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Gender-based changes in cognition and emotionality in a new rat model of epilepsy

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Summary. Epilepsy research relies heavily on animal models that mimic some, or all, of the clinical symptoms observed. We have previously described a new developmental rat model of epilepsy that demonstrates both behavioural seizures and changes in hippocampal morphology. In the current study we investigated whether these rats also show changes in cognitive performance as measured using the Morris water maze task, and emotionality as measured using the Elevated plus maze task. In the water maze, significant differences between male and female rats were found in several performance variables regardless of treatment. In addition, female but not male rats, treated neonatally with domoic acid had significant impairments in learning new platform locations in the water maze. In the elevated plus maze, a significant proportion of female rats spent more time in the open arm of the maze following prior exposure to the maze whereas this effect was not seen in male rats. We conclude that perinatal treatment with low doses of domoic acid results in significant gender-based changes in cognition and emotionality in adult rats.

Keywords: Domoic acid – Kainate – Brain development – Morris water maze – Elevated plus maze – Epilepsy

1. Introduction

Epilepsy is a family of debilitating neurological disorders that afflicts over 50 million people worldwide (Stables et al., 2002). The disease often begins in childhood and persists throughout the lifetime of the patient (Cole et al., 2002). Although a number of anti-convulsive therapies have been developed for the control of epilepsy, seizures remain uncontrolled in approximately one-third of patients with epilepsy (Schmidt and Rogawski, 2002) and treatment failures are common. Physicians, scientists and research-based organizations interested in the control and prevention of epilepsy have repeatedly stressed the need for new, clinically correlated animal models with which to study the aetiology of these disorders and to evaluate potential new therapeutants and therapeutic strategies (Stables et al., 2002).

To date, rodent models of epilepsy are restricted to either (a) chemically or electrically-induced acute status epilepticus, (b) spontaneously recurring seizures following chemical or electrical kindling, or (c) genetic models, such as inbred rodent strains that manifest a reduced seizure threshold but are also characterized by chronic systemic disease that limits their utility. Chemically or electrically induced acute neurotoxic insults, such as injection of kainic acid (KA) or pentylenetetrazol in mature rats, produces motor seizures, changes in cortical EEGs, and some neuroanatomical features that approximate human temporal lobe epilepsy (TLE) (Sarkisinian et al., 1997; Loscher, 2002), although permanent dysfunction is seen only subsequent to severe seizures (status epilepticus). Human epilepsy, however, is by definition a chronic disease, or at least a disease that manifests itself over a period of time, not simply at an instant in response to an acute insult (Cole et al., 2002). Epilepsy may begin at different moments in life and typically has both a developmental origin and a progressive history where seizures promote further seizures (Gowers, 1881) regardless of seizure type.

While existing animal models do not fully model the human condition, there is extensive evidence that many seizure disorders in experimental animals involve abnormal glutamatergic activity within the CNS and/or abherrant electrical connectivity within the limbic system. Injection of agonists for both NMDA and AMPA/kainate

subclasses of ionotropic glutamate receptors reliably induces motor convulsions in rats (for review see Loscher, 1998) and glutamate antagonists have been shown to prevent or reduce electrical seizures and motor convulsions (Loscher, 1998; Loscher et al., 1999). The neurochemical composition of glutamatergic systems within the brain is both dynamic and tightly regulated in late embryonic and early postnatal development in the rat (reviewed by McDonald and Johnston, 1990). Expression patterns for both NMDA and AMPA/kainate receptors fluctuate widely between embryonic day (E) 15 and postnatal day (PND) 18 and are subunit specific (Ritter et al., 2002). Moreover, the subunit composition of AMPA/kainate receptors is also subject to post-translational modifications and alternate splice variant expression during the period of PND 7–14 in the rat (reviewed by Ozawa et al., 1998) and changes in the relative expression of different AMPA receptor subunits have been shown to contribute to a reduction in glutamate-mediated Ca^{2+} permeability as a function of age up to PND 14 (Durand and Zukin, 1993). Thus, it is reasonable to speculate that subtle changes in glutamatergic tone, particularly changes in AMPA/kainate receptor activation during the second postnatal week, might result in changes of receptor expression that have immediate and long-term developmental consequences (Raol et al., 2001; Haberny et al., 2002).

In a series of recent papers we have reported that exposure of newborn rats to sub-convulsive doses of the AMPA/kainate receptor agonists domoic acid (DOM) and KA result in both immediate and permanent changes in brain development that correspond to many features of temporal lobe epilepsy. Rats treated in this paradigm demonstrate changes in neonatal learning as measured using odour conditioning in a standard conditioned place preference discrimination task (Doucette et al., 2003; Tasker et al., 2005). Moreover, we have reported (Doucette et al., 2004; Bernard et al., 2005) that when these rats are tested as both mature (PND 120) and aged (PND 510) adults in tests that are both novel and require spatial processing (Morris water maze, Novel water maze, Open field) the animals manifest a unique behavioural syndrome that is strikingly reminiscent of a stage 2 seizure (Racine, 1972). Post-mortem examination of the hippocampi from these rats reveals many of the ''hall-mark'' signs of animal epilepsy, including robust mossy fibre sprouting in the dentate gyrus and areas CA3 and CA1 and a significant loss of cells in the dentate hilus (Doucette et al., 2004).

The current study was designed to further explore this new model of epilepsy, by determining if treated rats also demonstrate subtle changes in cognition and/or emotionality, both of which are characteristic of human temporal lobe epilepsy (Stafstrom, 2006).

2. Materials and methods

2.1 Experimental animals, induction protocol and experimental design

Within 24 h of birth, offspring of untimed-pregnant Sprague Dawley rats (Charles River Laboratories, St. Constant, PQ) were culled to 10 pups/litter (5 males and 5 females). From PND 8–14, pups were weighed and given a single daily injection (s.c.; 10 ml/kg) of either saline, 5 μg/kg or 20 μg/kg domoic acid (DOM) (Diagnostic Chemicals Ltd., Charlottetown PEI) dissolved in saline. These doses of DOM have been previously shown to be well below those normally required to induce toxicity in animals of this age (Doucette et al., 2000, 2003). Pups were assessed daily until weaning (on PND 22) for overt signs of toxicity, and all pre- and post-weaning testing was conducted with the experimenter blind to treatment.

Following treatment, rats were housed with food and water available ad libitum and left undisturbed except for normal cage cleaning until behavioural testing at approximately 120 days of age (see below). Lighting in the colony room was maintained on a 14/10 h light/dark cycle (on at 06:00 h and off at 20:00 h) and all testing was conducted between 10:00 h and 16:00 h.

Spatial learning and memory, emotionality and arousal were tested in groups of male and female rats ($n = 6$–8 per group) over 9 days of daily testing beginning on PND 120. On Day 1, rats were tested using the elevated plus maze. From Day 2–8 testing continued in the Morris water maze, and on Day 9 rats were again tested in the elevated plus maze.

2.2 Morris water maze

Rats were tested in a Morris water maze (MWM) consisting of a circular tank measuring 1.5 m in diameter. Water temperature was maintained at approximately 21 °C and was made opaque by a layer of polypropylene pellets floated on the surface. Four points around the edge of the pool were arbitrarily designated as north (N), south (S), east (E), and west (W), allowing the apparatus to be divided into 4 corresponding quadrants (NE, SE, NW, SW). A clear plexiglass escape platform was submerged approximately 2 cm below the water surface and placed, initially, in the NE quadrant of the maze (alternate locations for the reversal task were implemented on Days 4 and 6). Extra-maze cues consisted of laboratory furniture and lights (held constant throughout the experiment). A video camera was mounted above the center of the pool and all performance was recorded for subsequent analyses. Rats were given 8 trials/day (administered as two consecutive blocks of 4 trials, with start locations pseudo-randomly selected without replacement within each block of 4 trials) for each of 6 test days (60 sec trial, 60 sec inter-trial interval during which time the rat remained on the escape platform). If the rat did not find the escape platform within the allotted time it was guided to the finish by the experimenter. A 60 sec probe trial was administered 24 h following the last test day. The time to reach the escape platform (escape latency) and inter-trial behaviour were recorded by observers blind to experimental treatment.

2.3 Elevated plus maze

Emotionality/anxiety were tested using an elevated plus maze. The maze consisted of 4 arms (2 open, 2 closed; 15 cm wide × 60 cm long) extending from a central platform and elevated 1.5 m above the floor. Rats were placed in the centre platform facing a closed arm and monitored from behind a partition. Time spent in the open arms was recorded during a 5 min trial.

2.4 Data analysis

To evaluate performance in the Morris water maze, mean latency data was determined for each animal for each block of 4 consecutive trials (i.e. four

start locations). Data were analyzed using 3-way ANOVA with treatment and sex as grouping variables in a repeated measures design using the General Linear Protocol in SAS (v. 6.2; SAS Institute, Cary NC). Post-hoc comparisons were done using Tukey's HSD test. Memory interference was analysed by comparing test blocks using Student's *t*-test. Elevated plus maze data were analysed using 2-way ANOVA for mean time in the open arm on each test day and the proportion of rats in each group that spent more time in the open arm on Day 9 versus Day 1 was analysed using Chi square. For all analyses significance was set a $\alpha = 0.05$.

All testing procedures were approved in advance by the institutional animal care committee and were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

3. Results

3.1 Morris water maze performance

Analysis of escape latency data over 12 consecutive blocks revealed a significant main effect for block ($F[11,429] = 46.29$, $p < 0.05$) as expected if rats are learning the maze, however there were no main effects for either sex or condition, nor were any interactions with treatment present (data not shown).

Two analyses were conducted to test for memory in the MWM. Firstly, time spent in the target quadrant during probe trial testing revealed a significant main effect for sex, with females spending significantly less time in the target quadrant than did male counterparts ($F[1,39] = 4.56$, $p < 0.05$) (Fig. 1A). However, male rats spent significantly less time engaged in thigmotaxis (circling the perimeter of the pool) during the probe trial than did female rats ($F[1,39] = 13.65$, $p < 0.05$) (Fig. 1B). In both analyses no significant differences were found between treatments.

Memory interference was assessed by comparing performance each time a new platform location was introduced (blocks 7 and 11). Mean escape latency of the last four trials on each platform location was compared with mean latency on the first four trials on the new platform location (i.e. block 6 vs 7 and block 10 vs 11). It was hypothesized that the recall of the previous day's location may be influenced by the increased memory load of previous platform locations, resulting in an increased latency to find the new platform. If so, this effect could be considered evidence of proactive interference. While no differences were found for male rats in any group (Table 1), females treated as neonates with 20 μg/kg DOM showed a significant increase in latency to find the new escape platform on both block by block comparisons (block 6/7: $t[7] = 2.8$, $p < 0.05$ and block 10/11: $t[7] = 6.07$, $p < 0.05$)

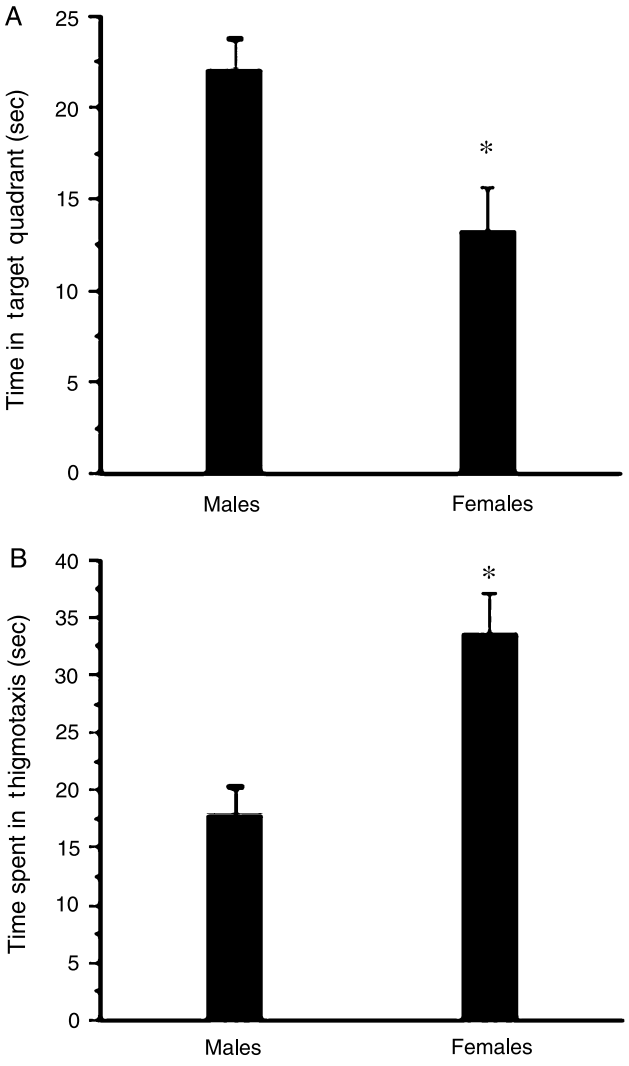

Fig. 1. Gender-based differences in Morris water maze performance. **A** Time (sec) spent in the target quadrant during probe trial. **B** Time (sec) spent in thigmotaxis during probe trial. $^{*}p < 0.05$

Table 1. Mean escape latency (sec) for male rats in Morris water maze reversal task

	Block 6	Block 7	*P*	Block 10	Block 11	*P*
Saline	8.29 (1.45)	13.73 (3.07)	n.s.	7.82 (0.73)	8.84 (1.84)	n.s.
5 DOM	17.62 (3.67)	14.89 (3.72)	n.s.	9.88 (2.38)	13.92 (5.05)	n.s.
20 DOM	12.09 (1.88)	13.09 (2.55)	n.s.	7.06 (1.08)	9.28 (1.50)	n.s.

Platform location was changed prior to blocks 7 and 11. Values in parentheses represent standard errors. *n.s.* Not significant

Table 2. Mean escape latency (sec) for female rats in Morris water maze reversal task

	Block 6	Block 7	P	Block 10	Block 11	P
Saline	14.25 (1.58)	19.5 (2.91)	n.s.	13.4 (2.77)	18.6 (2.49)	n.s
5 DOM	14.79 (3.86)	20.65 (3.1)	n.s.	15.93 (4.19)	17.55 (3.60)	n.s.
20 DOM	13.23 (2.65)	20.94 (4.3)	<0.05	10.97 (3.07)	18.62 (2.64)	<0.05

Platform location was changed prior to blocks 7 and 11. Values in parentheses represent standard errors. *n.s.* Not significant

(Table 2). No significant differences were found in either saline or 5 μg/kg DOM treated females (Table 2).

3.2 Elevated plus maze performance

Analysis of the amount of time spent in the "open" arm of the plus maze revealed no significant difference between groups on either Day 1 (PND 120) or Day 9 (PND 128) and no group by day interaction for either male or female rats, although it appeared female rats treated with either dose of DOM were spending more time in the open arm on Day 9 than on Day 1 (Fig. 2B). This was confirmed by conducting an analysis of the relative performance on Days 9 and 1 for each individual rat. While no group differences were found for male rats, the proportion of female rats spending more time in the open arm on Day 9 than on Day 1 was significantly greater for the 20 μg/kg DOM group (χ^2 [1] = 4.18, $p < 0.05$) (Fig. 3).

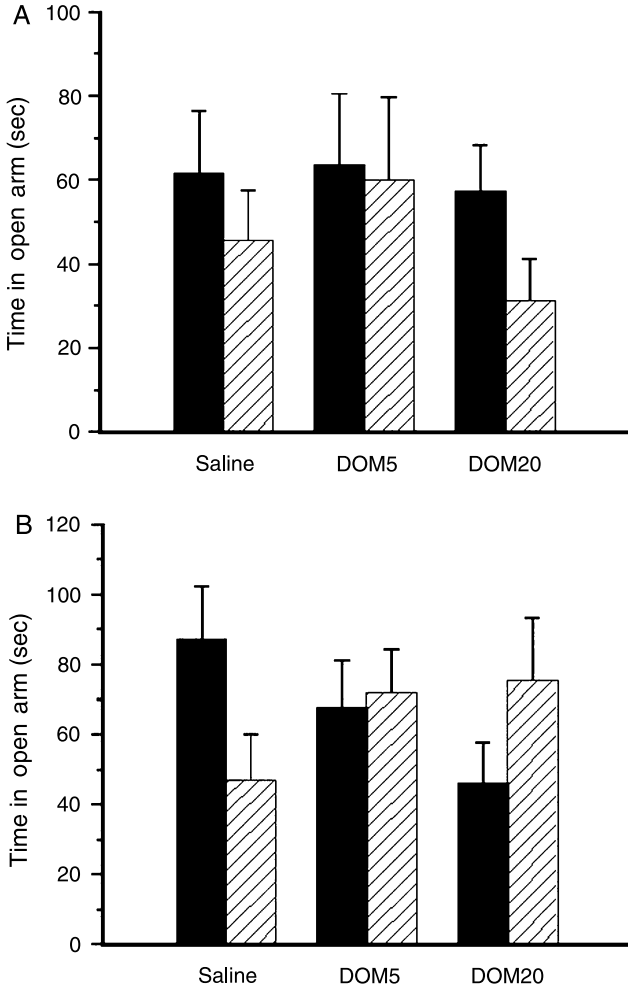

Fig. 2. Between-days performance in elevated plus maze. Time (sec) spent in the open arm of the maze on PND 120 (solid bars) and PND 128 (striped bars) for male (**A**) and female (**B**) rats treated neonatally with either saline, 5 μg/kg domoic acid (DOM5) or 20 μg/kg domoic acid (DOM20)

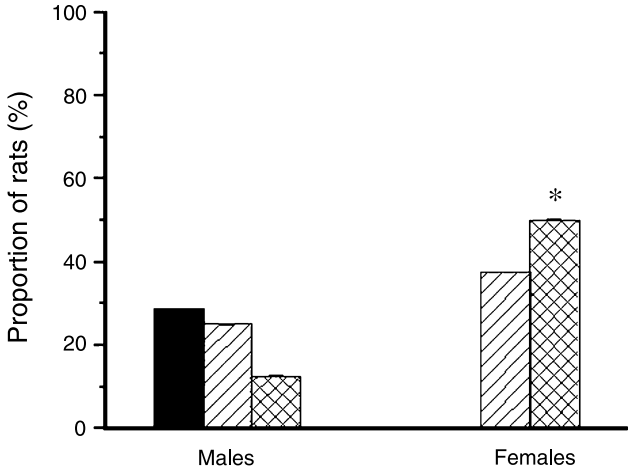

Fig. 3. Proportion of rats spending more time in the open arm of the elevated plus maze on PND 128 compared to PND 120. Rats were treated neonatally with either saline (solid bars), 5 μg/kg domoic acid (striped bars) or 20 μg/kg domoic acid (hatched bars). $^*p < 0.05$ relative to saline group

4. Discussion

The brain growth spurt, that lasts until about 2 weeks of age in the rat, is a period of considerable importance for assessing potential developmental neurotoxicants, as it is known that systems becoming functionally mature are most susceptible to teratogenic agents (Dobbing and Sands, 1979; Vorhees, 1986). Rather than producing a transient pharmacological effect, compounds administered to the developing organism during the brain growth spurt, have

the potential to cause permanent irreversible insult which may manifest immediately or be significantly delayed in onset (Rice and Barone, 2000). This insult can be expressed as either a permanent dysfunction of the neurotransmitter system involved, or may result in ''irreversible imprinting'' of receptor densities, which in turn results in lasting functional and/or structural changes to the nervous system (Kaufmann, 2000). Significantly, processes which characterize the brain growth spurt rely on appropriate glutamatergic signaling (McDonald and Johnston, 1990). We have previously reported (Doucette et al., 2003, 2004; Tasker et al., 2005) that administration of domoic acid (DOM), an environmental toxin that is known to be a selective kainate receptor agonist (Verdoorn et al., 1994; Tasker et al., 1996), to newborn rats during the second postnatal week results in permanent changes in brain development that manifest as both stage 2 seizure-like behaviour and cytoarchitectural changes in the hippocampal formation that are consistent with both conventional animal models and clinical cases of temporal lobe epilepsy (Houser, 1990; Wenzel and Schwartzkroin, 2006). In the current study we have extended these findings in this model, by providing evidence that as adults these rats also have subtle deficits in spatial cognition and emotionality. Changes in cognitive performance, altered emotional states and aberrant response to stress are all well documented in clinical cases of TLE and have also been reported in some existing animal models of epilepsy (Stafstrom, 2006).

While changes in cognition and emotionality in the current study are consistent with the notion that DOM-treated rats represent a developmental model of TLE, the finding that these changes are seen in female, but not male, rats is intriguing. In our previous reports of the behavioural sequelae seen in these rats we have noted that male rats are more prone to display stage 2 seizure-like behaviour than are female rats (Doucette et al., 2004). On post-mortem examination, however, increases in hippocampal mossy fibre sprouting (visualized with Timm's stain) and dentate hilar cell loss seem to occur equally in male and female treated rats (Doucette et al., 2004; Bernard et al., 2005). Collectively these data would suggest that perinatal treatment with domoic acid causes structural alterations in brain development that are equivalent in male and female animals, but that the behavioural consequences of those changes are gender-specific. It is well established that male and female rats perform differently in tests of spatial cognition (Roof and Stein, 1999; Silva-Gomez et al., 2003) and have differing responses to stress (Kitraki et al., 2004), but it is currently unclear as to whether the gender-based differences described herein arise from molecular changes in relevant neurotransmitter systems and/or reflect differences in the functional organization of the brain that are not apparent in routine histology. We are currently conducting studies to investigate these possibilities.

In conclusion, the results of the current study provide additional evidence that perinatal treatment of rats with low doses of domoic acid results in permanent changes in brain function as manifested by subtle changes in spatial cognition and altered response to an emotional challenge. Such deficits are also consistent with the notion that this treatment paradigm represents a new developmental model of epilepsy and related seizure disorders. However, the changes observed occur only in female, not male, rats. This suggests that future investigations of the neurobiology of epilepsy, and perhaps the development of new anti-epileptic therapies, should consider effects in both male and female animals, and that effective therapeutic strategies for seizure disorders could be gender-specific.

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