [$F(1,14)=4.792$, $p=0.0460$]. Similar analysis of the Slow rats failed to demonstrate a significant effect of caloric restriction within the strain. Thus, it appears as though caloric restriction had no discernible effect on our primary measure of proportional struggling, but it may be associated with a reduced number of vocalizations during the restraint trial, particularly in the Fast rats (Figure 4).

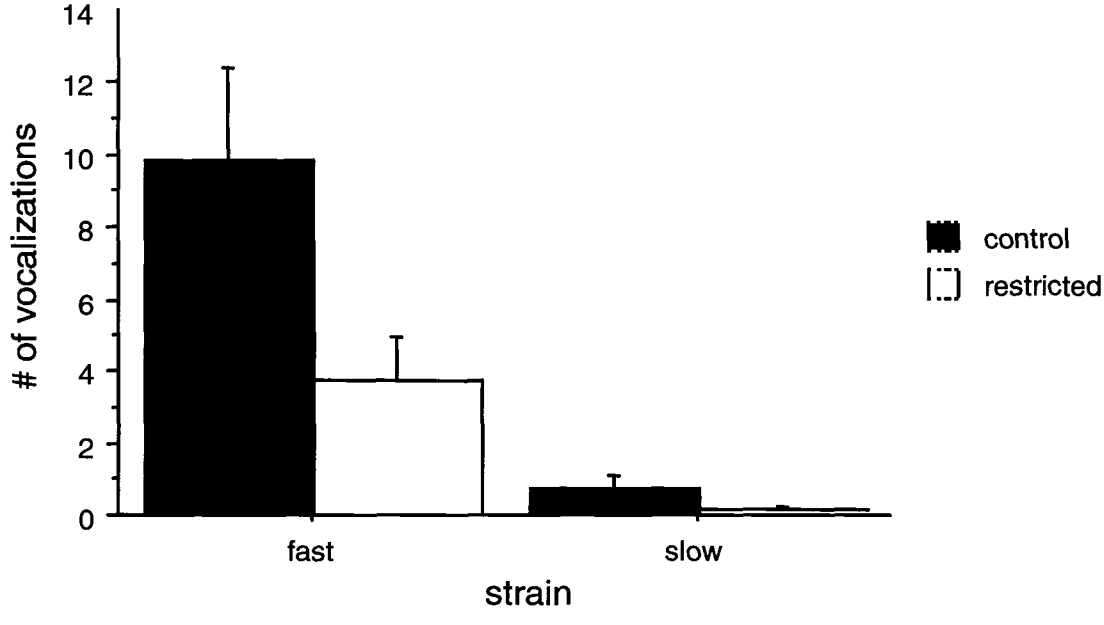

Figure 4: Vocalization during restraint trial (+/- 1 st. err). Calorically restricted Fast rats vocalize significantly less during restraint than Fast controls.

Morris Water Maze

The latency for each subject to locate and mount the submerged platform is the primary measure of interest in our analysis of the Morris water maze task. For each rat, this latency measure was averaged across the 4 trials run on each day of testing. For the first four testing days, which constituted the hidden platform testing period, repeated measures ANOVA revealed a significant main effect of strain [$F(1, 41)=9.651$, $p=0.0034$] demonstrating that Fast rats are inferior to Slow rats in this task (i.e. require longer swim latencies to locate the platform). Yet, a significant within factor effect of the testing day [$F(1, 123)=124.476$, $p<0.0001$] was also observed indicating that despite an inferior ability to execute the task, Fast rats, like Slow rats, perform better over days. No significant main effect of treatment or interaction between treatment and other factors was detected. An inferior overall performance by the Fast rats in this simple variant of the Morris water maze has been consistently observed in previous studies, and was expected within the control group (Anisman & McIntyre, 2002). Importantly, this longer latency in the Fast strain does not signify inferior mobility, as all groups performed similarly (figure 5) in the visible platform task (strain $p=0.2108$, treatment $p=.7860$, strain x treatment $p=0.3267$).

It is noteworthy that in spite of the fact that Fast rats demonstrated consistently longer average latencies in reaching the platform compared to Slow rats, both strains seemed to improve their performance over repeated exposure in a pattern statistically indistinguishable from one another (days x strain = $F(3,123)=0.465$, $p=0.7072$). In fact, in this study, Fast rats seemed to improve their performance similarly to Slows over subsequent days (figure 6), but it was the improvement within the first day that was significantly weaker in Fast versus Slow rats [$F(3,123)=2.982$, $p=0.0340$], irrespective of treatment (figure 7).

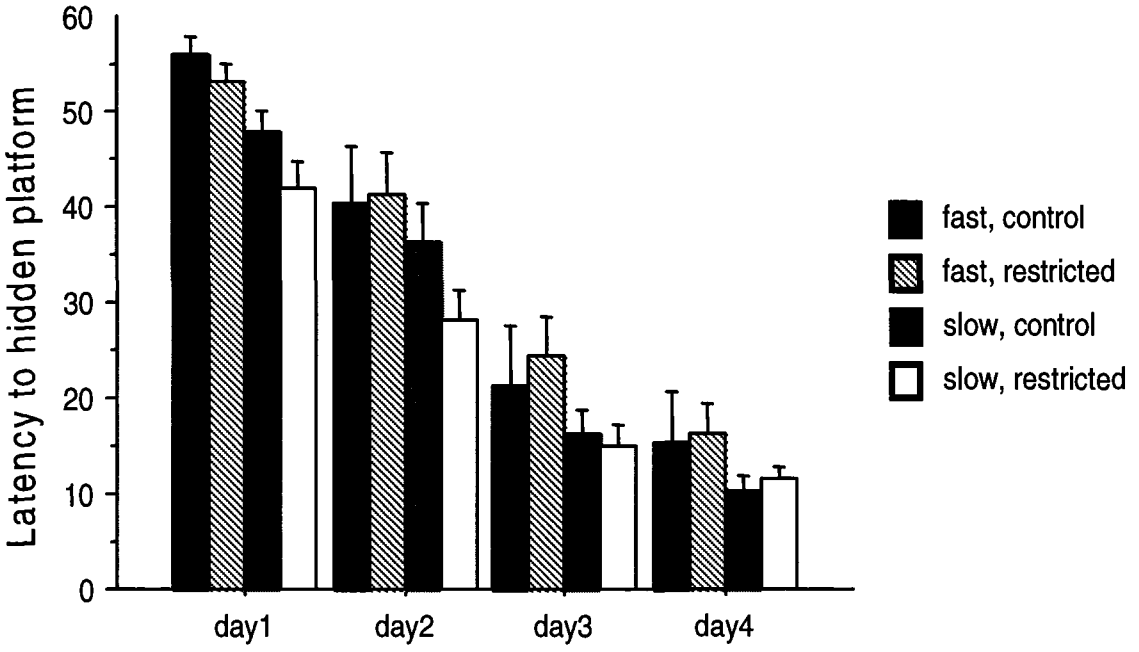

Figure 5: Latencies to hidden platform (in seconds) across all four hidden platform testing days (+/- 1 st.err).

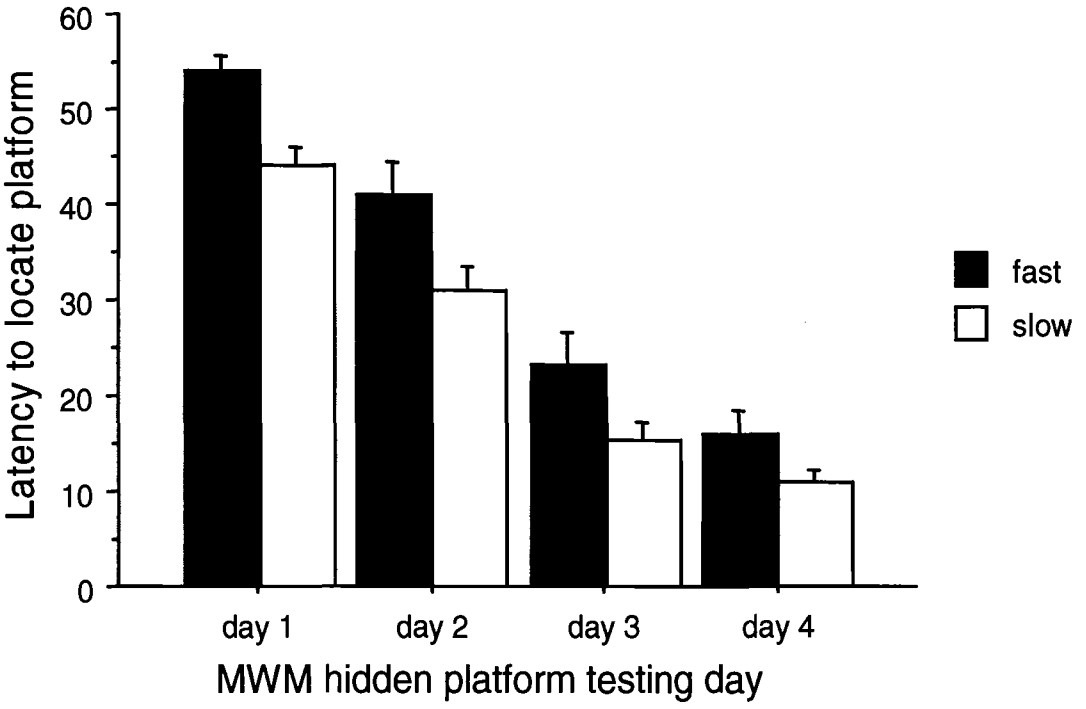

Figure 6: Comparison of mean daily latencies (in seconds) to hidden platform between strains (treatment groups collapsed) across all four hidden platform days (+/- 1 st. err)

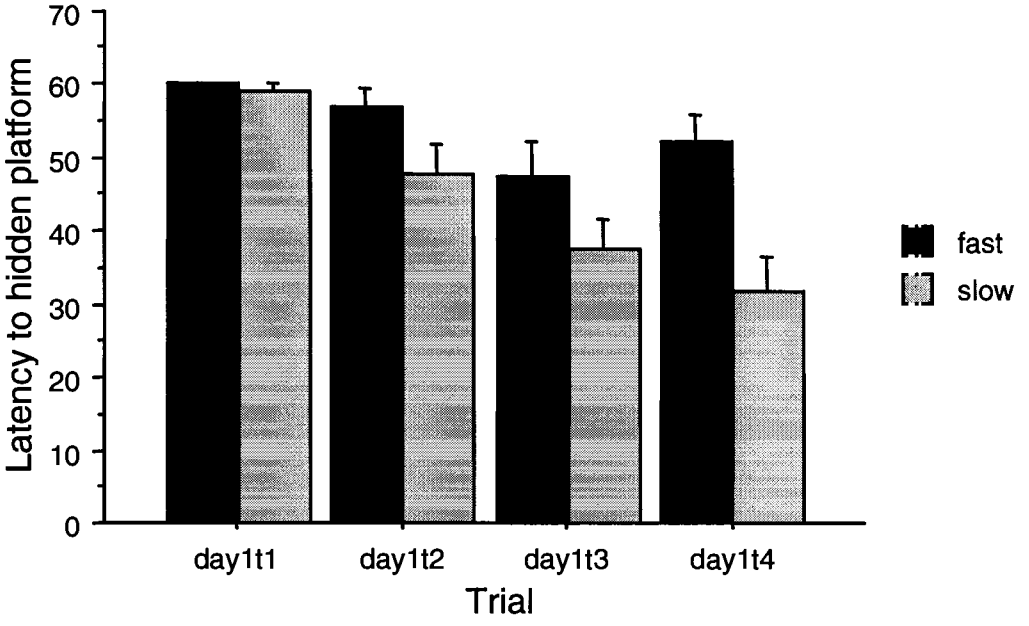

Figure 7: Latencies to platform (in seconds, treatment groups collapsed) across all 4 trials of the first MWM testing day (+/- 1 st. err). Slow rats improve their performance on the first day of MWM while Fast rats do not.

Finally, in order to assess the ability of subjects to recall and act on what had been learned the day before, a repeated measures ANOVA was performed using only the first trial of each day as the dependent measure. In this case, no significant interaction between strain and testing day was found, once again implying that both strains demonstrated a similar pattern of improvement over days (figure 8).

The longer average latencies per day, yet similar pattern of improvement over days exhibited by the Fast rats when compared to the Slows may benefit from some qualitative observations of note. In this study, Fast rats tended to utilize a different search pattern from that of Slow rats such that Fast rats were relatively thigmotaxic (tended to circle the pool) and would suddenly strike out straight across the water. Similar behavioral patterns have been reported previously by Anisman & McIntyre, (2002). In cases where the rat barely missed the platform, it would sometimes circle the immediate area, but more often would continue swimming straight to the other end of the pool before reorienting itself slightly and swimming back across to the platform. Moreover, it was repeatedly observed that several Fast rats would purposely jump off the platform once they mounted it. These animals would continue swimming, and have to be guided back to the platform, and in many cases, physically held there for 10 seconds, lest they jump off again. This has been observed anecdotally by the experimenters of several previous studies. No Slow rat in either treatment group of this study ever jumped off a successfully mounted platform. Considering the Morris water maze is a learning task based on an equal motivation for all subjects to get out of the water and onto the platform, this unique behaviour of Fast rats may engender difficulties in assessing the relative learning capabilities of these strains. In the interest of appropriate interpretation however, it should be noted that this apparent willingness to

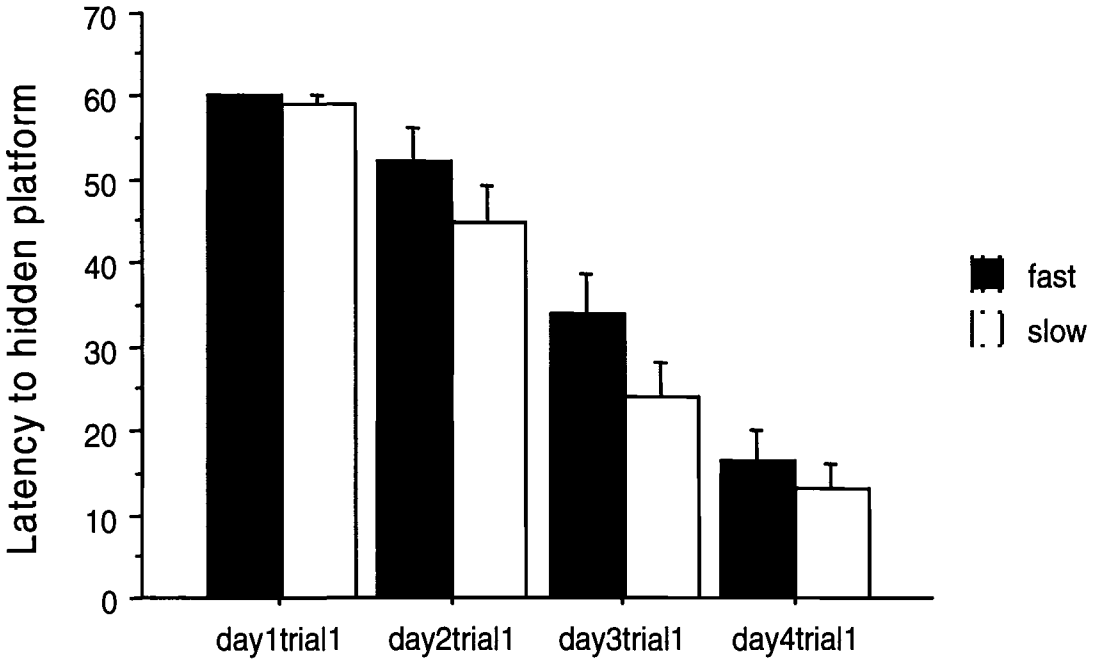

Figure 8: Latencies to platform for first trial of each testing day btw strains (treatment groups collapsed, +/- 1 st. err).

reenter the water from the platform displayed by several Fast rats may not necessarily have been maladaptive or imply insufficient motivation to escape the pool. Indeed, one calorically restricted Fast rat was clearly jumping towards the pool wall and actually reached its lip more than once. This rat would have escaped the maze if not for the experimenter's repeated intervention. The escape attempts made by this Fast rat (appropriately renamed Steve McQueen) serve as a particularly humorous reminder (especially if one were to watch the videotaped trial and the experimenter's reaction to the first incident) that the behavioural profiles of each strain may confer situation specific advantages. Regardless of these qualitative observations, it is clear that caloric restriction did not alter the typical behavioral patterns of Fast or Slow rats in any aspect of Morris water maze performance.

Water Consumption (Polydipsia)

Water consumption was initially assessed for every animal at the outset of the study when all subjects were fed *ad libitum.* It was then reassessed once all calorically restricted subjects had achieved 80% of their free feeding body weight. This allowed for each subject's consumption to be compared against itself prior to experimentation. Thus, a difference in water consumption for control animals would account for the effect of time/environment and any difference in the treatment groups would account for the effect of caloric restriction. In terms of the absolute volume of water consumed, a robust main effect of strain [$F(1, 25)=46.284$, $p<0.0001$] was detected, suggesting that Fast rats collectively drank significantly more water than Slow rats (figure 9). This is in accordance with previous research demonstrating that Fast rats consistently drink more water than Slow rats, as relative polydipsia may be an inherent characteristic of the Fast strain.

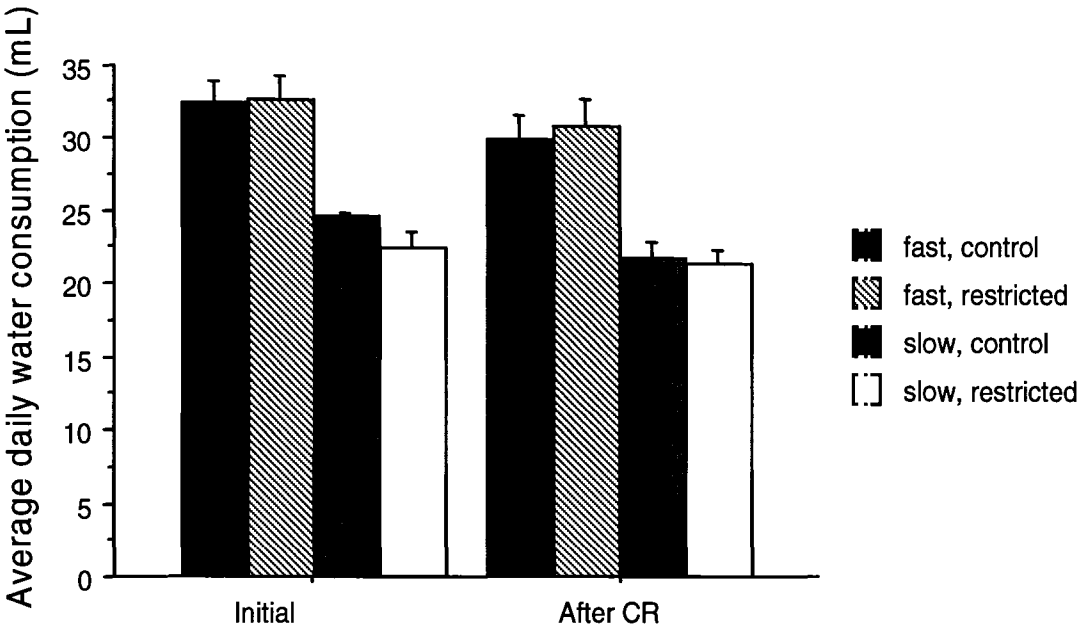

Figure 9: Absolute water consumption (mL/day) for all groups before caloric restriction with mean water consumption for all groups at time of achievement of 80% free feeding body weight by all CR animals (+/- 1 st. err, approx 4 months btw time points). Fast rats drink more water than Slow regardless of time or caloric restriction.

Considering the range of body weights within and between groups, however, water consumption also had to be analyzed as a function of body weight (mL water consumed/gram of body weight). In this case, repeated measures ANOVA showed the same main effect of strain [$F(1,25)=69.101$, $p<0.0001$] but also identified a treatment effect [$F(1,25)=10.059$, $p=0.0040$]. The main effect of strain, not surprisingly, resulted from the Fast rats drinking more water per unit of body weight than Slow rats, whereby Fast controls proportionally drank more water than Slow controls ($F(1, 6)=52.782$, $p=0.0003$) and the calorically restricted Fast group drank significantly more water than the calorically restricted Slow group ($F(1,19)=42.811$, $p<0.0001$). The main effect of treatment indicated that calorically restricted animals as a group drank more water relative to their body weight than did the *ad libitum* fed controls. From figure 10, it is evident that this difference between same strain calorically restricted and *ad libitum* groups was not present initially, suggesting that caloric restriction itself was responsible.

Interestingly, control animals significantly reduced water consumption/weight as they aged [$F(1,6)=28.582$, $p = 0.0018$]. Yet, analysis of the restricted animals, using oneway repeated measures ANOVA, demonstrated that both Fast and Slow rats displayed greater proportional intake of water when at 80% of their free feeding body weight than their respective control groups [$F(1, 19)=20.574$, $p=0.0002$ and $F(1,13)=17.944$, $p=0.001$ respectively]. This constituted a time by treatment interaction [$F(1,25)=31.951$, $p<0.0001$].

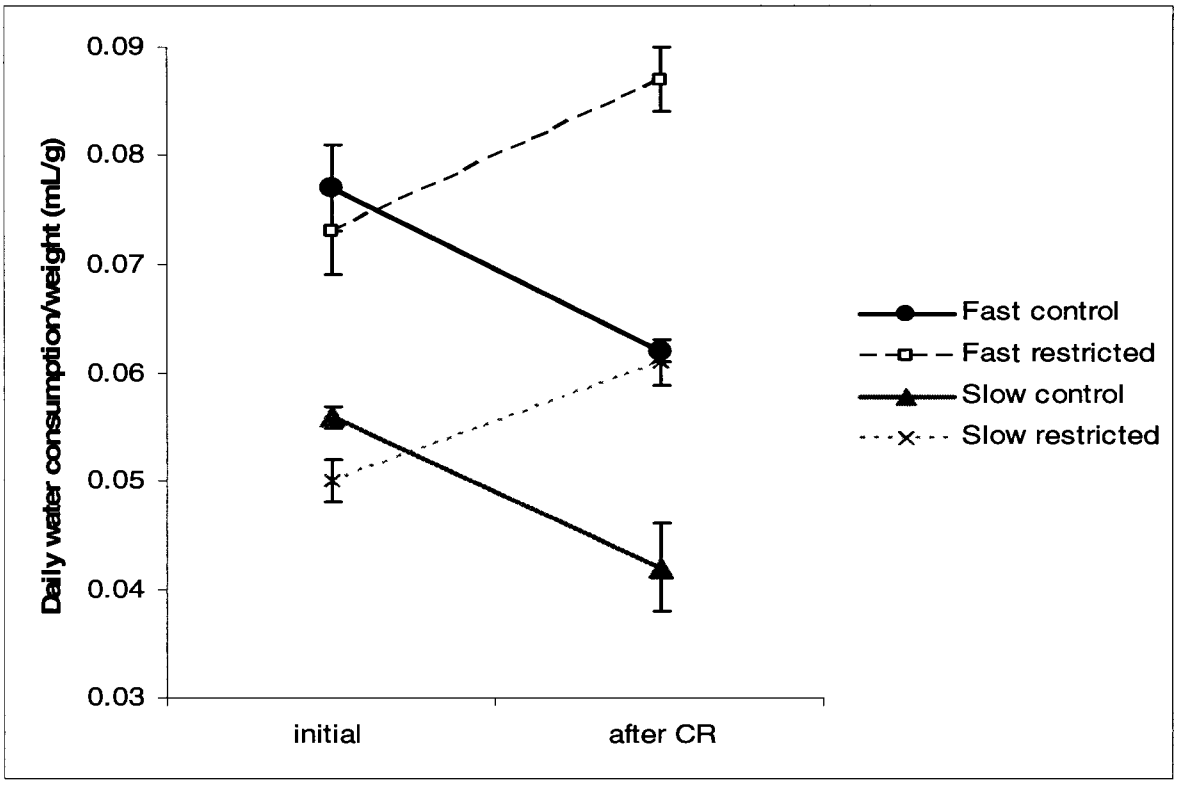

Figure 10: Initial and post-caloric restriction mean daily water consumption per weight (g/mL) (+/- 1 st. err, approx 3 months btw time points). For both strains, calorically restricted rats drink more water per weight than controls.

Kindling

Measures associated with local amygdala excitability

Prekindling After Discharge threshold (ADT); Stimulating Electrode

The two way omnibus ANOVA did not yield a significant main effect of treatment ($p=0.4494$), strain ($p=0.0778$), or a significant interaction between strain and treatment ($p=0.0742$). However, the near significance of an overall strain difference and an interaction between strain and treatment, suggested that one of the groups may be significantly different from the other three with respect to their initial ADT. Graphical representation of the groups (figure 11) further suggested that the calorically restricted Fast group tended towards a reduced ADT relative to the other groups. Post hoc testing revealed that the calorically restricted Fast group, indeed, had a significantly lower ADT than the Fast control group ($F(1,12)=10.630$, $p=0.0068$), and the Slow restricted group ($F(1,11)=8.863$, $p=0.0126$). As is evident from figure 11, no other groups differed significantly from one another with respect to their pre-kindling ADT's.

Prekindling After Discharge Duration: Stimulating electrode

Two way ANOVA of the pre-kindling after discharge duration (ADD) recorded from the stimulating electrode yielded a significant main effect of strain ($F(1,26)=8.956$, $p=0.0060$), such that the initial provocation of an AD was significantly longer in a Fast versus Slow brain, and an effect of treatment ($F(1,26)=4.994$, $p=0.0342$). It became evident upon subsequent analysis, however, that these main effects were largely due to the significantly longer after discharge durations (ADD) in the Fast restricted group (figure 12) compared to Fast controls [$F(1, 12)=5.387$, $p=0.0387$] and the two Slow groups [Slow controls ($F(1, 15)=9.998$, $p=0.0064$); calorically restricted Slows ($F(1, 11)=6.650$,

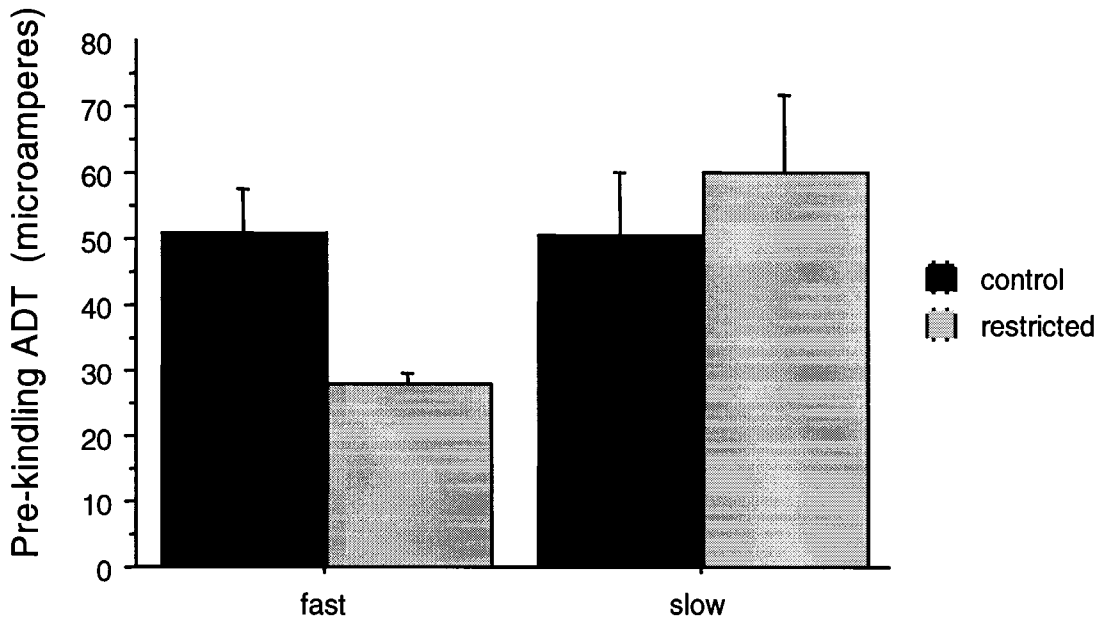

Figure 11: Pre-kindling afterdischarge thresholds as assessed from each animal's first stimulation (+/- 1 st. err). Calorically restricted Fast rats have lower amygdala ADTs than all other groups.

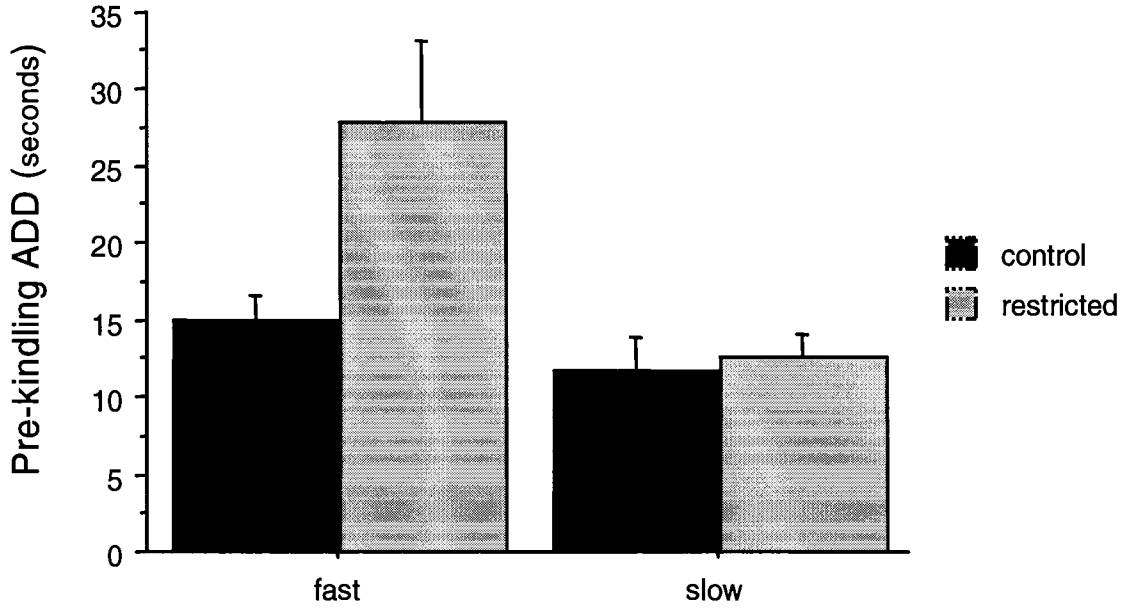

Figure 12: Pre-kindling afterdischarge durations as assessed from the first stimulation (+/- 1 st. err)

p=0.0256)]. As was observed in the pre-kindling ADTs, the remaining 3 groups showed no differences from one another that were of any significance (Slow control vs Fast control p=0.7756, Slow restricted vs Fast restricted p=0.6884, Slow control vs Slow restricted p=0.4957).

Measures associated with epileptogenic capacity (neuroplasticity)

Kindling rate

The number of stimulations required to elicit a fully generalized (stage 5) seizure is the accepted measure of epileptogenesis in the kindling model. Indeed kindling rate was the selection criteria on which the breeding of the Fast and Slow strains was based. For this measure, a two way ANOVA yielded a highly significant strain effect [$F(1, 27)=57.199$, $p<0.0001$], and a main effect of treatment that approached significance [$F(1, 27)=4.023$, $p=0.0550$]. Not surprisingly, the Slow strain collectively demonstrated significantly higher kindling rates than the Fast, which is a defining characteristic of this animal model and denotes relative seizure-resistance.

Since the overall treatment effect was nearly significant and graphical representation of the mean kindling rate for all four groups (figure 13) also suggested that there may be a significant treatment effect within the Fast strain, we analyzed the effects of caloric restriction independently within strain. Intuitively, it seems appropriate that a treatment expected to summon seizure-resistance would be less effective in an already resistant animal. Indeed, when Slow rats were removed from the analysis, one way ANOVA of the Fast groups detected that the calorically restricted Fast group exhibited a significantly higher mean kindling rate than ad libitum Fast controls [$F(1,13)=5.244$, $p=0.0394$].

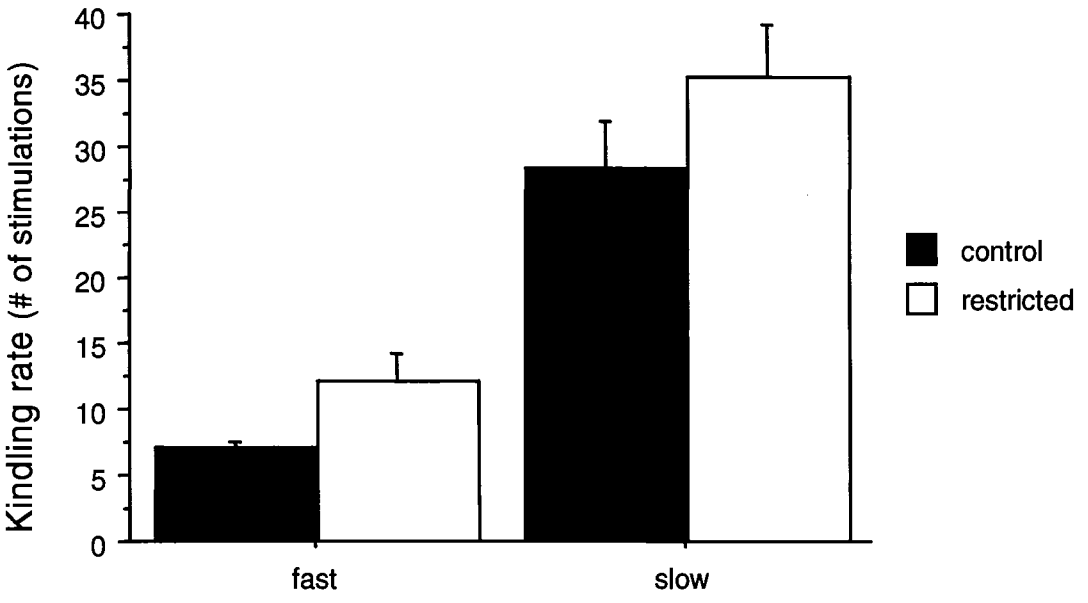

Figure 13: Kindling rate (# of daily elicited after discharges until expression of first fully generalized stage 5 seizure) (+/- 1 st. err). Calorically restricted Fast rats kindle more slowly than control Fast rats.

A similar analysis performed on the Slow data failed to demonstrate any significant difference in kindling rate between the control and calorically restricted Slow rats ($p=0.2267$). Hence, it appears as though caloric restriction retarded kindling in the Fast, but not the Slow, strain. Indeed, treatment appeared to correct the seizure disposition of Fast rats to that of a normal, outbred animal (Edwards et al., 1999; Loscher et al., 1998).

Measures associated with seizure severity in fully kindled animals

Latency to tonic/clonic state

During the elicitation of 6 stage 5 seizures in fully kindled animals, the latency from the time of stimulation until each animal entered a tonic/clonic convulsion was scored. Repeated measures ANOVA of the collected data did not reveal any significant treatment effects or interactions. Moreover, comparison of the two Fast groups alone yielded no significant difference in the latency to a tonic/clonic state ($p=0.2175$). Analysis of this measure did, however, replicate the previous findings of a main strain effect [$F(1,27)= 7.624$, $p=0.0102$] and one of trials [$F(5,135)=3.586$, $p=0.0045$] such that fully kindled Slow rats succumbed to a tonic/clonic state significantly faster than fully kindled Fast rats (figure 14).

Interestingly, graphical representation of each group's average latency per stage 5 seizure (figure 15) suggested that the Fast restricted group may not have differed

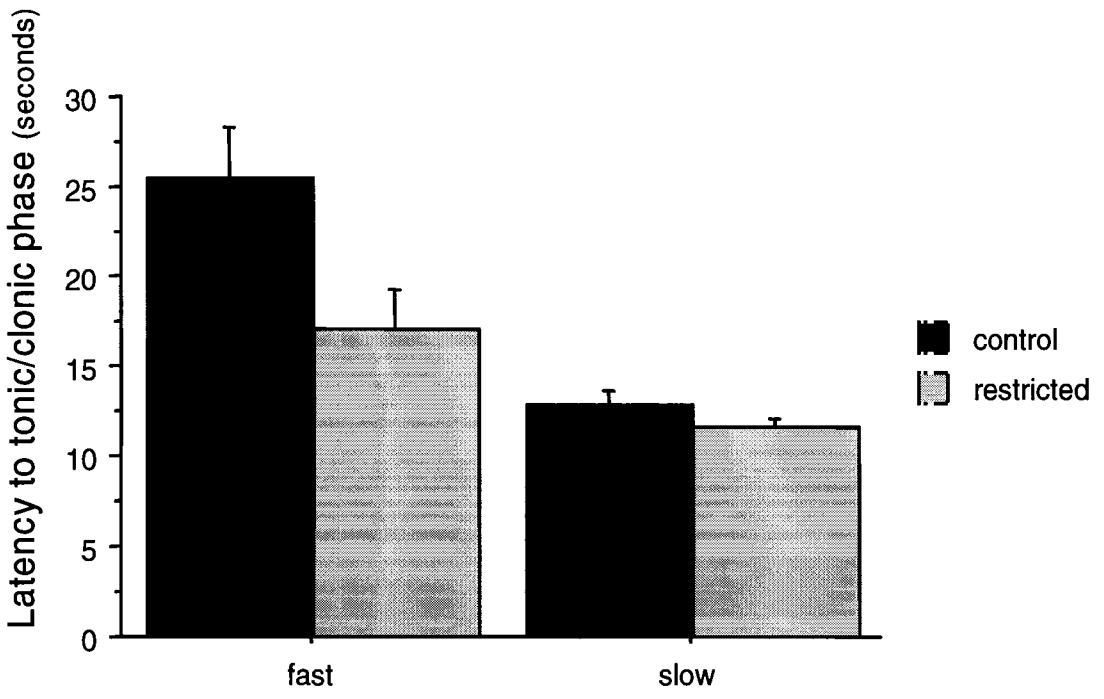

Figure 14: Latency to tonic/clonic phase of fully generalized seizure (+/- 1 st. err)

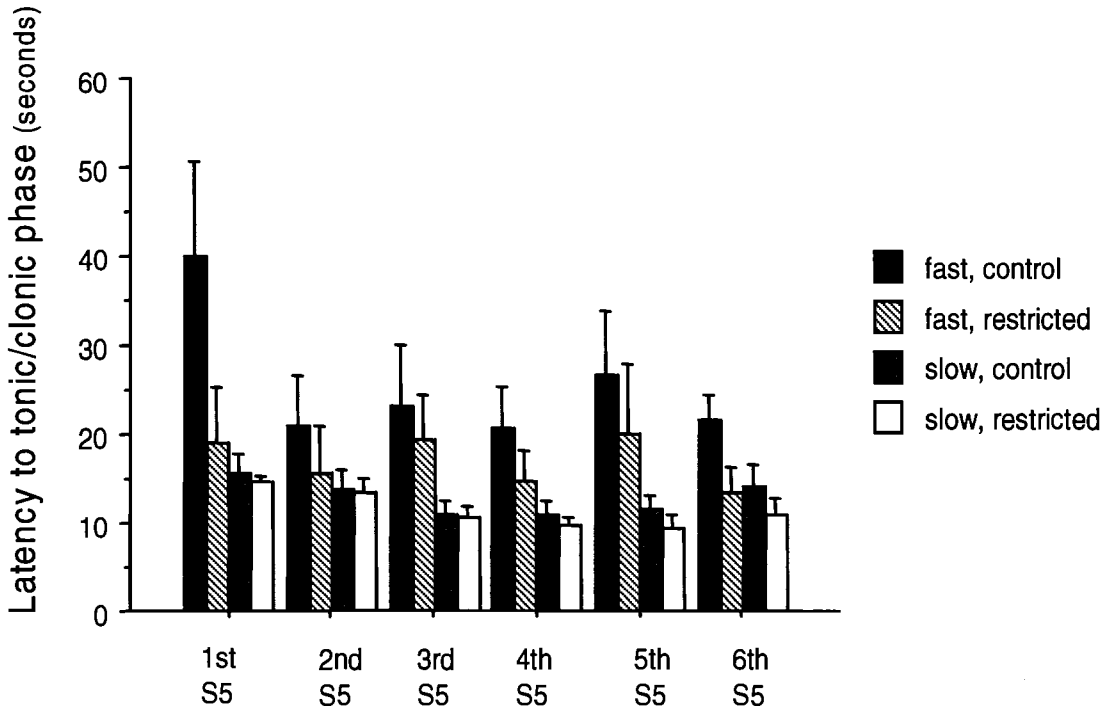

Figure 15: Latency to tonic/clonic phase of seizure across all six elicited stage 5 seizures (+/-1 st. err).

significantly from the two Slow groups. Indeed, post hoc analyses confirmed that the latency to tonic-clonic convulsion did not differ significantly between calorically restricted Fast rats and calorically restricted or *ad libitum* fed Slow rats ($p=0.2971$, $p=0.3124$ respectively). Thus, it appears as though the driving force behind the main effect of strain that was initially detected by ANOVA originated in the Fast control group. Ultimately, latencies in the calorically restricted Fast group did not significantly differ from the *ad libitum* Fast group ($p=0.2175$) but instead only tended to be shorter, which left them statistically indistinguishable from the two Slow groups.

Duration of Tonic/Clonic convulsions

As with the majority of kindling measures, comparisons of tonic/clonic activity during the 6 stage 5 seizures revealed a main effect of strain [$F(1, 27)=27.279, p<0.0001$] and one of trials [$F(5,135)=4.683, p=0.0006$]. In agreement with previous studies within the laboratory, Slow rats demonstrated consistently shorter tonic/clonic convulsive seizure durations than Fast rats (figure 17) and this effect was statistically independent of caloric restriction. This effect is congruent with their relative resistance to seizure.

The reported trials effect on this measure speaks to a commonly observed phenomenon, whereby a general increase in the duration of tonic/clonic activity is associated with repeated stage 5 seizure induction (figure 16).

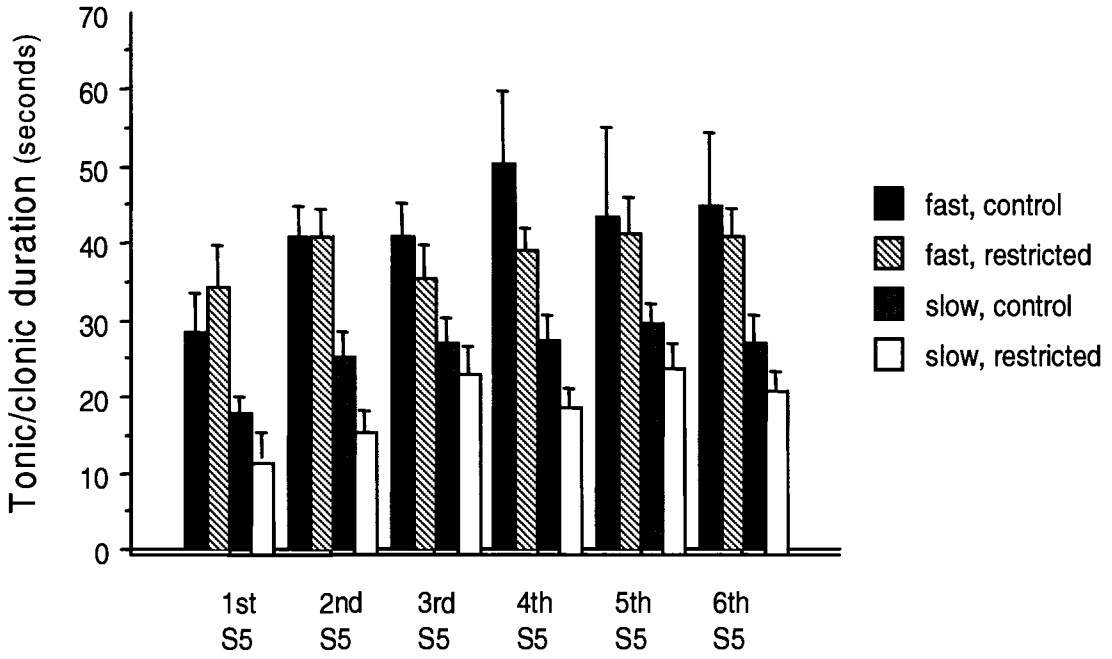

Figure 16: Duration of tonic/clonic phase across all 6 elicited stage 5 seizures (+/- 1 st. err)

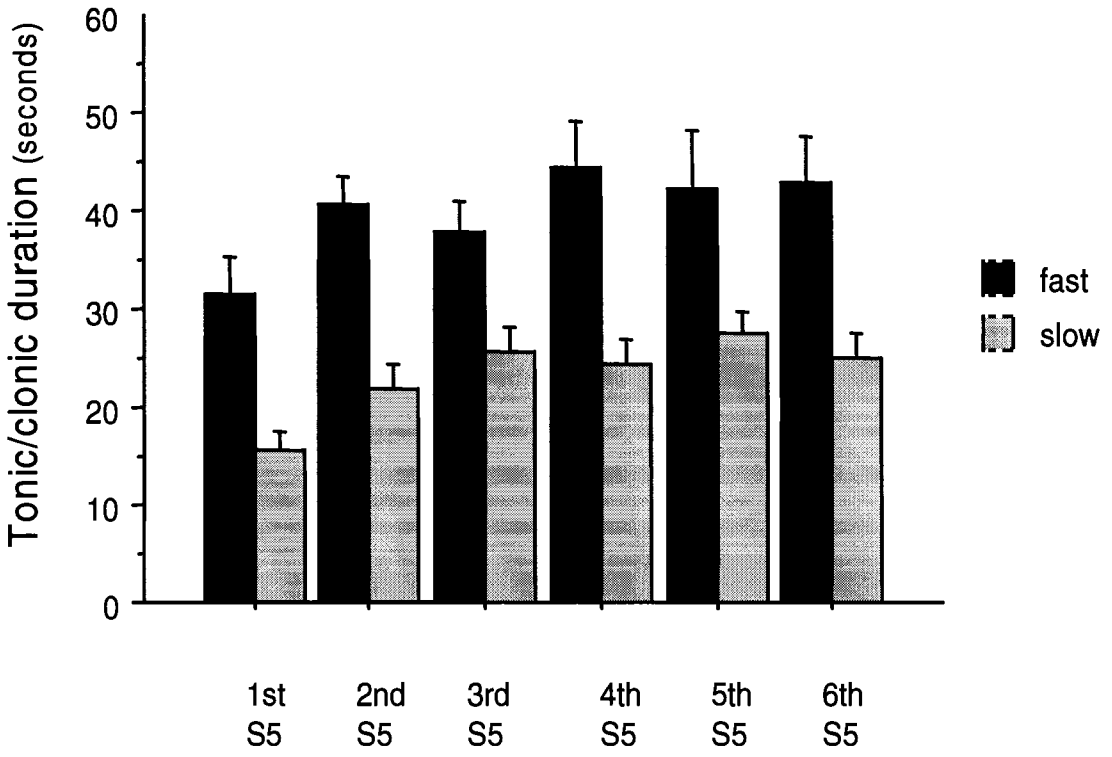

Figure 17: Duration of tonic/clonic phase (treatment groups collapsed) across all 6 elicited stage 5 seizures (+/- 1 st. err)

Importantly, the calorically restricted Fast group did not differ significantly from their respective *ad libitum* control group ($p=0.6369$) in the time spent in tonic/clonic activity within fully generalized seizures (figure 18), implying no effect of caloric restriction on the Fast strain. Independently compared Slow group data was, however, suggestive of a trend towards less tonic/clonic activity in calorically restricted than in *ad libitum* fed Slow rats, but this was not found to be significant ($p=0.0865$). Taken together, analysis of the tonic/clonic seizure durations in fully kindled rats did not demonstrate a significant effect of caloric restriction on either strain.

After discharge durations associated with stage 5 seizures

The afterdischarge durations elicited during each of the six stage 5 seizure trials from the stimulated and contralateral (unstimulated) amygdalae were recorded and analyzed as a measure of electrographic seizure duration. In line with the behavioral (tonic/clonic) seizure durations, and data from previous studies, a robust main effect of strain was detected for this measure in the stimulated [$F(1, 26)=47.085$, $p<0.0001$] and contralateral amygdala [$F(1, 22)=36.121$, $p<0.0001$]. Specifically, upon provocation Slow rats demonstrated significantly shorter after discharge durations from both amydalae than were observed in Fast rats, regardless of treatment group or trial (Figures 19 & 20). Ultimately, caloric restriction did not have a demonstrable effect on the duration of electrographic seizures (ADDs) elicited from either strain (Fast $p = .8472$; Slow $p=0.1003$) in their fully kindled state (figures 21 & 22).

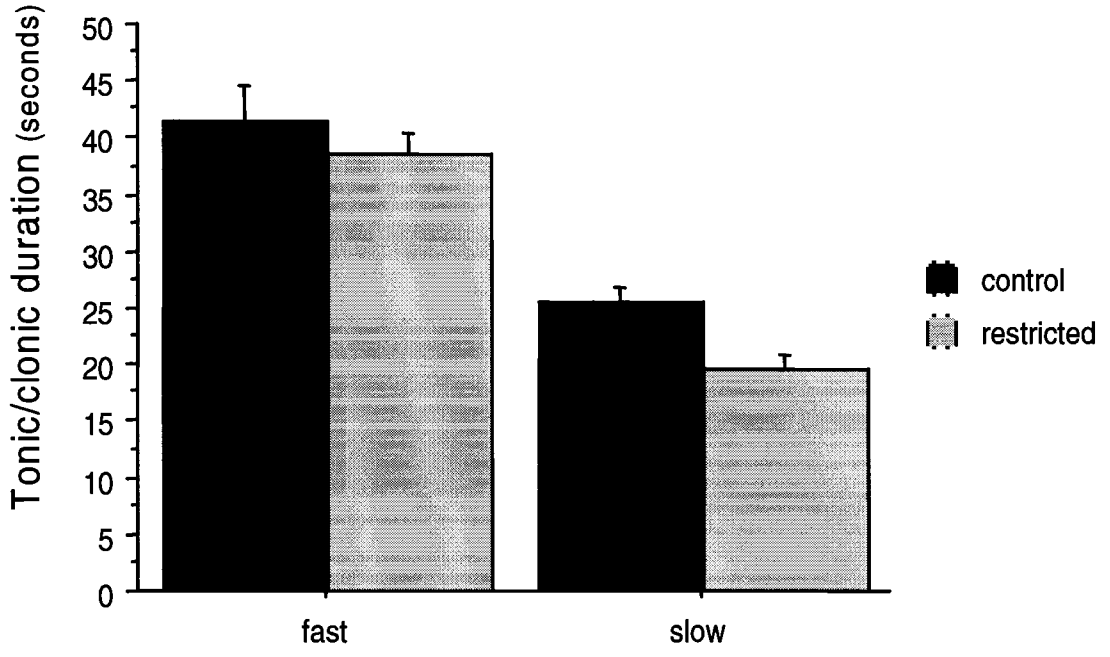

Figure 18: Overall duration of tonic/clonic seizure phase (+/-1 st. err)

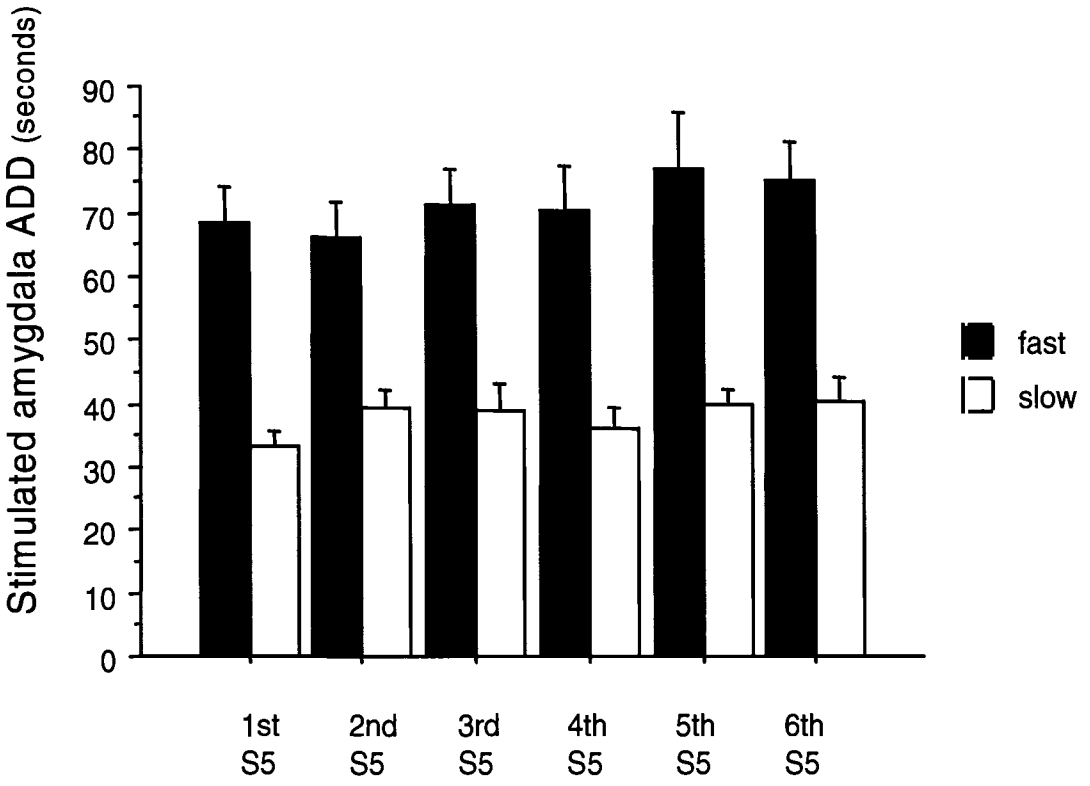

Figure 19: Afterdischarge duration recorded from stimulated amygdala (treatment groups collapsed) across all 6 elicited stage 5 seizures (+/- 1 st. err). Fast rats demonstrate significantly longer after discharges than Slow rats.

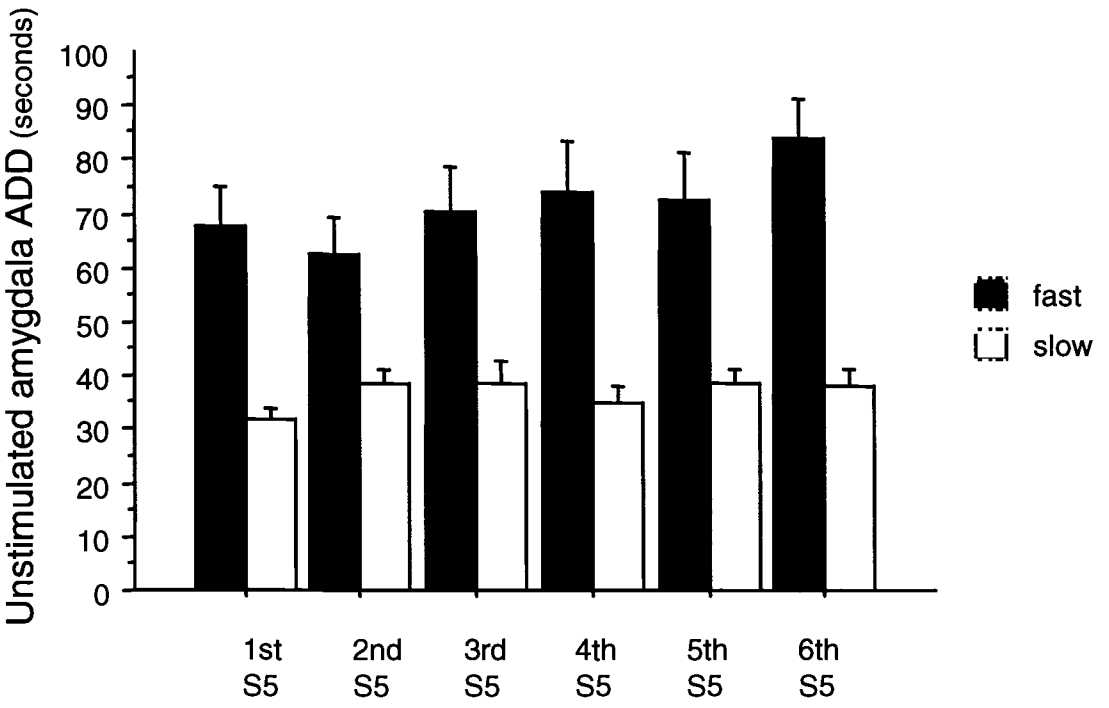

Figure 20: Afterdischarge duration (treatment groups collapsed, recorded from amygdala contralateral to stimulation) across all 6 elicited stage 5 seizures.

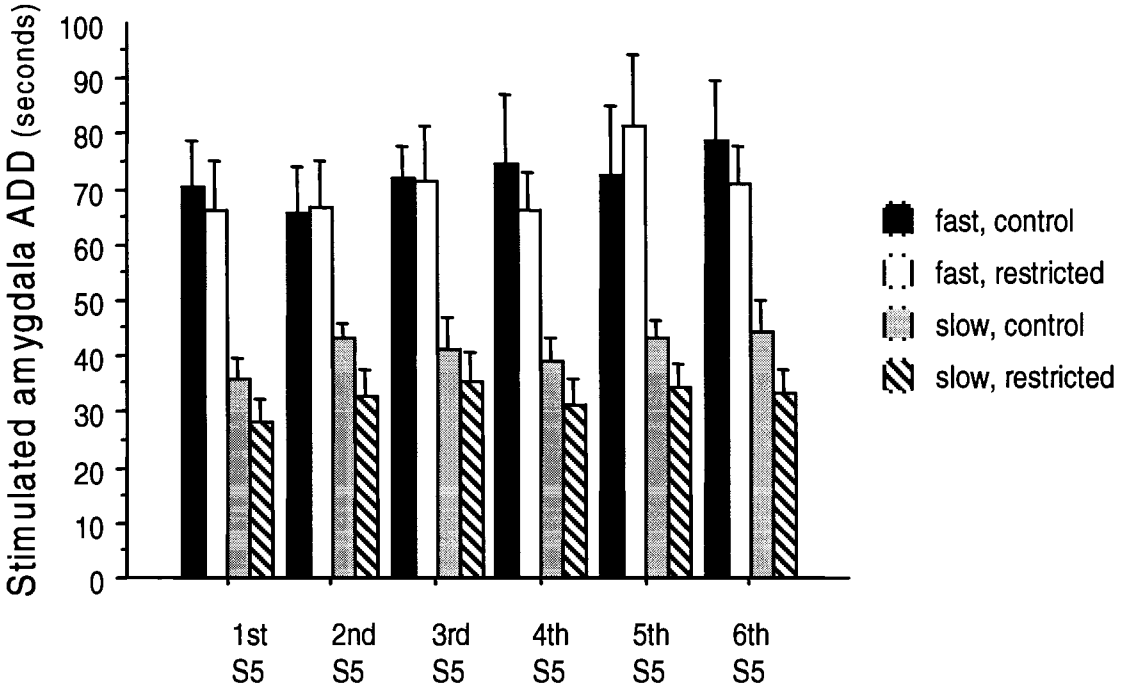

Figure 21: Afterdischarge duration (+/- 1 st. err, recorded from stimulated amygdala) for all 6 elicited stage 5 seizures.

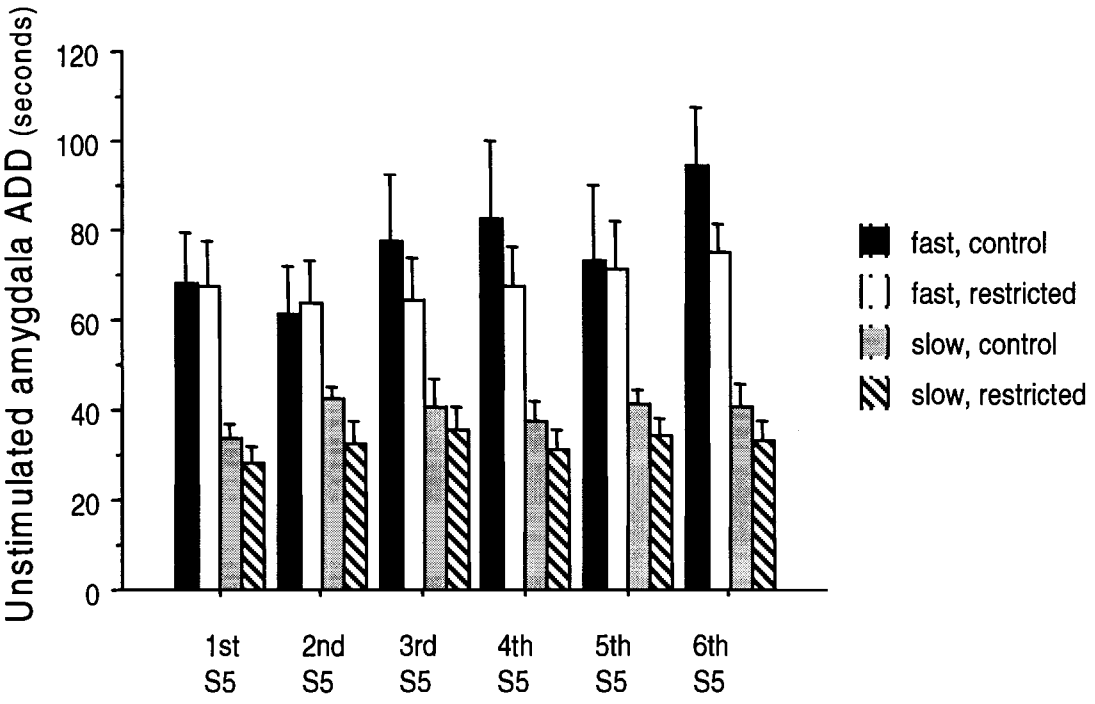

Figure 22: Afterdischarge duration (+/- 1 st.err, recorded from amygdala contralateral to stimulation) for all 6 elicited stage 5 seizures.

Afterdischarge threshold re-determination

Upon redetermination of the ADTs in the kindled (stimulated) amygdala at the conclusion of the kindling paradigm, ANOVA found no significant difference between the ADTs of calorically restricted Fast rats and those of the *ad libitum* Fast group ($F(1, 13)=3.092$, $p=0.8472$). Thus, the reduced thresholds detected in calorically restricted Fast rats at the outset of kindling were no longer significantly lower than those in Fast control rats (figure 23).

DISCUSSION

At the outset of this study, we proposed that an age old modulator of epilepsy, namely caloric restriction, might ameliorate the heightened seizure sensitivity and comorbid ADHD-like behaviours that are innate to the Fast rat strain. In this study, the open field, morris water maze, restraint, and water consumption provided a battery of behavioral measures in which Fast rats are known to reveal their ADHD-like characteristics (i.e. hyperactivity, impulsivity, learning deficits). The construct validity of any of these measures is naturally of some debate, particularly as they pertain to ADHD-like symptoms (Sagvolden et al., 1992), but this is the cross borne by the majority of animal models used for such purposes to date. Indeed, these behavioral tests and others have mounted considerable support for the Fast strain as a rat model predisposed towards ADHD-like behaviours (Anisman & McIntyre, 2002; McIntyre & Gilby, 2007; Gilby et al., 2007[b]) and the testing performed in this study has been similarly used to assess ADHD-like characteristics in other animal models of ADHD (Laming et al., 1989; Sagvolden et al., 1992; Ferguson & Cada, 2004). Relative seizure susceptibility in this study was appropriately assessed using the amygdala kindling

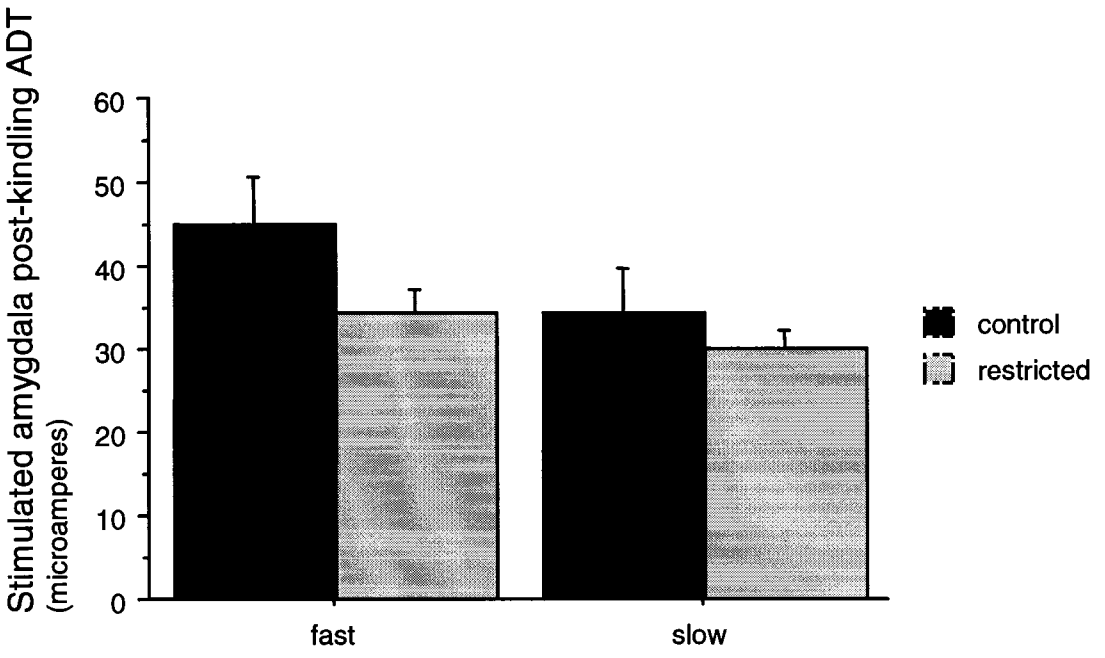

Figure 23: Afterdischarge threshold redetermined post-kindling (+/- 1 st. err).

model since kindling rate was the measure by which the seizure-prone (Fast) and seizure-resistant (Slow) strains were originally derived.

It is also important to note that although treatment studies are often primarily concerned with animals like our Fast rats that model a disease or risk phenotype, as these seem the most clinically relevant to their manipulation, it is important not to overlook the contribution of the Slow strain to this study. Slow rats are not to be confused with a 'normal' control strain for the Fast. Both strains were selectively bred to represent opposing ends of the seizure disposition spectrum; thus, they demonstrate seizure and behavioural profiles markedly different from each other (Racine et al., 1999; McIntyre et al., 1999; McIntyre & Gilby, 2007; Anisman & McIntyre, 2002) but also from most outbred rat populations (Hort et al., 2000; Loscher et al., 1998; Edwards et al., 1999; Pedrazza et al., 2007; Weijers & Weyers, 1998).

Open Field

Open field activity is one of the most frequently used measures of hyperactivity in rats. In the most widely used variant of this test, subjects are repeatedly exposed to the open field over days, rather than utilizing a single trial to assess the levels of activity as the environment becomes less novel to the animal. One of the most consistently reported characteristics of the Fast strain has been relative hyperactivity in the open field compared to the Slow strain (Mohapel & Mcintyre, 1998; McIntyre & Gilby, 2007). Specifically, like outbred rats, the Slow strain reduces activity in the novel environment over repeated trials (habituation), yet Fast rats do not (Weijers & Weyers, 1998; Herman et al., 1986; Pedrazza et al., 2007). As mentioned previously, maintenance of high activity levels over repeated exposures to the open field is thought to represent abnormally high levels of activity

(hyperactivity), which is a hallmark characteristic of ADHD (Laming et al., 1989; Sagvolden et al., 1992; American Psychiatric Associaton, 2000). In this study, the *ad libitum* fed Fast controls demonstrated the same lack of habituation for which the Fast strain are known. However, calorically restricted Fast rats habituated to the open field in a manner that was statistically indistinguishable from both Slow groups. Thus, we can only presume that some aspect of caloric restriction ameliorated the hyperactive tendency of Fast rats.

It seems unlikely that reduced energy levels or relative sedation were responsible for this therapeutic effect in Fast rats given that both Fast groups demonstrated similarly high levels of activity on the first day of this task, that the Slow CR group demonstrated similar levels of activity to their *ad libitum* controls throughout the study, and that several CR studies in rodents and primates report higher levels of activity and an apparent energy boost associated with CR (Ingram et al., 2001; Greene et al., 2001; Smith & Metz, 2005; Wu et al., 2003). In fact, there is a growing concern that *ad libitum* fed animals are poorly suited for many studies due to their high food intake, making them unintentionally representative of the overweight human population (Keenan et al., 1997), which is a population that often reports lower energy levels than those at a fraction of their body weight and caloric intake (Paddon-Jones et al., 2008). With little to suggest that the uncharacteristic low activity levels of CR Fast rats was due to undernourishment, we consider the possibility that a CR-induced metabolic shift somehow abolished one of the most characteristic expressions of abnormal hyperactivity in Fast rats, one which has contributed to their role as a natural animal model for ADHD.

The lack of an apparent effect of caloric restriction on the open field behaviour of the Slow rats contributes to our interpretation of this effect. CR did not demonstrate a

universal suppression of activity, which itself stands to reason when one considers that CR studies with outbred rat strains have not found more pronounced reductions in open field activity, and have even demonstrated that CR ameliorates aging-related decreases in open field activity (Means et al., 1993). With known metabolic differences between the strains, particularly in lipid handling (Gilby et al., 2007[a]; unpublished data) which itself is influenced by CR(Weindruch et al., 2001), it seems more likely that CR shifted the Fast rats from a metabolic state that may have been directly or indirectly responsible for their lack of habituation in the open field paradigm.

Regardless, our findings lend support to the body of literature advocating metabolic treatments for ADHD, as well as our own research program's growing impression that energy utilization strategies play a significant role in the differences observed between these strains. With these results warranting further investigation, it would be interesting to assess whether CR confers a similar effect to the open field activity of Spontaneously Hypertensive Rats (SHR). The SHRs are another selectively bred natural animal model for ADHD that demonstrate behaviour (Sagvolden et al., 1992) and kindled seizure susceptibilities reminiscent of the Fast strain (Greenwood et al., 1989). Considering that neither the SHR nor our Fast rats were selectively bred for ADHD-like behaviours (Racine et al., 1999; McIntyre et al., 1999), comparative studies may isolate common metabolic characteristics that lead to the similar abnormal behavioral/seizure profiles in these strains.

Restraint

Impulsivity is one of the behavioural dimensions with which the DSM-IV-TR (American Psychiatric Association, 2000) operationally defines ADHD. In the restraint paradigm, Fast rats typically demonstrate a pattern of behaviour thought to represent

impulsivity (Gilby et al., 2007[b]; McIntyre & Gilby, 2007). The "impulsive" nature of Fast rats manifests as long bouts of struggling behavior compared to the Slow rats, which spend most of that time in a frozen posture (Anisman et al., 1997; Merali et al., 2001). Interestingly, while the absolute struggling percentages may vary within each strain across different studies, the proportional difference between Fast and Slow groups is highly consistent. That is to say, struggling behaviour of both strains can fluctuate in the same direction due to factors like age or humidity, but the relative difference is always maintained (unpublished data, Anisman et al., 1997; Merali et al., 2001; Gilby et al., 2007[b]). Not surprisingly then, this strain difference was readily apparent in our study. Less apparent, however, was an effect of caloric restriction. In this task, while a trend for reduced struggling was observed in the Fast calorically restricted group, neither the Fast nor Slow treatment groups demonstrated a significant difference in the time spent struggling compared to their respective *ad libitum* controls. Consequently, it appears as though these data do not support a significant effect of caloric restriction on this measure of relative impulsivity.

Caloric restriction did, however, affect a seemingly peripheral measure that is routinely taken during this task, namely the number of vocalizations produced by restrained animals. Despite consistently observing more frequent and vehement vocalizations in restrained Fast compared to Slow rats, previous research from our laboratory has not emphasized or published these findings, presumably because this measure has always been closely associated with the extent of struggling activity. Likewise, we supposed that any effect caloric restriction would have had on proportional struggling activity would also similarly affect the number of vocalizations. However, in this study, there was a significant reduction in vocalizations associated with caloric restriction that did not accompany any

demonstrable reduction in struggling behaviour. In considering the implications of this effect in isolation from struggling activity, some qualitative elaboration on the vocalization behaviour itself is warranted.

Vocalizations emitted during restraint trials do not overtly resemble those produced when an uneasy animal is handled or in pain. Rather, the vocalizations have been described as more aggressive and likely to reflect frustration. Interestingly, studies that have investigated play behaviors in Fast versus Slow rats have shown Fast rats to be much more aggressive which may be relevant to their increased frequency for vocalization in this task (Reinhart et al., 2006). While it is, of course, difficult to infer what a change in vocalization frequency suggests about the animal, particularly when their activity level is unchanged, it is conceivable that caloric restriction had its effect on the emotional state of the animal. The behaviour of Fast rats in this task demonstrates a high level of stress. Alongside the higher number of vocalizations, Fast rats demonstrate significantly more frequent incidences of porphyrin secretion from the nose (unpublished data) than Slow rats during the restraint trial, which itself is an indication of acute stress (Harkness & Ridgeway, 1980). Taken together, the vocalization patterns, porphyrin secretions, and increased struggling behaviours of Fast rats in this paradigm may imply that it is a particularly stressful test for the Fast strain, though caution should be taken with this interpretation, as the Slow rats may be expressing their stress level differently. Even so, the intense struggling and apparent frustration/stress level demonstrated by Fast rats is reminiscent of the negative responses children with ADHD display when restrained or made to sit still (McIntyre & Gilby, 2007). Perhaps, then, CR's effect on vocalization frequency is a consequence of stress amelioration.

Studies of CR's longevity and associated health benefits often propose a profound effect on stress response (Dubey et al., 1996; Keenan et al., 1999; Klebanov et al., 1995). Central to this theory are the findings that CR protects rodents of all ages from the damaging actions of acute stressors (reviewed in Masoro, 1998; Klebanov et al., 1995). More specifically, it has been found that caloric restriction protocols such as ours, where food availability is limited to the light cycle, increase morning corticosterone levels to a significant extent (Belda et al., 2005) which may chronically mobilize mechanisms that protect against more acute stressors (Klebanov et al., 1995). Thus, caloric restriction may have simply blunted their emotional or outward response to the struggle itself. However, due to the trend toward reduced struggling and the significantly reduced vocalization frequency that was observed in calorically restricted Fast rats compared to their controls, further study of a potentially positive effect of caloric restriction on the impulsive nature of Fast rats should be conducted and be expanded to include other established measures of their relative impulsivity, such as their propensity to mount non-estrous females (Michaud et al., 1999; McIntyre & Gilby, 2007).

Morris Water Maze

Results from this task primarily demonstrated the characteristic strain differences oft reported between the Fast and Slow rats, with no evidence to suggest that CR influenced strain-specific behaviours in the Morris Water Maze. As such, Fast rats demonstrated significantly longer latencies to the platform over days, inferior performances across trials on the first day and strikingly different search strategies. However, all four groups demonstrated similar latencies to a visible raised platform, indicating that any observed group differences in the hidden platform trials were not due to relative motor impairment.

The significantly longer latencies exhibited by Fast rats when compared to the Slow groups has been consistently observed in previous studies utilizing the Morris Water Maze (Anisman & McIntyre, 2002; Gilby et al., 2007[b]), as has their remarkably different search strategy. Even with repeated exposure to the task, Fast rats demonstrated more thigmotaxic behavior (swimming along the edge of the pool) and straight line searching. This pattern appeared inefficient since even in situations where the animal "remembered" the location of the platform (as indicated by a slight miss) that trial's latency would be increased by the time it took to reach the other end of the pool and try again. Thus, as suggested by qualitative observations, unsuccessful approaches to the platform would often result in Fast rats leaving the target quadrant altogether. Conversely, Slow rats tended to swim with frequent changes in direction. This strategy allowed for quick discovery of the platform upon a slight error in localization. Caloric restriction did not seem to influence strain-specific search strategies of the Fast or Slow rats.

This difference in search strategy may actually explain why the Fast rats demonstrated significantly longer average latencies to the platform than the Slow rats, even in the absence of a noticeable difference in their learning curves over days. In this study, Fast rats did not demonstrate a significantly different pattern of latency reduction over repeated days from that of Slow rats, suggesting that both strains were similarly able to improve their performance with experience. Moreover, when analysis was restricted to only the first trial of each testing day, there was again no significant difference in the pattern of performance improvement, suggesting that neither Slow group demonstrated a superior ability to utilize what was learned the day before from either Fast group. Assuming that the lack of a significant difference in improvement over time is evidence that both Fast and Slow

rats were equally capable of learning and remembering the platform's approximate location, the approach pattern each strain predominantly used to reach the platform may have put the Fast strain at a relative disadvantage for task completion. This disadvantage may be due to more time spent in thigmotaxis, the greater time cost of straight line platform misses, and the seemingly greater frequency of these misses when compared to Slow rats (Anisman & McIntyre, 2002), although this data was not quantified in the present study.

Importantly, however, our analysis of the latencies to platform across the four trials conducted on the first day only is suggestive of a relative working memory impairment in both groups of Fast rats relative to the Slows. While both Slow groups demonstrated significant improvement with repeated trials, calorically restricted and *ad libitum* fed Fast rats did not. The complete lack of improvement demonstrated by both Fast groups on the first day suggests that their relative impairment may not have been due to strategy alone. This phenomenon has been repeatedly observed (Anisman & McIntyre, 2002; Gilby et al., 2007[b]) and it is suggested that the inferior performance of Fast rats may be indicative of their greater distractibility (Anisman & McIntyre, 2002).

Water Consumption

Our finding that caloric restriction does not have a discernible effect on absolute water consumption, in tandem with our finding that calorically restricted animals consumed significantly more water per gram of body weight than *ad libitum* rats of the same strain, posed an interesting question. With both *ad libitum* groups steadily gaining weight over time, and both calorically restricted groups necessarily losing 20% of their body weight, some may argue that the effect is simply a mathematical byproduct of their bidirectional weight changes. This is true, and yet it suggests something particularly relevant. If a lack of

a treatment effect had been observed, it would imply that rats modulated their water intake as a function of their weight. This was not the case. Neither weight gain nor weight loss engendered a change in absolute water consumption. In fact, the only detectable influence on absolute water consumption seemed to be time, as all groups slightly reduced their consumption; this is consistent with the finding that rats decrease water consumption as they age (McGivern et al., 1996).

Importantly, however, by the conclusion of the study, many *ad libitum* controls outweighed calorically restricted rats of the same strain by hundreds of grams (data not presented). The fact that much smaller rats (CR groups) maintained similar water consumption to that of their larger control counterparts should be considered. Specifically, maintaining a similar level of absolute water intake, which to the smaller animal means a significantly elevated water supply relative to their body, was actually a response to caloric restriction. This effect was profound yet it is difficult to speculate why it occurred. Perhaps the relatively high consumption level was maintained because of the absence of food, in order to attenuate hunger in the calorically restricted group (Belda et al., 2005; McKiernan et al., 2008, Penn State, 1999). An alternate hypothesis however might be that there is some degree of genetic control over absolute water consumption as a metabolic requirement.

Nevertheless, one cannot exclude the possibility that water intake is a behaviour completely unrelated to caloric restriction. This lack of an apparent relationship between weight and water drinking in the strains is not unprecedented. Changes in body weight created in Fast rats as a result of vagal nerve stimulation relative to within-strain controls were not associated with any alteration in water consumption (Dedeurwaerdere et al.,2006). Regardless, in this study both strains reacted similarly to CR, as evidenced by our

proportional and absolute measures of water consumption, and intricate extrapolation is outside the scope of this study. Therefore, the significantly greater water consumption of Fast rats compared to Slows, for which polydipsia is implicated (Gilby & McIntyre, 2002; Gilby et al., 2007[a]) was maintained and seemed unaffected by CR.

Kindling

Pre-kindling local excitability (ADT and ADD)

It has been previously reported that the Fast and Slow strains do not differ significantly with respect to the threshold required to elicit their first afterdischarge (Racine et al, 1999; McIntyre et al., 1999). Consequently, our finding that calorically restricted Fast rats demonstrated significantly lower thresholds than all three groups implies a state of greater local amygdala excitability that is specific to that group. The lack of a discernable effect of caloric restriction on the prekindling ADTs of the Slow group serves as yet another indication that this treatment was more (or only) influential in the Fast rats. Indeed, this appears to be a recurring pattern spanning most of the paradigms we utilized.

At the outset of this study we did not expect that caloric restriction, a treatment found to proffer seizure resistance via threshold increases in several epilepsy models, would increase local excitability in the kindling model. However, the Fast restricted group's elevated local excitability was echoed by an increased duration of the initial after discharge. Once again, the Fast restricted rats were significantly different in this measure from all other groups. With significantly lower ADTs and longer ADDs than Fast controls and both Slow groups, it seems clear that caloric restriction distinctively increased local amygdala excitability in the Fast rats. Although increases in ADT in the kindling model are often taken to predict efficacy of anticonvulsant medication (Reismuller et al., 2000; Gilbert, 1988), the

reduction in ADT observed in this study does not necessarily imply an epileptogenic effect for caloric restriction, particularly considering the results from our other kindling measures as discussed below.

Epileptogenic capacity (kindling rate)

Our finding that the calorically restricted Fast group kindled at a significantly slower rate than *ad libitum* Fast rats was somewhat unprecedented. To date, no metabolic manipulations have demonstrated a retardation of kindling rate. Even the ketogenic diet, with its overwhelming clinical evidence of seizure control and suggested anti-epileptogenic properties (Stafstrom & Bough, 2008; Livingston & Berman, 1972; Freeman et al., 2000; Hartman & Vining, 2007; Bough et al., 2003), has never been found to affect kindling rate in outbred strains (Hori et al., 1997; Nylen et al., 2006).

Caloric restriction is actually the first manipulation that has significantly retarded kindling rates in our seizure-prone Fast strain. Kindling rates in calorically restricted Slow rats also tended to be higher than Slow controls but that effect was ultimately found to be non-significant, which may be due to the already high degree of seizure resistance resident to that strain. It would be premature to presume that the known metabolic differences between our Fast and Slow strains are responsible for the inferior treatment effect in Slow rats, but it is certainly a distinct possibility. For example, consistently lower concentrations of circulating plasma non-esterified fatty acids (NEFA) have been observed in Fast versus Slow rats (Gilby et al., 2007[a]; Gilby et al., in press) and both caloric restriction and the ketogenic diet are known to significantly increase circulating NEFA levels in rodents and humans (Sugden et al., 1999; Kua, 2006; Fraser et al., 2003; Gonzalez et al., 2004; Greene et al., 2003). If the therapeutic effect of these treatments is, indeed, borne from NEFA elevation it

would be far more valuable to the strain that is naturally deficient in NEFA (Fast) than the strain that already has uncharacteristically high circulating levels of NEFA (Gilby et al., 2007; unpublished data).

The reduced efficacy of caloric restriction observed in Slow compared to Fast rats may also offer some insight as to the reason why the ketogenic diet has not been found to significantly retard kindling. The majority of models used to test the ketogenic diet and caloric restriction involve imposing an epileptic state upon outbred 'normal' rat strains with unknown and potentially variable seizure susceptibilities. Interestingly, however, both caloric restriction and the ketogenic diet have been shown to delay the onset of stress-induced seizures in EL mice, demonstrating an anti-epileptogenic effect on a strain inherently susceptible to seizures (Greene et al., 2003; Greene et al., 2001; Mantis et al., 2004). Hence, while caloric restriction does possess anti-convulsant properties in outbred strains using chemical induction models (Bough et al., 1999; Harney et al., 2002; Eagles et al., 2003), it is possible that its anti-epileptogenic capability is reliably detectable in only those subjects with naturally high epileptogenic capacities, such as the EL mice and our Fast rats.

Measures associated with seizure severity in fully kindled animals

Latency to tonic/clonic state

It has generally been reported that Slow rats exhibit shorter latencies to the tonic/clonic state during fully kindled seizures than Fast rats (Racine et al., 1999; McIntyre et al., 1999). This strain difference in latency was upheld by our study but no effect of treatment was evident for within-strain comparisons leaving treatment groups from both strains statistically indistinguishable from their control groups on this measure. Still, the calorically restricted Fast group did not differ significantly from either Slow group on this

measure. Thus, the Fast *ad libitum* rats are unique in the fact that only their latencies are consistently higher than those from the two Slow groups. Clearly, these data do not evidence a profound caloric restriction-induced effect, since the Fast restricted group was also not statistically distinct from the Fast *ad libitum* group. However, this may suggest a trend towards more Slow-like latencies in calorically restricted Fast rats, which pushed them to an intermediary level indistinct from either of the control groups.

Duration of tonic/clonic convulsions

It seems intuitive that longer tonic/clonic convulsions are indicative of more severe seizures. By this measure, our seizure-prone Fast strain has repeatedly demonstrated more severe seizures in their fully kindled state than the Slow strain (Racine et al., 1999; McIntyre et al., 1999; McIntyre & Gilby, 2007). In this study, Fast rats again showed longer tonic/clonic activity during seizure than Slow rats regardless of treatment. Moreover, the often observed pattern of increasing tonic/clonic activity upon repeated seizures was present in all groups. Both of these phenomena have been repeatedly reported by previous studies (McIntyre et al., 1999; McIntyre & Gilby, 2007), and so we can only conclude that caloric restriction had no discernible effect on seizure severity in either strain.

This lack of an effect is not surprising, as caloric restriction has generally not been effective in reducing seizure duration (Raffo et al., 2008; Eagles et al., 2003; Bough et al., 2003) in other seizure models. In fact, the clinically efficacious ketogenic diet is also generally reported not to affect seizure severity once the seizure has started (Raffo et al., 2008; Hori et al., 1997; Bough et al., 2003; Thavendiranathan et al., 2000). Considering the retarded kindling rate observed in the restricted Fast rats, this may imply that caloric

restriction, like the ketogenic diet, affords protection against seizure onset and recruitment rather than seizure severity.

After discharge durations associated with fully generalized (stage 5) seizures

When comparing the kindling parameters between the seizure-resistant Slow strain and the seizure-prone Fast strain, several characteristics reflect their relative resistance. The much slower kindling rate implies a resistance to epileptogenic mechanisms and their significantly shorter tonic/clonic seizure durations signifies a resistance to the behavioural convulsion. This differential susceptibility also extends to internal expressions of seizure, namely the electrographically recorded seizure duration. In this case, Slow rats demonstrate significantly shorter afterdischarge durations following stimulation than Fast rats, even in a fully kindled state (Racine et al., 1999; McIntyre et al., 1999; McIntyre & Gilby, 2007). This difference was once again evident in this study, regardless of caloric restriction. Thus, both Fast groups demonstrated significantly longer ADDs than either Slow group, and this trend was observed in ADDs recorded from the stimulated amygdala and the recruited contralateral amygdala. Thus, once again caloric restriction did not impact the characteristic seizure profile of fully kindled Fast and Slow rats.

CONCLUSION

As mentioned above, Fast rats consistently demonstrate a predisposition towards seizure that is comorbid with ADHD-like behaviours, modeling two human afflictions that not only are comorbid themselves, but are also ameliorated by metabolic treatments known to alter lipid handling. Results from this study showed that caloric restriction, itself known to alter several metabolic processes, not only ameliorated abnormal hyperactivity and to some degree the impulsive nature of the Fast strain, but also retarded their predilection towards

rapid epileptogenesis in the kindling model. Thus, three of the more robust characteristics of our seizure prone Fast strain, each reminiscent of a different (but comorbid) affliction, were positively impacted by a treatment as simply performed as caloric restriction. Importantly, however, the metabolically distinct Slow rats seemed largely unaffected by caloric restriction in each of our behavioural measures and with respect to their typical kindling profiles. Given that the multifactorial effects of caloric restriction associated with longevity are profoundly evident in organisms that range in complexity from nematode to primate, it is highly unlikely that the Slow rats were completely unaffected by caloric restriction. Rather, the metabolic changes induced by caloric restriction may have corrected for a state present only in the Fast strain. In this way, our use of testing paradigms designed to highlight behavioral patterns associated with the seizure-prone state may have insufficiently reflected any of the benefits afforded to Slow rats by caloric restriction.

Kindling results from this study were, however, somewhat paradoxical in calorically restricted Fast rats given the apparent increase in local amygdala excitability which occurred in the face of an obvious reduction in epileptogenic capacity, as indexed by kindling rate. The fact that anti epileptic drug testing in the kindling paradigm attributes anti-convulsant efficacy to elevations in ADTs (Loscher & Fiedler., 2000), makes it tempting to conclude that such reductions in local thresholds as were observed in this study would be pro-convulsant. This, however, may be an oversimplification. For instance, the Fast strain is remarkably more seizure prone than the Slow strain, and this differential susceptibility spans several seizure induction models (McIntyre et al., 1999; Xu et al., 2004; Racine et al., 1999), yet the two strains generally do not naturally demonstrate significant differences in ADTs. Thus, the seizure resistance in the Slow strain is not proffered by relatively elevated

thresholds, nor is the abnormal sensitivity in the Fast strain resultant from relatively reduced thresholds in the kindling paradigm. Particularly relevant to our consideration of any proconvulsant implication is the significantly retarded kindling rate displayed by our calorically restricted Fast group. It is difficult to overlook the fact that these animals were selectively bred for amygdala kindling rate, and that it is in this measure they differ most significantly (Racine et al., 1999; McIntyre et al., 1999). Therefore, it is the kindling rate and ultimate seizure severity that appears to be inextricably linked to their differential seizure susceptibility, not their relative ADTs.

Ultimately, results from this study have provided reason to delve further into investigations of the possible benefits to be gained from metabolic manipulation in both ADHD and epilepsy. Jump right in, the water is fine...and if you see Steve McQueen, tell him that I want my watch back.

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