A high fat diet does not exacerbate CA1 injury and cognitive deficits following global ischemia in rats

Anastasia P. Arvanitis\textsuperscript{a}, Dale Corbett\textsuperscript{b}, Frederick Colbourne\textsuperscript{a,c,*}

\textsuperscript{a}Department of Psychology, University of Alberta, Edmonton, AB, Canada
\textsuperscript{b}BioMedical Sciences, Memorial University of Newfoundand, St. John’s, NL, Canada
\textsuperscript{c}Centre for Neuroscience, University of Alberta, Edmonton, AB, Canada

\textbf{ARTICLE INFO}

Article history: Accepted 18 November 2008 Available online 28 November 2008

Keywords: High fat western diet Hippocampus Morris water maze Global ischemia Stroke

\textbf{ABSTRACT}

A diet high in saturated fat and similar in composition to western diets (WD) has been shown to exacerbate injury following traumatic brain injury. Thus, we investigated the effects of a WD on cell death and functional outcome following global ischemia. First we assessed the effects of a 60-day WD regimen on temperature, activity and glucose levels in normal rats (Experiment 1). Second, we evaluated the influence of a 60-day WD regimen on hippocampal CA1 injury and learning and memory impairments following global ischemia in rats (Experiment 2). Male Sprague–Dawley rats, obtained at \textasciitilde{} 50 g, were randomly assigned to either the WD or the low-fat control diet (CD). Animals were fed for 30 days, then subjected to surgery (body temperature probe implantation in experiment 1; forebrain ischemia in experiment 2), and then they stayed on the same diet for another 30 days. Two and 4 weeks following surgery, learning and memory were assessed using the Morris Water Maze. At 60 days, rats were killed and viable hippocampal CA1 cells were quantified. Results from experiment 1 revealed no differences in glucose or temperature profiles between animals fed the WD and CD; however, WD animals were significantly less active than CD animals. Eight minutes of ischemia in experiment 2 induced severe hippocampal CA1 cell loss (\textasciitilde{} 90\%) and learning and memory impairments relative to non-ischemic controls. However, the WD did not exacerbate CA1 injury or behavioural deficits. These findings suggest that a 60-day WD regimen does not significantly influence recovery following global ischemia.

© 2008 Elsevier B.V. All rights reserved.

* Corresponding author. Department of Psychology, University of Alberta, P217 Biological Sciences Building, Edmonton, AB, Canada T6G 2E9. Fax: +1 780 492 1768. E-mail address: fcolbour@ualberta.ca (F. Colbourne).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved. doi:10.1016/j.brainres.2008.11.058
potentially harmful to the brain (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Morris et al., 2004; Winocur and Greenwood, 1993, 1999), via an indirect effect of a high-fat diet on cardiovascular dysfunction (e.g., atherosclerosis), and a direct effect of a high-fat diet on neural function (Molteni et al., 2002). Epidemiological stroke studies on diets high in saturated fat have been inconclusive. With increased saturated fat intake, some reported a lower risk of cerebral ischemia (Gillman et al., 1997; Reed, 1990). Similarly, several studies have found a lower mortality (McGee et al., 1985; Reed, 1988; Sauvaget et al., 2004), whereas others report no significant association between incidence and mortality from cerebral ischemia and consumption of saturated fat (He et al., 2003).

In addition to affecting stroke risk, dietary manipulations have the capacity to attenuate or aggravate injury. Thus, outcome following stroke may be improved by following particular dietary regimens and/or avoiding others. For instance, Yu and Mattson (1999) showed that caloric restriction attenuated injury and improved functional outcome after focal ischemia in rats. Bobyn et al. (2005) found that protein energy malnutrition impaired functional outcome in an open field test in gerbils as long as 10 days following global ISC. Wu et al. (2003) showed that a western diet (WD) further exacerbated injury and cognitive impairments following traumatic brain injury. Specifically, they found that relative to controls, rats fed a WD for 4 weeks had longer escape latencies in the Morris water maze (MWM) and significant reductions of brain-derived neurotrophic factor (BDNF) mRNA in the CA3 and dentate gyrus one week following traumatic brain injury. These findings are in line with additional animal research on WDs, which have been shown to compromise neuronal function by increasing levels of oxidative stress, reducing levels of BDNF, and consequently impairing spatial learning and memory (Molteni et al., 2002). Indeed, Greenwood and Winocur (1990) and Winocur and Greenwood (1993) showed that providing postweanling rats a WD for 3-months caused severe and widespread learning and memory impairments on numerous tests. Specifically, these authors found that rats fed the largest amount of saturated fatty acids performed the worst relative to rats fed a poly- or mono-unsaturated fatty acid diet (Greenwood and Winocur, 1996). Molteni et al. (2004) also showed that a 2-month WD impaired spatial learning performance in the MWM.

The effects of a WD in global cerebral ISC have yet to be established. Thus, in the present study we sought to delineate the impact of a WD in a well established 2-vessel occlusion (2-VO) model of global ISC (Smith et al., 1984). This study consisted of 2 experiments. In both experiments rats were placed either on a WD or a low-fat control diet (CD). Since outcome following ISC can be influenced by changes in temperature (Colbourne et al., 1997), glucose (Li and Siesjö, 1997) and activity levels (Gerhardt and Boast, 1988; Weber et al., 1989), it was imperative to establish these profiles on non-ischemic (NO-ISC) animals fed the WD and CD. Thus, experiment 1 evaluated the influence of each diet on temperature, activity, and glucose levels, spatial learning and memory, and hippocampal CA1 cells. Experiment 2 assessed the impact of a WD on the severity of hippocampal CA1 damage and spatial learning and memory following 8 min of forebrain ISC. We predicted that a WD alone would have no significant effects on temperature, activity, and glucose levels. However, based upon previous literature (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Winocur and Greenwood, 1993), we predicted that a WD would impair spatial learning and memory. In experiment 2, based upon findings from Wu et al. (2003), we predicted that a WD would affect residual CA1 neurons along with other hippocampal regions (e.g., CA3), and thus impair spatial learning and memory even further.

2. Results

2.1. Experiment 1

2.1.1. Protocol violations and mortality

No rats were excluded from this experiment. The group sizes were as follows: WD (n=8) and CD (n=8) group.

2.1.2. Body weight measurements

Rat body weight (Fig. 1A) was analyzed using a 2-factor ANOVA (Diet; Time: arrival, days 7, 14, 21, 28, surgery, 35, 42, 56 and euthanasia). There was no significant Diet×Time interaction. Both NO-ISC and ISC animals gained a significant amount of weight over time. In the ISC group only, animals fed the WD were significantly heavier than animals fed the CD at euthanasia (*p<0.05).

Fig. 1 – Body weight (mean±SEM) for NO-ISC (A) and ISC (B) animals at several times throughout the study (“Surg” = surgery). Both NO-ISC and ISC animals gained a significant amount of weight over time. In the ISC group only, animals fed the WD were significantly heavier than animals fed the CD at euthanasia (*p<0.05).
There was a significant Time effect \((p<0.001)\) as animals gained weight as they aged.

### 2.1.3. Glucose values

Three weekly venous blood samples were collected from rats to measure whether there were any differences in blood glucose levels between animals consuming the WD and CD. There was no significant main effect of diet at any time \((\text{data not shown})\) with the average values being very similar at 9.89±0.24 and 9.14±0.24 in the WD and CD groups, respectively \((t\text{-test}: p=0.058)\).

### 2.1.4. Temperature and activity

Temperature \((\text{Fig. 2A})\) and activity \((\text{Fig. 2B})\) were analyzed using an independent student’s \(t\)-test, which revealed that the overall 30-day temperature profile was not significantly different \((p=0.726)\) between animals fed the WD \(37.4 \pm 0.1°C\) versus the CD \(37.3 \pm 0.1°C\). However, activity levels were significantly different \((p<0.001)\) between the WD \(2.0 \pm 0.1\) and CD \(3.2 \pm 0.2\). A Pearson’s correlation analysis revealed that temperature and activity were significantly correlated in both the WD \((r=0.840, p<0.001)\) as well as the CD \((r=0.707, p<0.01)\).

Thus, as expected, it is likely that activity changed core temperature, as the more active animals were, the warmer they became.

#### 2.1.5. Morris Water Maze

A repeated measures ANOVA was used to analyze the swim latency data. Collapsing across trials, learning acquisition \((\text{Figs. 3A and B})\) was analyzed using a 3 factor ANOVA \((\text{Diet; Week; 2 and 4 weeks post surgery; and Day: 4 days of testing})\). There was a significant Week×Day interaction \((p<0.001)\), but no significant interaction effect involving Diet \((p≥0.092)\), and no main effect of Diet \((p=0.368)\). Memory retention \((\text{Fig. 3C})\), assessed via a probe trial, was analyzed using a 2 factor ANOVA \((\text{Diet, Week})\). There was no significant Week×Diet interaction \((p=0.468)\), or a main effect of Week \((p=0.492)\) or Diet \((p=0.561)\). Animals fed the WD and CD spent an equal amount of time swimming in the target quadrant, which did not vary considerably between weeks. Collapsing across trials, the visible platform \((\text{Fig. 3D})\) was analyzed using a 2 factor ANOVA \((\text{Diet, Week})\), which revealed no significant Week×-Diet interaction \((p=0.647)\), and no main effect of Week \((p=0.146)\) or Diet \((p=0.103)\). Thus, as per the visible platform, the WD and CD did not significantly influence motivation and swim speed.

### 2.1.6. Histopathology

No hippocampal CA1 pathology was observed in non-ischemic control rats given either CD or a WD \((\text{Fig. 4A})\), and cell counts were not significantly different between the two diet groups \((p=0.762)\).

#### 2.2. Experiment 2

##### 2.2.1. Protocol violations and mortality

Five rats were excluded owing to surgical problems with isolating and catheterizing the tail artery. The remaining group sizes were as follows: WD \((n=11)\) and CD \((n=8)\).

##### 2.2.2. Body weight measurements

Rat body weight \((\text{Fig. 1B})\) was analyzed using a 2-factor ANOVA \((\text{Diet factor; Time factor: arrival, days 7, 14, 21, 28, surgery, 35, 42, 56 and euthanasia})\). There was no overall main effect of Diet \((p=0.140)\); however, there was a significant Time effect \((p<0.001)\) and Diet×Time interaction \((p<0.001)\). Owing to the significant interaction, the data were analyzed for each time point to determine when diet affected body weight. Significant differences occurred at day 7 \((p=0.019)\), day 14 \((p=0.019)\) and euthanasia \((p=0.016)\). At euthanasia, animals fed the WD weighed ~60 g more than animals fed the CD.

##### 2.2.3. Physiology

Physiological variables during surgery are given in Table 1. Blood pH, pCO\(_2\), P\(_{O_2}\), concentration of total hemoglobin (ctHb), glucose (ctGlu), skull temperature \((T_s)\) and mean arterial blood pressures during ISC \((\text{MABP})\) were the same for animals maintained on the WD and CD \((p≥0.358)\).

##### 2.2.4. Morris Water Maze

A repeated measures ANOVA was used to analyze the swim latency data. Collapsing across trials, learning acquisition
Author's personal copy

Fig. 3 – Spatial learning and memory for NO-ISC and ISC animals. Learning acquisition (A, B; s, mean ± SEM), memory retention (C; %, mean ± SEM), and visible platform latencies (D; s, mean ± SEM) at 2 and 4 weeks post surgery were not influenced by diet in either the NO-ISC or ISC animals. However, 8 min of ISC induced spatial learning deficits at day 13 and 14 post-stroke as compared to NO-ISC animals at the same time point. Forebrain ISC also significantly decreased the amount of time animals spent swimming in the target quadrant as compared to NO-ISC animals. However, visible platform latencies were not different between ISC and NO-ISC animals. See Results for statistics.

(Figs. 3A and B) was analyzed using a 3 factor ANOVA (Diet, Week and Day). There was a significant Week×Day interaction (p < 0.001), but no influence of Diet (p ≥ 0.545) and no main effect of Diet (p = 0.177). Memory retention (Fig. 3C), assessed via a probe trial, was analyzed using a 2 factor ANOVA (Diet, Week). There was no significant Week×Diet interaction (p = 0.140), or a main effect of Diet (p = 0.864). However, there was a significant main effect of Week (p = 0.043). Animals

Fig. 4 – Average (±SEM) CA1 sector cell counts (A) in NO-ISC (B) and in rats subjected to 8 min of ISC (C) at a 30 day survival time following the insult. Ischemia caused massive CA1 sector injury that was not significantly affected by diet. Scale bar = 50 μm. The medial CA1 sector is shown at −3.60 mm to Bregma.
spent more time swimming in the target quadrant on day 16 post-surgery than on day 29 post-surgery. Collapsing across trials, the visible platform (Fig. 3D) was analyzed using a 2 factor ANOVA (Diet, Week), which revealed no significant Week×Diet interaction (p = 0.113), and no main effect of Week (p = 0.274) or Diet (p = 0.121). Thus, as per the visible platform, the WD and CD did not significantly influence motivation and swim speed.

We were also interested in examining any differences in latency scores between ISC and NO-ISC animals. Collapsing across trials, learning acquisition was analyzed using a 3 factor ANOVA (Diet, Week, Day). There was a significant Week×Day×Group interaction (p < 0.001). Accordingly, the data were analyzed separately for each time point to determine when differences between the two groups existed. On day 13 (p < 0.001) and 14 (p = 0.035) post-surgery, NO-ISC animals were able to locate the platform much faster than ISC animals. By day 15 this effect was no longer present (p = 0.845), and did not reappear thereafter. The probe trial, analyzed using a 2 factor ANOVA (Group; Week), revealed a significant Group main effect (p < 0.001) with a non-significant Week main effect (p = 0.553) and a non-significant interaction (p = 0.089). NO-ISC animals spent more time swimming in the target quadrant than ISC animals, indicating that the NO-ISC animals retained more information regarding the location of the hidden platform obtained over the 4 days of learning acquisition training. A 2 factor ANOVA (Group, Week) for the visible platform training data revealed no main effect of Week (p = 0.388) or Group (p = 0.484), nor a significant Week×Group interaction (p = 0.651). Swim speed and motivation did not differ significantly between NO-ISC and ISC animals for the duration of testing.

### 2.2.5. Histopathology

Eight minutes of ISC induced severe CA1 sector necrosis (~92%; p < 0.001 for ISC vs. NO-ISC animals), which was not different between rats fed the WD and CD (Fig. 4A; p = 0.363).

### 3. Discussion

This is the first study to assess whether a WD, in the absence of other risk factors associated with cardiovascular dysfunction (e.g., atherosclerosis, hyperglycemia and altered BP), influences functional recovery and hippocampal CA1 injury following global ISC. We showed that a WD did not potentiate the deleterious effects of an 8 minute insult, which killed approximately 92% of hippocampal CA1 neurons resulting in transient spatial learning deficits (e.g., up to 14 days post-stroke), and long-lasting memory impairments (e.g., up to 30 days post-stroke) in rats. On days 13 and 14 post-stroke, ISC animals took significantly longer to locate the hidden platform, which assesses spatial learning; by day 15 post-stroke, ISC animals’ latency scores did not differ from NO-ISC animals. This indicates that ISC animals successfully learned the location of the hidden platform. We assessed memory retention in a probe trial, which involved removing the hidden platform from the pool and measuring the amount of time the animals spent swimming in the target quadrant. In the present study, the probe trial revealed that ISC animals spent significantly less time swimming in the target quadrant up to 30 days post-stroke. Therefore, ISC resulted in long-lasting spatial memory deficits. A WD did not exacerbate spatial learning and memory impairments or cell loss as compared to animals maintained on the CD. These findings suggest that a WD, initiated 30 days prior to ISC, and provided for a total of 60 days, does not aggravate histological and functional outcome following global ISC.

Our findings are different from those of Wu et al. (2003), who showed that a high-fat and sucrose diet aggravated injury and spatial learning following traumatic brain injury. Upon closer examination, the disparity between the WD used in the present study and the high-fat and sugar diet used in a study of Wu et al. (2003) may have contributed to the different outcome. In the present study, the approximate energy from fat and sucrose was 40% and 7%, respectively; in the study of Wu et al. (2003), the approximate energy from fat and sucrose was 39% and 40%, respectively. A diet with high amounts of refined sugar may have induced hyperglycemia, and research over the past two decades has already established that preischemic hyperglycemia aggravates damage following ISC by enhancing intra- and extracellular acidosis (Myers, 1979; Siesjö, 1981, 1984), edema, and post-ischemic seizures (Siesjö, 1988, 1985). Our goal was to establish the effects of a high saturated fat diet in global ISC whilst controlling for factors such as hyperglycemia, which is already known to exacerbate injury. Indeed, Wu et al. (2003) did not measure peri-insult glucose levels, which may have been a factor in their study. In addition, the different models of brain injury, and their varying pathophysiology, must also be taken into consideration.

We also found that a sustained 60-day WD regimen alone, beginning post-weanling, did not notably influence the number of hippocampal CA1 sector neurons or spatial learning and memory. These findings do not mirror previous work (Greenwood and Winocur, 1990, 1996; Molteni et al., 2002; Winocur and Greenwood, 1993), which showed that a high fat diet alone induced severe learning and memory impairments. However, animals in Greenwood and Winocur’s (1990, 1996) and Winocur and Greenwood’s (1993)
experiments were fed for approximately 4.5 months before any cognitive testing was initiated, whereas in our study animals were only fed for 1.5 months at the time of behavioural testing. The aforementioned authors used post weaning rats and a diet high in saturated fat (e.g., 40% energy from fat), and found that rats fed a high fat diet performed poorly on the radial arm maze (Greenwood and Winocur, 1990), a standard hippocampus-sensitive test of spatial memory. The present study also used post-weaning rats, along with using a test reliant primarily on hippocampal function (e.g., MWM), and provided a diet similar in fat composition. Thus, it is possible that the duration of feeding in our study was too short to have an appreciable effect on neuronal plasticity and cognitive function. A pilot study with longer feeding durations (e.g., 3 months prior to ISC) was aborted due to difficulties with doing the 2-VO on very obese rats (e.g., 1 kg; Arvanitidis and Colbourne, unpublished data). However, Molteni et al. (2002) found significant impairments in spatial learning and memory retention in the MWM task as early as 1 month following the consumption of a diet high in saturated fat and refined sugar, a deficit that was even more pronounced at 2 months. In addition, they found that a high-fat, high sucrose diet significantly reduced levels of hippocampal BDNF mRNA and protein, which correlated significantly with longer escape latencies and greater cognitive impairments. Although we did not measure hippocampal BDNF mRNA or protein levels, the present study did not detect spatial learning and memory impairments in the animals fed a WD as compared to animals maintained on a CD at 1.5 months into the diet regimen. Again, this may be due to nutritional differences in the diets, particularly the large amount of refined sugar in the study of Molteni et al. (2002), which was not the main nutritional constituent of the diet provided in the present study.

It is also possible that the MWM protocol used in the present study was unable to detect small treatment effects. For instance, ISC animals in experiment 2 located the hidden platform as quickly as NO-ISC animals as early as 3 days into testing (day 15 post-surgery). A moving platform paradigm, incorporating more trials per day (e.g., 10 trials per day), may have been a better measure to detect subtle, but very real differences in mean latency scores between the animals on the WD versus the CD. As suggested by Winocur and Greenwood (1999), the Olton’s radial arm maze, the Hebb–Williams complex maze series, and a variable-interval delayed alternation test are all sensitive to the deleterious effects of high-fat diets. Thus, future work should consider incorporating one or more of these tests as they have been shown on numerous occasions (Greenwood and Winocur, 1990, 1996; Winocur and Greenwood, 1993) to be sensitive not only to high-fat diets, but also sensitive to differences between types of fats (e.g., polyunsaturated versus saturated fats).

In summary, a WD provided for 60 days failed to notably influence both histological and cognitive outcome in the 2-VO model of global ISC. While nutrition has been shown to interact with different forms of brain injury to exacerbate injury in animals (global ischemia and protein energy malnutrition by Bobyn et al. (2005); traumatic brain injury and high fat diet by Wu et al. (2003)), and humans (e.g., Alzheimer’s disease and high fat diets by Kalmijn et al. (1997) and Morris et al. (2003)), we showed that a WD did not enhance brain damage and cognitive impairments following global ISC. Even with the majority of CA1 cells killed, one would still expect the WD to have deleterious physiological effects on the remaining CA1 neurons and on other hippocampal regions and thus aggravate learning and memory. Indeed, CA1 sector injury following ISC is less severe at more posterior levels of the hippocampus, and therefore injury should have been assessed there. However, we know that the MWM is a task of spatial learning and memory, which relies primarily on the hippocampus. Since we did not observe any spatial learning and memory impairments between the WD and CD in either experiment, this lends support to the notion that a 60-day WD did not have deleterious effects on neural plasticity and cognition. Nevertheless, before any definitive conclusions can be made regarding the impact of a WD in stroke, several factors must be delineated. First, an effective model of global ISC for obese rats must be established so that longer feeding protocols (e.g., 4 months and greater) can be evaluated. Note that potentially-important physiological confounds, including glucose and temperature, must be considered in such studies as the duration of the diet and interactions with ischemia might lead to important differences from that found in our first experiment. Second, the effects of a WD must also be assessed in other types of strokes since the pathophysiology varies substantially between them (e.g., global ISC, focal ISC, hemorrhagic stroke). Lastly, tasks that have been previously shown to be sensitive to the negative effects of a high-fat diet should be used.

4. Experimental procedures

4.1. Animals

Thirty-five male Sprague–Dawley rats (Biosciences Breeding Colony, Edmonton, Alberta, Canada), weighing 45–55 g at the time of arrival, were used in this study. Animals were housed in groups of 4 to 5 until surgery and maintained on a reverse 12-h light/dark cycle (lights on at 10:00 PM) with free access to water. All procedures followed the Canadian Council for Animal Care guidelines and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta.

4.2. Diet

Immediately upon arrival, animals in all experiments were placed on a WD (STJN. Western diet for rodents, TestDiet, Richmond, IN) or CD (STJS, low fat control for WD, TestDiet, Richmond, IN). The two diets were isocaloric, but differed slightly in energy density. Percent energy (kcal/g) from protein was similar between the WD and CD (16%). The CD contained 72% energy from carbohydrate and 12% energy from fat. Conversely, the WD contained 44% energy from carbohydrate and 40% energy from fat. In addition, energy density of the WD was 4.49 (kcal/g) and 3.84 (kcal/g) for the CD. However, the amount of vitamins, minerals, fiber and cholesterol per kilocalorie remained equal between the two.
diets. Animals were fed their respective diets ad libitum throughout the entire experiment, unless otherwise stated. Food intake, however, was not measured throughout the study.

4.3. Body weight measurements

Body weight (g) was measured everyday for 7 consecutive days after arrival, and then on a weekly basis until euthanasia. Body weight was also measured at the time of surgery (e.g., core-probe implantation or ISC) and everyday thereafter until animals returned to their pre-surgical weight.

4.4. Experiment 1

We examined the effects of a 60-day WD regimen on core temperature, activity, glucose levels and cognition. The NO-ISC animals were fed their respective diets for 30 days, at which time a core telemetry probe was implanted.

4.4.1. Core temperature telemetry probe implantation

Aseptic surgical techniques were used throughout (e.g., autoclaved or hot-bead sterilized instruments, autoclaved surgical drapes). Animals had sterilized core telemetry probes (model TA1OTA-F40; Data Sciences, St. Paul, Minn.) implanted into the peritoneal cavity. Briefly, rats were anesthetized (∼20 min) with isoflurane (1.5%–2% maintenance in 30% O₂ and 70% N₂O) and a 2 cm incision was made in the abdomen into which the sterilized probe was implanted. The muscle was sutured closed and then infiltrated with Marcaine (Sanofi Canada, Markham, ON, Canada). The skin was then closed and treated with a topical antibiotic. These probes remained in situ until euthanasia. For 30 consecutive days after core probe implantation, temperature and activity were sampled every 5 min by an automated system (DQ3 System, Data Sciences) previously described (Colbourne et al. 1993). The 24-hour periods for temperature and activity were averaged and analyzed.

4.4.2. Blood glucose

Following core-probe surgery, animals’ blood glucose levels were measured each week for 3 weeks. Animals were briefly anesthetized (∼1–3 min) with isoflurane (1.5%–2% maintenance in 30% O₂ and 70% N₂O) and venous blood samples (approximately 100 μL) were collected in heparinized capillary tubes to measure blood glucose levels via a Radiometer ABL810 blood gas analyzer (Radiometer, Copenhagen, Denmark).

4.4.3. Morris Water Maze (MWM)

At 2 and 4-weeks after surgery, rats were tested in a modified version of the MWM task (Morris et al., 1982). Animals were placed snout towards the black circular pool (∼140 cm diameter) containing water (∼27±1 °C; Brown and Whishaw, 2000). The submerged black platform (∼1.5 cm below the surface of the water; ∼14 cm in diameter) was maintained in the same location throughout the hidden platform training allowing for the assessment of learning over four days. Following the last trial on the last day, a 60 s probe trial was conducted whereby the platform was removed from the pool and the amount of time spent swimming in each quadrant was measured allowing for the assessment of memory retention. Animals that learn the task will spend most of the 60 s trial searching for the platform in the target quadrant. After completion of the hidden platform training and probe trial, we administered one day of visible platform training to assess motivation and swim speed. Rats received 4 trials a day and were released from a different quadrant around the perimeter of the pool in a random sequence. The maximum trial length for the hidden and visible platform trials was 90 s, with an inter-trial time of 1 min. If the rat failed to locate the platform within the given time, the rat was removed from the water and placed on the platform for 10 s before returning to the holding cage. Animals that successfully found the platform also remained on the platform for 10 s before being returning to the holding cage. Latency to locate the platform was measured.

4.5. Experiment 2

We examined the effect of a 60-day WD diet regimen on the severity of hippocampal CA1 injury and functional outcome following ISC in 19 rats. These animals were fed their respective diets for 30 days at which time ISC was induced using a modified 2-Vessel Occlusion (2-VO) model developed by Smith et al. (1984).

4.5.1. Forebrain Ischemia (ISC)

Animals were subjected to food deprivation (∼12 h) prior to surgery in order to lower glucose levels into a consistent range (4–8 mmol/L). Rats were anesthetized (∼45 min) with isoflurane (1.5%–2% maintenance in 30% O₂ and 70% N₂O) and placed on a heated water blanket (model TP3E, Gaymar, NY) with feedback control from a thermocouple probe (HYPO-33-1-T-G-60-SMG-M, Omega, Stanford, CT) placed subcutaneously on the skull (centre). A model CSC-32 (Omega) feedback regulator maintained skull temperature (Tₜ) near 37.5 °C via an infrared lamp (175 W) to minimize an unwanted drop in brain temperature during ISC, which would have lessened injury. A 2-cm incision was made along the ventral midline of the neck and the common carotid arteries were isolated. Mean arterial blood pressure (MABP) was measured via a tail artery cannula kept patent by heparinized saline. Ischemia was achieved by transient bilateral carotid artery occlusion and systemic hypotension (35–45 mmHg; BP-1, World Precision Instruments, Sarasota, FL). Systemic hypotension was produced by withdrawing blood from the jugular vein into a heparinized syringe. Following an eight minute 2-VO, clamps were removed and blood was slowly re-infused. Arterial blood samples (approximately 100 μL each) were taken to measure blood pH, pCO₂, pO₂, hemoglobin (ctHb), and glucose levels before and after ISC.

4.5.2. Morris Water Maze

Behavioural testing was identical to that described in experiment 1.

4.6. Histopathology

Rats were euthanized 60 days after the initiation of feeding using an overdose of sodium pentobarbital (100 mg/kg i.p.) and...
transcardially perfused with 0.9% saline followed by 10% neutral-buffered formalin. Extracted brains were embedded in paraffin. Subsequently, 6-μm coronal sections were cut and stained with hematoxylin and eosin. One section at ~3.60 mm to Bregma (Paxinos and Watson, 1998) was chosen in which CA1 sector injury was determined. Injury at this level has been shown to correlate highly with injury at more anterior and posterior injury (Colbourne and Corbett, 1995). Viable (healthy-looking, non-eosinophilic) CA1 sector pyramidal neurons were counted in medial (next to the subiculum), middle, and lateral regions (next to the CA2 zone) of the CA1 zone of both hemispheres. The number of CA1 sector neurons was summed over the left and right hemispheres and expressed as a percent of normal (e.g., non-ischemic animals in experiment 1). The researcher was blinded to the group identity.

4.7. Statistics

Temperature, activity, and glucose levels (experiment 1 only) were analyzed using a Student’s t-test. Correlations between temperature and activity were achieved by using a Pearson’s correlation. Physiology (e.g., glucose levels, MABP), histology, body weight, and MWM data were analyzed using ANOVA (SPSS, v. 15.0. Chicago, Ill.). A p-value of 0.05 was considered statistically significant. All data are reported as mean±SEM.

Acknowledgments

Research supported by a grant from the Canadian Stroke Network (CSN) to FC who is an Alberta Heritage Foundation for Medical Research (AHFMR) Senior Medical Scholar. APA is supported by the department of Psychology at the University of Alberta. The authors gratefully acknowledge M. Pennner and L. Tong for their technical assistance.

REFERENCES


gerbils from forebrain ischemia. In: Hartmann, A.,
Kuschinsky, W. (Eds.), Cerebral Ischemia and Calcium.
conditional discrimination learning in rats. Psychobiology 21,
286–292.
Winocur, G., Greenwood, C.E., 1999. The effects of high fat diets
and environmental influences on cognitive performance in
diet aggregates the outcome of traumatic brain injury on
hippocampal plasticity and cognitive function by reducing
brain-derived neurotrophic factor. Neuroscience 119,
365–375.
administration reduced focal ischemic brain damage and
improve behavioural outcome: evidence for a preconditioning
Zola-Morgan, S., Squire, L.R., Amaral, D.C., 1986. Human amnesia
and the medial temporal region: enduring memory impairment
following a bilateral lesion limited to field CA1 of the
hippocampus. J. Neurosci. 6, 2950–2967.