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Chronic stress prior to hippocampal stroke enhances post-stroke spatial deficits in the ziggurat task

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ABSTRACT

Stress is one of the most important variables to determine recovery following stroke. We have previously reported that post-stroke exposure to either stress or corticosterone (CORT) alleviates hippocampal ischemic outcome. The present experiment expands previous findings by investigating the influence of exposure to stress prior to ischemic event. Rats received either daily restraint stress (1 h/day; 16 consecutive days) or CORT (0.5 mg/kg; 16 consecutive days) prior to focal ischemic stroke in the hippocampus induced by bilateral injection of endothelin-1 (ET-1). All experimental groups were then tested in the ziggurat task, a new task for spatial cognition. The stress + stroke group showed significant deficits in both hippocampal structure and function. No deleterious effect of pre-stroke exposure to CORT was found in the CORT + stroke group. Our results indicate that a history of chronic stress sensitizes hippocampal cells to the damaging consequences of focal ischemia. The opposing effects of CORT-related experiences in this study not only reflect the diversity of glucocorticoid actions in the stress response, but also provide evidence that elevated CORT in the absence of emotional disturbance is not sufficient to produce hippocampal deficit.

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1. Introduction

Previous studies investigating hippocampal function have revealed both permissive and suppressive actions of glucocorticoid hormones. On the one hand, glucocorticoids protect the brain against adverse events, induce structural recovery and are essential for cognitive performance (de Kloet, Oitzl, & Joels, 1999; Faraji, Lehmann, Metz, & Sutherland, 2009; Roozendaal, 2000). On the other hand, the central action of corticosteroids has mostly been portrayed as damaging and disruptive to learning and memory (McLaughlin, Gomez, Baran, & Conrad, 2007; Sapolsky, 2000; Wright & Conrad, 2008). Generally, it is believed that corticosteroid effects on cognition and the respective brain regions can turn from protective into maladaptive when actions via the two corticosteroid receptor types (MR or mineralocorticoid receptors and GR or glucocorticoid receptors) are imbalanced for an extended period of time (de Kloet et al., 1999). Under these conditions, both chronic stress and glucocorticoids may reduce hippocampal dendritic complexity (Conrad, Magariños, LeDoux, & McEwen, 1999; Kleen, Sitomer, Killeen, & Conrad, 2006; McLaughlin et al., 2007; Watanabe, Gould, & McEwen, 1992) and can even cause hippocampal cell death (Landfield, Waymire, & Lynch, 1978; McDonald, Craig, & Hong, 2008; Sapolsky, 2005; Uno, Tarara, Else, Suleman, & Sapolsky, 1989).

The structural and functional alterations in the brain by stress or glucocorticoids (e.g., corticosterone; CORT) have become the focus of further experimental considerations about the dynamic biological dialogue between neural and hormonal systems. Several investigations in animal studies indicate that psychological stress induces an effect, either structural or functional that may be different than mere glucocorticoid treatment (Diamond, Macintosh, Fleshner, & Woodson, 2002; Jamieson, Fuchs, Flugge, & Seckl, 1997; Kim, Lee, Han, & Packard, 2001). These findings not only reveal the different profiles of stress and CORT-related changes, but also highlight the central role of psychological conditions (e.g., emotional disturbances) in the development of the brain structure and function.

Because little is known about the contribution of CORT in stress-dependent challenges before the ischemic insults, the primary purpose of this experiment was to determine whether a history of chronic stress and glucocorticoid elevations modulate dentate gyrus (DG) damage after hippocampal stroke. There have been two rationales for the present study to induce stroke in the hippocampus: (1) the hippocampus is a structure intimately involved in the processing, learning and storage of certain types of new information (O'Keefe & Nadel, 1978; Scoville & Milner, 1957; Sutherland, Kolb, & Whishaw, 1982; Sutherland & Rudy, 1989), and (2) stroke and other neuropathological conditions are frequently associated with learning and memory deficits (Gainotti et al., 2004; McDonald, 2002). More important, evidence suggests that hippocampal function is extremely sensitive to stress and its hormonal consequences (McEwen & Sapolsky, 1995). With two

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types of corticosteroid receptors (MRs and GRs), the hippocampus represents a key structure in the stress response. The profile of the hippocampal involvement in stress response, however, is different for dorsal and ventral hippocampus (Venero et al., 2002). It has been shown that stress may alter the relationship between hippocampal neuronal function in the dorsal and ventral hippocampus (Muchimapura, Fulford, Mason, & Marsden, 2002).

The secondary purpose of this study was to investigate the effects of chronic stress and CORT elevations before hippocampal stroke on the magnitude of spatial learning and memory deficits. Studies investigating the effects of chronic glucocorticoid exposure on spatial performance have reported mixed results, with some studies showing intact spatial memory under conditions that should produce hippocampal damage (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003; Conrad et al., 2007; Luine, Spencer, & McEwen, 1993; Magariños, Orchinik, & McEwen, 1998), whereas other investigations show spatial memory deficits (Dachir, Kadar, Robinzon, & Levy, 1993; McDonald et al., 2008; McLay, Freeman, & Zadina, 1998).

The present study examines the differential effects of stress and glucocorticoids using a dry land maze, the ziggurat task (ZT). The nature of this task avoids the stress associated with a water task or other aversively motivated tasks and therefore may produce novel insights on stress-induced structural and spatial memory changes and recovery after hippocampal stroke.

2. Materials and methods

2.1. Subjects

Twenty-six adult male Long-Evans rats, weighing 330–360 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22 °C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. Animals were food-restricted prior to baseline training and testing in the ZT, and maintained at about 90% of their initial body weight throughout the experiment. To maintain body weight, rats were given an additional amount of food in their home cage at least 3–4 h after completion of the behavioural training and testing. Because animals were housed in pairs, they were weighed daily throughout the experiment in order to monitor their food consumption. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Rats were divided into four groups: Sham ($N = 6$), stroke ($N = 7$), CORT + stroke ($N = 7$) and stress + stroke ($N = 6$). Rats in CORT and stress groups received daily CORT and restraint stress before the ET-1-induced stroke in the hippocampus. Bilateral injection of ET-1 into the hippocampus was used to induce stroke. In order to assess baseline levels of circulating CORT, all groups were subjected to blood sampling 1 day before and on day 16th of the CORT and stress treatment. All four groups were subjected to the ziggurat-task training for spatial performance. Following the behavioural tests, rats were euthanized and the brains processed for histological analysis to determine lesion extent and location. Fig. 1 illustrates the time course of all experimental manipulations.

2.2. Blood samples

Blood samples were taken at baseline, i.e. the day prior to CORT and stress treatment. Blood samples were also taken 1 h after CORT and stress on day 16 of treatment (or day 17 of the experiment). All samples were collected in the morning hours. Rats were transported individually to the surgical suite and anesthetized with 4% isoflurane. During the 3–4 min of anesthesia, 0.70 mL of blood was collected from the tail vein. Blood was sampled using a heparinized butterfly catheter. Blood samples were then transferred to centrifuge tubes and plasma was obtained by centrifugation at 5000 rpm for 5 min. The plasma samples were stored at −20 °C until analyzed for CORT concentration using commercial radioimmunoassay kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA). All procedures for blood sampling were the same as those previously reported by Metz, Jadavji, and Smith (2005).

2.3. CORT administration

Each animal in the CORT + stroke group was orally administered 0.5 mg/kg CORT (Sigma–Aldrich, St. Louis, MO, USA) daily (16 consecutive days; Faraji et al., 2009) in the morning hours between 10:30 and 11:30 am before the injection of ET-1 into the hippocampus. The CORT was mixed with 0.30–0.40 mg crushed banana-flavoured pellets (Bio-Serv, USA) and one-two drops peanut oil (Planters, JVF Canada Inc, Toronto, ON, Canada) (Metz et al., 2005). All rats readily consumed the mixture.

2.4. Restraint stress

The stress procedure used was the same as that previously reported by Faraji et al. (2009) with the exception that the restraint tubes were manually vibrated for 5–10 s every 15 min of that stress phase in order to prevent habituation. For restraint stress, the animals in the stress + stroke group were maintained in custom-made transparent Plexiglas tube (6 cm inner diameter) of adjustable length, from 10:30 am to 11:30 am for 16 consecutive days. The tubes allowed the complete restriction of the animals while at the same time allowing them to breathe through perforated ends of the tube. The tubes maintained the animals in a standing position without compression of the body. Following the 16-days of restraint stress, and in order to assess spatial performance of the animals, all groups were trained and tested in the standard or non-cued version of the ZT for spatial performance.

2.5. Surgery: ET-1 injection into the hippocampus

All animals except shams were subjected to bilateral hippocampal injection of ET-1 (Sigma–Aldrich, St. Louis, MO, USA). Briefly, twenty rats in three groups received two injections of a low concentration (7.5 pmol) of ET-1 (0.5 μl; 0.1 μl/min) in each hippocampus through a 23-gauge cannulae attached to a Harvard infusion pump (model 22) and using the coordinates *AP*: −4.1, −5.3; *ML*: ±3.0, 5.5; *DV*: −3.7, −6.3 in millimetres relative to the bregma-lambda distance (Faraji et al., 2009). The cannulae were left in place for 5 min after each injection. The scalp was sutured after surgery and the animals were monitored until they became active before being returned to their home cages. Sham group received all surgical procedures up to the skull opening. Skull trephination was not performed in sham-operated animals because it has been previously reported to produce behavioural and neurochemical asymmetries (Adams, Schwarting, & Huston, 1994). Rats were allowed to recover for 6–7 days before the beginning of ZT testing.

2.6. Ziggurat task (ZT)

In order to assess spatial performance of the animals, all rats were tested in eight trials per day for nine consecutive days in the ZT (Fig. 2). The training and testing procedures were previously published in detail (Faraji, Lehmann, Metz, & Sutherland, 2008).

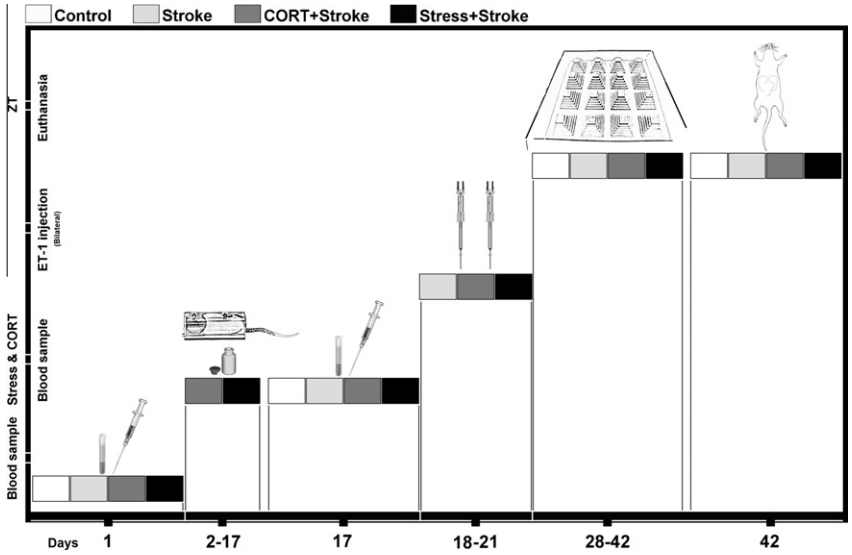

Fig. 1. Schematic presentation of the experimental protocol. CORT, corticosterone; ET-1, endothelin-1; ZT, ziggurat task.

Briefly, all behavioural testing was carried out in a white rectangular room with different distal cues (pictures and signs) on each wall. For data acquisition, a ceiling-mounted camera recorded the movements of the rats. The standard version of the task for spatial function consisted of an open field box measuring 179 cm × 179 cm × 25 cm in height. The environment contained sixteen pyramidal ziggurats, arranged in a four by four matrix. The ziggurats were identical and the distance between them within the environment was 11 cm. A circular hole was drilled in the centre of the highest level of each ziggurat. The hole held pieces of uncooked pasta, 1–2 cm long. They were food-restricted 1-week prior to habituation sessions and behavioural testing. After habituation, the testing sessions were conducted over nine consecutive days and began the day immediately following the last session of habituation. The cycle consisted of alternating *different-goal* or learning days (odd days or days 1, 3, 5, 7) and *same-goal* or memory days (even days or days 2, 4, 6, 8). On the odd days, the goal ziggurats were located in a new location, and rats had to find and learn the location of the goal ziggurat in the new place. The goal ziggurats remained in the same place on the even days. Thus, the rats were required to remember the location they had learned previously.

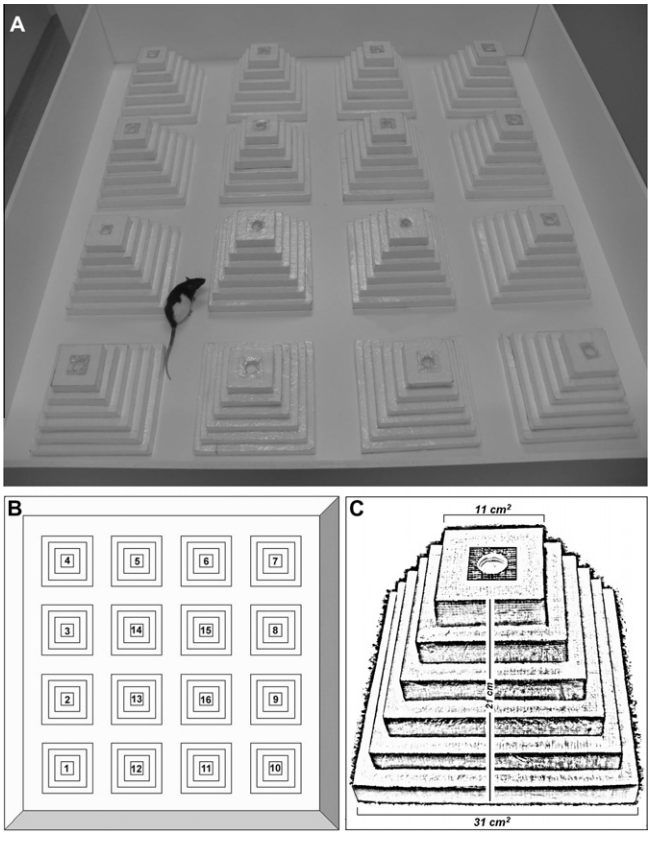

Fig. 2. (A) Standard or non-cued version of the ziggurat task (ZT) for spatial learning which is subjected to the present study. The task requires rats to learn and remember that the top of 1 of 16 ziggurats in the open field is baited with a food reward. (B) A vertical-view graph of the ziggurat task contained sixteen pyramidal ziggurats, arranged in a four by four matrix. (C) A photograph of an individual ziggurat, 31 cm × 31 cm in base, by 21 cm in height.

Two sets of ziggurats were defined in the environment. First, "start" ziggurats, located in each corner, and second, the rest of ziggurats or "goal" ziggurats. On the testing days, the rats, released from each starting point, were allowed to explore the environment. One goal ziggurat (peripheral or central) was baited with spaghetti for each trial. During each testing day, the exploration took place in eight trials per rat and at four different starting points at a randomized position. Across trials, the starting location varied among the four corners of the apparatus, and on each trial, animals navigated in the environment for 60 s or until they found the goal ziggurat. To start, rats were placed facing the wall on the starting point (for instance, ziggurat number 1), and were required to explore the environment until they found the goal ziggurat to consume the food. Since the location of the goal ziggurat remained constant from trial to trial every 2 days, the subjects had to learn and remember the new locations of the goal ziggurat following each 2 days. Normally, the animals are able to find the goal ziggurat using the distal and/or proximal cues following investigation of some non-goal ziggurats in the first trials. In order to minimize olfactory cues, both the box and ziggurats were cleaned with 5% alcohol after testing each group.

Probe trial-dependent behaviours in the ZT were measured on the 9th day as an additional measure for spatial memory performance. The ZT environment had four quadrants. Each rat was given three consecutive 60-s probe trials, released from different starting points to reach the goal ziggurat. On the first trial, the goal ziggurat, located in the former location (quadrant 2 in SE; target

quadrant) had 3–5 pieces of spaghetti. On the second and third trials, however, there was no food on it. Rats were allowed to navigate freely in the environment during the specified time. The percentage of time rats spent in trial two in each quadrant of the ziggurat task was recorded.

Furthermore, each investigation of non-goal ziggurats (i.e., non-baited ziggurats) was considered an error. In other words, behaviours such as climbing onto incorrect ziggurats and touching the circular holes with the nose have been defined as errors. It should be pointed out that rats in ZT can make two kinds of errors: (1) errors *type 1* in which rats investigate non-goal ziggurats once and (2) errors *type 2* in which rats re-investigate non-baited ziggurats. This categorization of errors can be useful for distinguishing processes related to working and reference memories (Faraji et al., 2008).

The movements of the animals including latency or time to find the goal ziggurat, path speed and percentage of time spent in each quadrant of the ZT were recorded and analyzed by a video tracking system (HVS Image 2020, UK) and an Acer computer (Travel Mate 225X).

2.7. Histology

All animals were euthanized by an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline followed by 4% paraformaldehyde. Each brain was removed from the skull and stored in 30% sucrose–formalin solution. The brains were then cut in 40 μm coronal sections on a cryostat microtome. Every fourth section was mounted on glass slides and stained with cresyl violet. The stained sections were examined under a microscope (Zeiss, Germany) and images were captured using an AxioCam camera (Zeiss, Germany) to quantify the extent of the lesions in both dorsal and ventral hippocampus. The amount of hippocampal lesion in each ischemic rat was estimated according to the Cavalieri method (Schmitz & Hof, 2005). In this Experiment, five images were captured, corresponding approximately to −2.12, −2.80, −3.60, −4.30 and −5.20 mm relative to bregma. After capturing an image of each section under 1× and 10× magnification, a systematic sampling grid with an area per point of 20,000 pixels was randomly thrown over each image and the number of points hitting intact hippocampal tissue were counted. Grids were generated using Image J software (http://rsb.info.nih.gov/ij/). The total number of hits in each rat was then divided by the average number of hits obtained by three control rats. The complement proportion was used as the percentage hippocampal lesion estimate (Lehmann, Lacanilao, & Sutherland, 2007). This assessment was intended to indicate an overall difference in hippocampal damage and tissue loss in different experimental groups in the present study.

2.8. Statistical analysis

Statistical analysis was performed using SPSS for Windows 11.5.0 (Standard Version, 1982–2002; SPSS Inc., USA). Four behavioural indices within the ZT (i.e. latency, path speed, percentage of time spent in target quadrant on the probe trial day and errors) were averaged and analyzed for each odd and even day. Repeated measures analysis of variance (ANOVA) was conducted with group (control, stroke, CORT + stroke, stress + stroke), day (days 1–8), trial (trials 1–8) and goal location (1–4) for the independent measures. Latency, path speed, percent time spent in the target quadrant as well as the number of errors served as the dependent variables. *Post-hoc* (Tukey HSD) test was used to adjust for multiple comparisons. Differences in between-group and within-group comparisons were also assessed with independent and dependent sample *t*-tests, with $P < 0.05$ set as the significance level. All data are presented as mean ± standard error of the mean.

3. Results

3.1. Histological results

Figs. 3 and 4 illustrate an intact hippocampus from a control animal and compare it to the extent of ET-1-induced damage to the hippocampus in all stroke groups (stroke, CORT + stroke, stress + stroke). In addition, Fig. 5 illustrates the estimate of the percentage hippocampal damage and tissue loss for different groups. ET-1 produced tissue loss in both dorsal and ventral regions of the hippocampus in all rats of the ischemic groups. In all ischemic groups, ET-1-induced damage was mostly limited to the dorsal CA1, CA2 and the DG. The extent of tissue loss in the ventral hippocampus, however, was typically restricted to the CA1 and CA2 regions. No detectable major tissue damage was observed in the ventral DG, except in two animals in the stroke-only and CORT + stroke groups that showed slight damage in this area. In addition, rats in the stress + stroke group showed extensive tissue loss in most regions of the hippocampus particularly CA1 and the DG when compared with the CORT + stroke group. The additional damaging effects of CORT and stress were not found in the ventral hippocampus. An analysis performed on the percent tissue loss in the dorsal hippocampus, using volumetrics, indicated a significant main effect of group [$F(2, 17) = 11.39$, $P < 0.05$] indicating that CORT and stress treatment prior to stroke could induce structural alteration in the dorsal hippocampus. *Post-hoc* analysis (Tukey HSD) for dorsal damage revealed that stroke-only group had less tissue loss compared to stress + stroke group (19.08% ± 1.26 *vs.* 25.61% ± 1.33; $P < 0.031$). No significant difference was found between stroke-only and CORT + stroke group (19.08% ± 1.26 *vs.* 21.33% ± 1.49; $P > 0.69$). An analysis also showed a significant difference between CORT + stroke and stress + stroke groups (21.33% ± 1.49 *vs.* 25.61% ± 1.33; *Post-hoc* $P < 0.033$) suggesting that the stress + stroke group showed more tissue loss in the dorsal hippocampus when compared to the CORT + stroke and stroke-only groups. Furthermore, the extent of damage to the ventral hippocampus was more consistent across stroke groups. No significant difference was found between stroke groups in terms of the extent of the tissue loss in the ventral hippocampus (all $P > 0.05$). That is, only the dorsal hippocampus was affected by stress prior to the stroke.

3.2. CORT levels

Fig. 6 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline and day 16th of CORT and stress treatment. As can be seen, rats in the CORT + stroke and stress + stroke groups, showed elevated levels of CORT on day 16. An ANOVA conducted for CORT levels in baseline showed no significant between-groups difference ($P > 0.86$) while this effect for chronic point (day 16 of CORT and stress treatment) was significant [$F(3, 22) = 6.41$, $P < 0.05$] suggesting that CORT administration and stress procedure caused in elevated levels of plasma corticosterone. *Post-hoc* analysis for chronic point showed a significant difference between control and CORT + stroke groups (185 ± 49.12 ng/mL *vs.* 323 ± 52.56 ng/mL; $P < 0.048$), and control and stress + stroke groups (185 ± 49.12 ng/mL *vs.* 376 ± 53.81 ng/mL; $P < 0.042$). This suggests that the CORT + stroke and stress + stroke groups were significantly involved in more levels of circulating CORT when compared with controls. In addition, a significant difference was found between stroke-only (168 ± 50.22 ng/mL) and other stroke groups (*Post-hoc* both $P < 0.05$). This means that mere CORT treatment and stress could significantly enhance the levels of plasma CORT before stroke. No significant difference was found between the

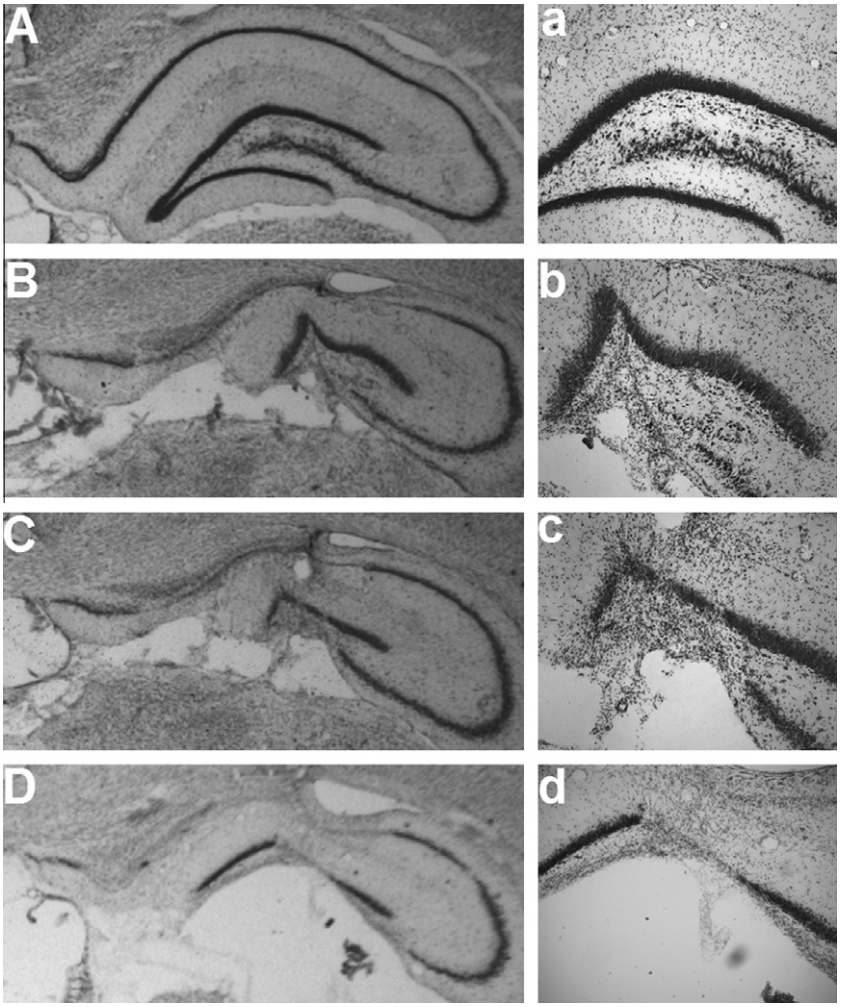

Fig. 3. Photomicrograph of a coronal section of a dorsal region of the hippocampus (left panel, magnification 1×) for a control (A), stroke only (B), CORT + stroke (C) and stress + stroke (D) rat. Higher magnification (10×; right panels) of the CA1 and the DG shows the hippocampal damage in the stroke only (b), CORT + stroke (c) and stress + stroke (d) rats. Stress–stroke combination was significantly associated with more tissue loss than stroke-only and CORT + stroke groups.

control and stroke-only groups ($P > 0.1$), and CORT + stroke and stress + stroke ($P > 0.82$). An additional dependent samples *t*-test conducted for baseline and day 16 in each group showed a significant difference between the CORT values of baseline and chronic time points only in the CORT + stroke ($t = 3.11$, $P < 0.04$) and stress + stroke groups ($t = 11.59$, $P < 0.02$). Hence, both exogenous CORT administration and restraint stress produced significantly elevated levels of circulating CORT prior to the ET-1-induced hippocampal stroke.

3.3. Behavioural results

Because latency and path length always reveal the same profile of spatial navigation (Faraji, Metz, & Sutherland, 2010; Kapoor, Kostaki, Janus, & Matthews, 2009; Vorhees, Reed, Skelton, & Williams, 2004) we have considered and reported only latency and path speed in the ZT.

3.3.1. Latency

Fig. 7A and C shows latency or the average time spent to find the goal ziggurat in the ZT for all groups over the acquisition (learning) and retrieving (memory) days. A repeated measures ANOVA was performed with group, goal location, training days and trials as independent variables, and latency to find the goal ziggurat over 64 trials of the ZT testing as the dependent variable. Our analysis revealed a significant main effect of group [$F(3, 22) = 14.26$, $P < 0.05$], trial [$F(7, 63) = 13.13$, $P < 0.05$], goal location [$F(1, 22) = 9.08$; $P < 0.05$] and day [learning: $F(3, 22) = 4.31$, $P < 0.05$; memory: $F(3, 22) = 5.80$, $P < 0.05$]. Group difference in latency likely stemmed from the fact that the animals in experimental groups had different cognitive abilities during the spatial navigation within the ZT. This may be attributed to the effects of stroke, stress and CORT administration. The significant effect of trial, goal location and day, on the other hand, suggest that animals were able to acquire and retrieve the spatial location of the goal ziggurats in different trials and days regardless of their experimental conditions. An additional repeated measure ANOVA also conducted for latency on learning and memory days showed a significant difference between learning and memory days [$F(1, 22) = 7.12$, $P < 0.05$] indicating all groups spent less time to find the goal ziggurat on memory days compared to learning days (Fig. 7A). No interaction effects of group by trial [$F(21, 184) = 2.08$, $P > 0.093$], group by day [$F(3484) = 6.58$, $P > 0.057$] and group by goal location [$F(3, 22) = 5.19$, $P > 0.052$] were observed. *Post-hoc* comparison revealed significant difference between controls and stroke-only, CORT + stroke and stress + stroke (all $P < 0.05$) suggesting that controls spent less time to find the goal ziggurat than other groups. In addition, *Post-hoc* comparison indicated a significant difference between the stress + stroke group when compared with the CORT + stroke group ($P < 0.046$). That is, the latency to

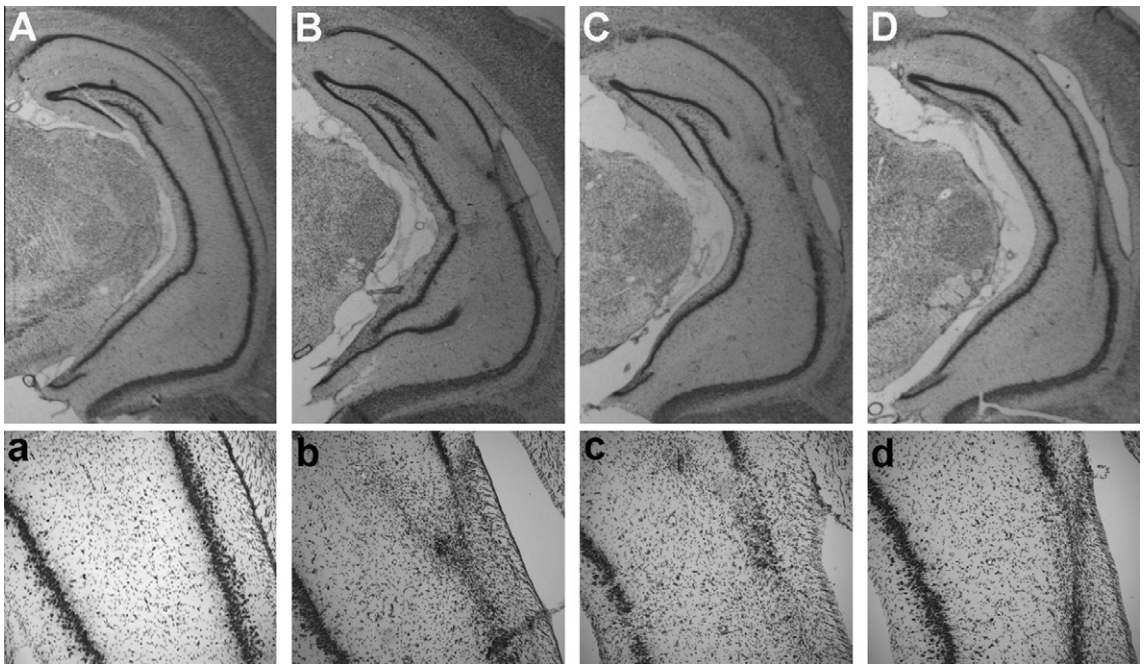

Fig. 4. Photomicrograph of a coronal section of a ventral region of the hippocampus (top panel, magnification 1×) in a control (A), stroke only (B), CORT + stroke (C) and stress + stroke (D) rat. Higher magnification (10×; below) panels show that all ischemic rats (b–d) had an extensive tissue loss in the CA1 area. No destructive effects of CORT and stress were found in the CORT + and stress + stroke (c and d respectively) groups.

Group	HPC Damage (%)	
	Dorsal	Ventral
Stroke-only	*19.08 (± 1.26)*	*27.12 (± 1.42)*
CORT+Stroke	*21.33 (± 1.49)*	*26.91 (± 1.28)*
Stress+Stroke	*25.61 (± 1.33)**	*26.34 (± 1.27)*

HPC, hippocampus; CORT, corticosterone; ★: p<0.05

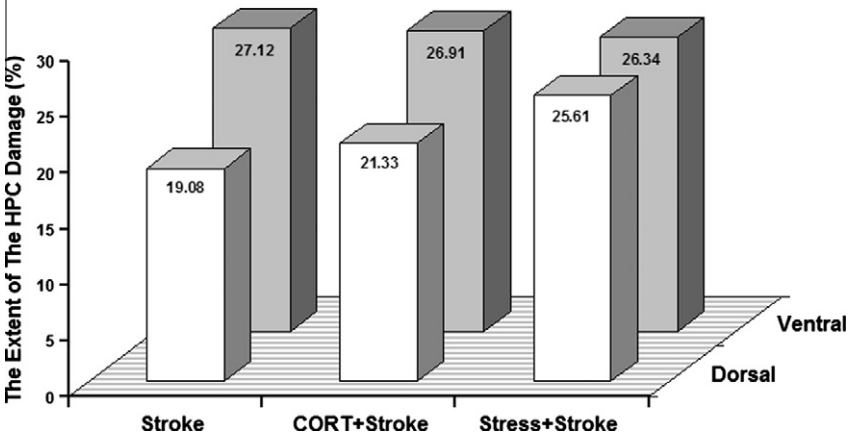

Fig. 5. Estimate of the percentage hippocampal damage for the stroke-only, CORT + stroke and stress + stroke groups. ET-1 produced tissue loss in both dorsal and ventral regions of the hippocampus in all rats of the ischemic groups. However, the additional damaging effects of CORT and stress were not found in the ventral hippocampus.

locate the goal ziggurat by the stress + stroke group was different (slower) from rats that have been given CORT prior to the stroke. No significant difference was found between stroke-only and CORT + stroke groups ($P > 0.66$).

3.3.2. Path speed

Path speed during acquisition and retrieving in the ZT is depicted in Fig. 7B and D. All four groups showed relatively constant speeds across the eight testing days in the ZT. No significant main effect of group in terms of path speed was found in the task ($P > 0.73$) supporting the idea that the observed behavioural deficits in the ZT following CORT and stress treatment and ET-1 injection may be attributed to the cognitive outcomes of the rats' exposure to CORT, stress and ET-1-induced ischemia in the hippocampus and not due to a simple motor or motivational effect.

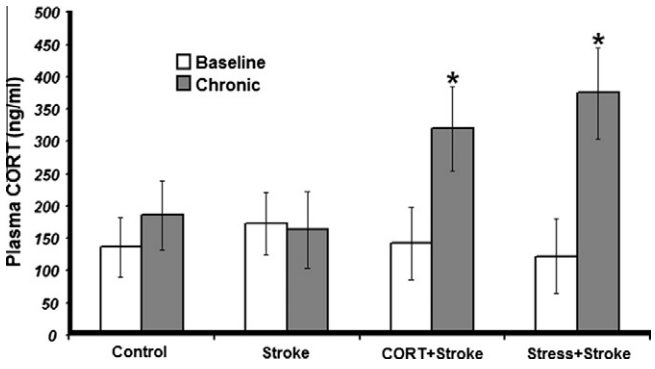

Fig. 6. Plasma CORT concentration prior to (baseline) and at chronic levels (day 16) of daily CORT and stress administration. No significant difference was found between the CORT + stroke and stress + stroke. $^{*}P < 0.05$; dependent samples *t*-test for within-subject comparison. Error bars show ± SEM.

3.3.3. Probe trial

The percentage time spent in the testing (target) and opposite quadrants of the ZT during the probe trial (day 9th) is depicted in Fig. 8A. Analysis of the 60 s of the probe performance in the ZT revealed that only rats in the control and stroke-only groups spent a considerable proportion of their time searching in the target quadrant. A repeated measures ANOVA conducted for the percentage of time spent at the target quadrant (quadrant two) within the ZT showed significant main effect of group [$F(3, 22) = 3.06$, $P < 0.05$]. This suggests that the experimental groups in the present study influenced by stroke, CORT and stress had different abilities to express their knowledge of the goal ziggurat during the probe trial. Specifically, stroke groups receiving CORT or stress spent significantly less time in the target quadrant than both the controls and the stroke-only group (*Post-hoc* both $P < 0.05$). No significant difference was found between control and stroke-only groups (39.04% ± 1.87 *vs.* 35.12% ± 1.83; *Post-hoc* $P > 0.57$) and between CORT and stress + stroke groups (22.72% ± 1.29 *vs.* 24.55% ± 2.48; *Post-hoc* $P > 0.81$). This finding is consistent with the idea that both CORT and stress groups did not acquire or retain a strong bias for the previous location of the goal ziggurat as compared to the control and stroke-only groups.

3.3.4. Errors

Examination of spatial learning and memory in terms of the number of errors revealed a gradual decrease in the numbers of errors of all groups during 8 days spatial navigation in the ZT (Fig. 8B). However, an ANOVA conducted for between-groups comparison showed a significant difference between groups in terms of the numbers of errors [$F(3, 22) = 12.51$, $p < 0.046$]. Control, stroke-only and CORT + stroke groups showed fewer errors than stress + -stroke group (12.75 ± 1.12, 13.87 ± 1.18 and 12.49 ± 1.13 *vs.* 19.25 ± 1.28; *Post-hoc* $P < 0.05$) indicating that a combination of stress and stroke may induce more cognitive disturbance than CORT + stroke. There were no differences between groups in term of the type of errors (types 1 and 2).

4. Discussion

The results of the present experiment indicate that ischemic stroke localized to the hippocampus using ET-1 had clear structural and functional effects. Rats who experienced chronic restraint stress prior to hippocampal stroke, however, displayed significantly more structural damage compared to the stroke-alone rats or those who received corticosterone before stroke. Chronic stress treatment prior to stroke caused an enhanced spatial impairment (e.g. high latency and errors) in the ziggurat task, an appetitive, dry-land task for measuring spatial learning and memory. These

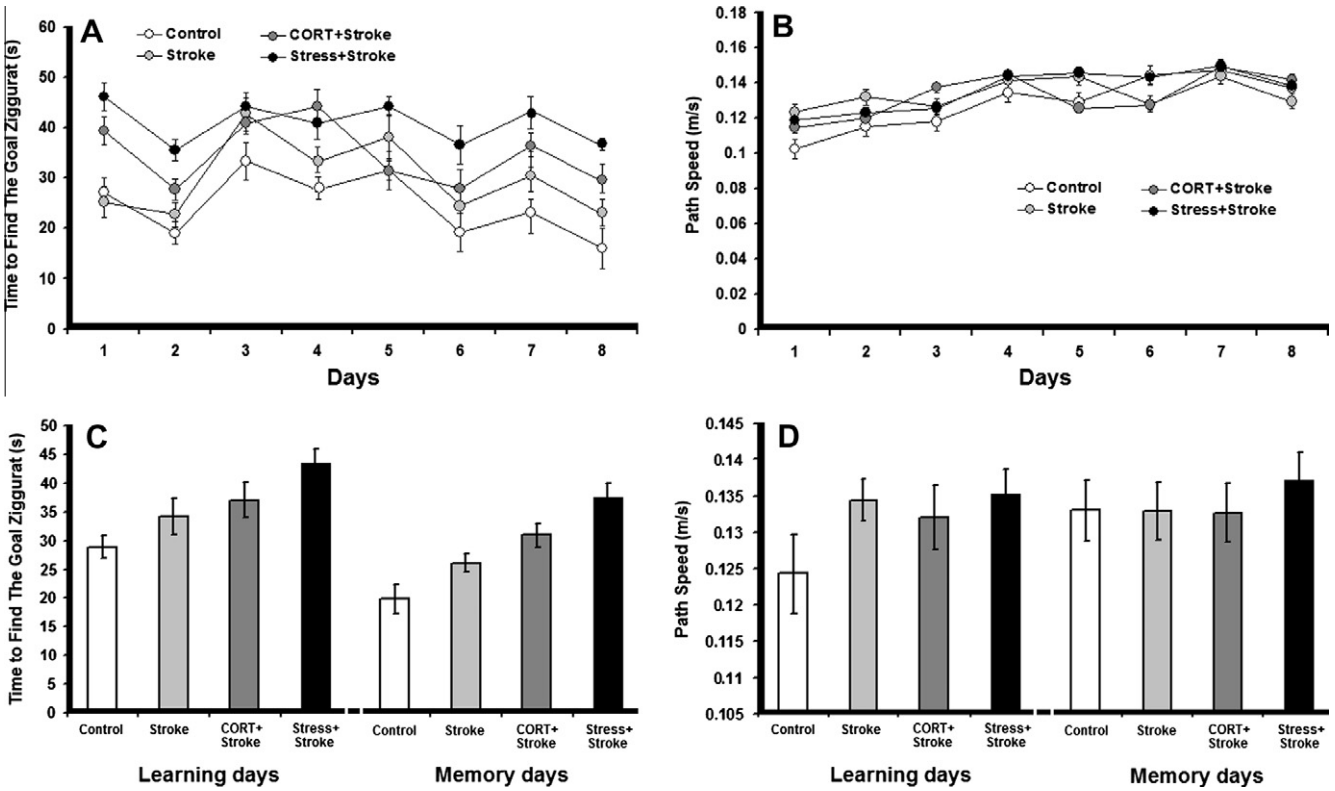

Fig. 7. Testing in the non-cued (standard) version of the ZT for spatial learning. (A) Latency or time to find the goal ziggurat during 8 days of testing. (B) Mean path speed averaged across 8 days of testing. (C) Average latency and (D) path speed to find the goal ziggurat, on learning and memory days in the ZT for all groups. Error bars denote average ± SEM for each group.

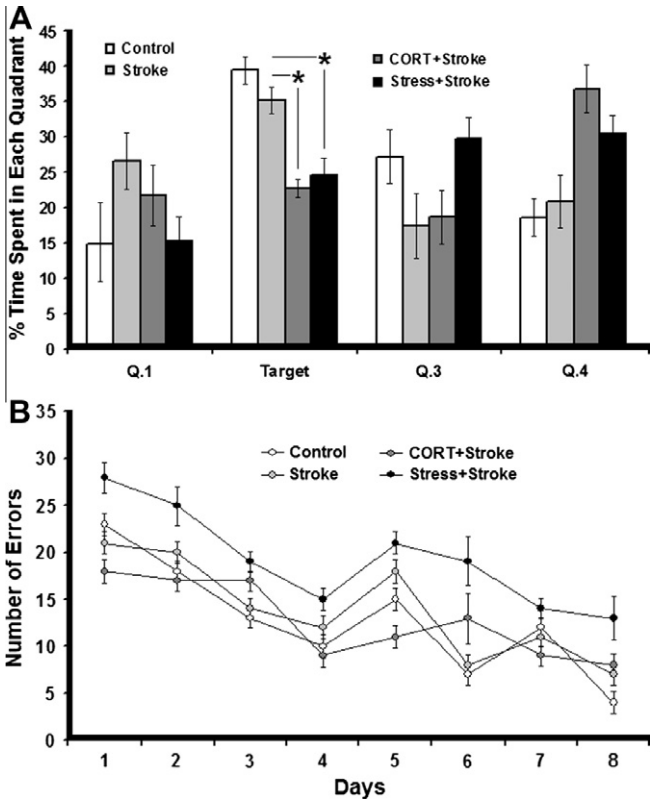

Fig. 8. (A) The mean percentage of time spent in four quadrants of the ZT during the 60 s of the probe trial conducted on day 9. Both CORT + stroke and stress + stroke groups spent significantly less time searching the goal ziggurat in the target quadrant relative to control and stroke groups. (B) Averaged number of errors in the ziggurat task during 8 days of testing. Control, stroke-only and CORT + stroke groups significantly showed fewer errors than stress + stroke group. $^{*}P < 0.05$. Error bars show ± SEM. Q, quadrant.

results suggest that a chronic history of stress sensitizes hippocampal cells to injury associated with focal ischemia. These deleterious effects of stressful experiences on the hippocampus can markedly impair post-stroke hippocampus-dependent memory processes. Despite the deleterious consequences of pre-stroke stress on rats' latency, probe performance and errors, our results showed significant effects of trial, goal location and day within the ZT. Specifically, the significant effect of trial in the present experiment indicates that different groups of animals were able to locate the spatial goal within the ZT on different trials. The significant effect of trial in both Morris water task (MWT) or ZT has been previously reported for stressed animals and animals with focal ischemic stroke (Faraji et al., 2009; McDonald et al., 2008).

4.1. Pre- vs. post-stroke stress can have opposite effects

It has been previously shown that post-stroke exposure to either mild stress or CORT may improve recovery both in hippocampus infarct volume and in spatial memory (Faraji et al., 2009). In contrast, in the current experiment, exposure to even mild chronic stress or exogenously administered CORT prior to stroke presented a different profile of anatomical and behavioural effects. In the literature, chronic exposure to glucocorticoids or stress has been shown to be associated with exacerbation of several neurological disorders (Kirkland, Coma, Colwell, & Metz, 2008; Smith, Jadavji, Colwell, Katrina Perehudoff, & Metz, 2008; Sugo et al., 2002; Zigmond & Stricker, 1984). For instance, McDonald et al. (2008) showed that rats with hippocampal focal stroke that had previously experienced stress showed enhanced hippocampal cell death and spatial deficits in the MWT when compared to a non-stressed group with the same kind of hippocampal stroke. Interestingly, this finding is consistent with the current results at both structural and functional levels within the ZT, a dryland task that has been recently developed for measuring spatial performance (Faraji et al., 2008). Hence, the deleterious effects of stressful experiences are not limited to a single behavioural task or other laboratory assessment procedures.

4.2. Neurohormonal mechanisms of the deleterious effects of stress

It is commonly believed that stress is a key factor in the etiology of stroke (DeVries et al., 2001; McDonald et al., 2008) although there are some reports that have concluded that there is no effect of emotional factors on stroke incidence (Macko et al., 1996). Despite disagreement regarding the effects of prior exposure to stress on stroke incidence, several studies have provided evidence that high emotionality before a stroke is associated with elevated concentrations of glucocorticoid hormones and adversely affects stroke outcome (DeVries et al., 2001; Sugo et al., 2002; Zucchi et al., 2009). These deleterious effects of stress and also enhanced levels of corticosterone are often attributed to compromised antioxidant enzyme defenses (McIntosh, Cortopassi, & Sapolsky, 1998), inhibitory effects of CORT on local cerebral glucose utilization and glucose transport in neurons and consequently ATP depletion in the neurons (Horner, Packan, & Sapolsky, 1990; Kadekaro, Ito, & Gross, 1988; Sugo et al., 2002) or diminished neurotrophic factor supply (Knapman et al., 2009; van Donkelaar, van den Hove, Blokland, Steinbusch, & Prickaerts, 2009). In addition, it has been shown that pre-ischemic exposure to stress may affect infarct size by suppressing endogenous expression of *bcl-2* which promotes cell survival and protects against apoptosis and cellular necrosis in neurological disorders such as stroke (DeVries et al., 2001). Furthermore, both dysregulation of peripheral noradrenergic neural networks and elevated hippocampal corticotropin-releasing hormone (CRH) caused by chronic stressors have been linked to the structural and cognitive impairments (see O'Donnell et al. (2004) for review; Contarino et al., (1999), Ivy et al. (2010)). Kerr, Campbell, Thibault, and Landfield (1992) also proposed the Ca^{2+} hypothesis of glucocorticoid-related hippocampal damage. On this hypothesis, the excessive glucocorticoid-receptor activation and resultant increased Ca^{2+} influx may contribute to the hippocampal impaired structure and function (Kerr et al., 1992).

In an alternative line of research on stress-induced cognitive deficits in the absence of any gross morphological changes, some findings, on the other hand, highlight the effect of CORT-associated experiences on neuritic capacities (Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). It is well-known now that exposure to stress results in alterations in hippocampal dendrites, axons and synapses (Magariños, McEwen, Flügge, & Fuchs, 1996; Watanabe et al., 1992). In this perspective, therefore, these potential neuritic alterations may underpin stress-induced functional impairment in the present study. Interestingly, these biological alterations in turn may feature a different profile of structural and functional consequences in the presence of stress compared with corticosterone administration (Fuchs & Flügge, 2003; Kim & Diamond, 2002).

4.3. Stress can have more detrimental effects than mere elevated corticosterone

Elevated level of plasma CORT is a prominent neurohormonal correlate of stress-related emotionality in rodents (Metz et al., 2005; Sutanto & de Kloet, 1994). There is a corollary, however, that should be noted with respect to stressed animals and neurohormonal responses to stress: stressed organisms may experience high emotionality that can modulate the normal biobehavioural

responses via unknown neurohormonal pathways (Herman & Cullinan, 1997; Ramos & Mormède, 1998). Our results revealed a unique feature of stress effects on hippocampal structure and function when compared with the effect of pre-stroke CORT treatment. Rats that had experienced stressful episodes prior to stroke showed enhanced tissue loss and functional deficit that did not occur in rats with pre-stroke exogenous CORT. This observation suggests that, compared with other groups, only stressed rats are vulnerable to insults to the hippocampus. The differing consequences of stress and CORT alone in the present study provide some support for the previous suggestions that some of the effects of stress on the brain may occur through a non-adrenocorticotropin-mediated mechanism (De Souza & Van Loon, 1982). For instance, stress may affect hippocampal function via a mechanism by which some growth factors such as brain-derived neurotrophic factor (BDNF) are decreased (Duric & McCarson, 2005; Pizarro et al., 2004; Smith, Makino, Kvetnansky, & Post, 1995). In addition to BDNF, it has been shown that stress decreases the hippocampal progenitor cells, an evidence for suppressed neurogenesis in the hippocampus (Rosenbrock, Koros, Bloching, Podhorna, & Borsini, 2005). Because neurotrophic factors are the key elements for neuronal plasticity in the adult brain, stress-induced deficits in hippocampal function after stroke clinically emphasizes on the importance of reducing stress and stressful experiences among high-risk populations.

In addition to stress-induced physiological changes, it is now well established that stress effects are generally associated with high emotionality characterized by the subjective experience of strong feelings, anxiety, frustration and fear (Greenberg, Carr, & Summers, 2002; Lupien, McEwen, Gunnar, & Heim, 2009). Stress-induced high emotionality particularly after uncontrollable and unpredictable stress as well as fearful experiences during stress can simply make stressed animal more susceptible to devastating structural changes than when it experiences only elevated plasma CORT. Similarly, different profiles of stress and CORT effects on the basolateral amygdala (BLA) have also been described (Kavushansky & Richter-Levin, 2006). Therefore, it seems unjustified to define a stress state based upon only the hypothalamic–pituitary–adrenal (HPA) activity or elevated plasma CORT.

Interestingly, the profile of stress-induced structural deficits in the present study indicates that dorsal and ventral hippocampus may respond differently to stress experiences. It has been previously shown that dorsal and ventral hippocampus differ in their anatomical organization (Weible, O'Reilly, Weiss, & Disterhoft, 2006), as well as in their diverse role during locomotor activity (Zhang, Bast, & Feldon, 2002), spatial navigation (Gallagher & Holland, 1992; Jung, Wiener, & McNaughton, 1994; Zhang, Pothuizen, Feldon, & Rawlins, 2004, see also Bannerman et al. (2004) for review), stress and anxiety (Bannerman et al., 2002; Maggio & Segal, 2009; Muchimapura et al., 2002; Venero et al., 2002) and stroke (Daisu, Hatta, Sakurai-Yamashita, Nabika, & Moritake, 2009). Our findings revealed that the dorsal hippocampus is structurally more affected by stress-related disturbances when compared to ventral part of the hippocampus. This structural dorsal–ventral diversity and patterns of alterations induced by stress history might be related to differential neural systems involved in stroke and stress. In this context, one can consider the various connections of the dorsal and ventral hippocampus with different sets of extrahippocampal structures (Siegel & Tassoni, 1971; Swanson & Cowan, 1977) particularly the amygdala that has been implicated in the regulation of central emotional processing (Fuchs & Flügge, 2003). Hence, clarification of these differences might provide clues as to the hippocampal connections that are involved in the pathophysiology of cognitive deficits following stroke and stress-dependent diseases. Taken together, future studies should determine the mechanisms by which stress history produces regional effects within the hippocampus following ischemic stroke.

5. General conclusion

Our results emphasize the existence of deleterious effects of pre-stroke stress on hippocampal structure. These effects have not been found with treatment with corticosterone alone prior to ischemic stroke. Stress history also impaired the hippocampal function after stroke, as measured by performance in the ZT. That the pre-stroke exposure to stress enhances tissue loss in the hippocampus and impairs hippocampus-dependent functions in the ZT stands in marked contrast to the facilitative effects of post-stroke stress and CORT-related experiences that have been previously reported (Faraji et al., 2009). The opposing effects of pre- and post-stroke CORT and stress not only attest to the diversity of glucocorticoid actions in the stress response (Sapolsky, 2000), but also provide some firm evidence for the dynamic interplay of neuroendocrine and behavioural mechanisms (Johnson, Kamilaris, Chrousos, & Gold, 1992). The most important aspect of destructive and facilitative consequences of stress and CORT in such studies is that they provide a provocative hypothesis in which the detrimental effects of glucocorticoids and/or emotional disturbances may occur through mechanisms that are potentially different than the neurobiological mediators underlying facilitative effects of stress.

One limitation for the results in the present study that need to be acknowledged and addressed is the lack of separate groups for CORT and stress. A four-group design has been chosen in the present experiment because the ZT requires longer time to train and test the subjects compared to the other open-field tasks such as MWT (Faraji et al., 2008). Hence, more and larger groups in this study could potentially impose the effects of other confounding and unwanted factors to our final results. While the authors cannot ignore this methodological shortage, they still believe that the four-group design could sufficiently provide a preliminary answer for the questions presented in this study.

The second limitation refers to employing ET-l model of stroke in the present experiment. Because ET-1 can indirectly affect post-stroke recovery through the regulation of oligodendrocyte development (Gadea, Aguirre, Haydar, & Gallo, 2009) and activity (Kallakuri, Kreipke, Schafer, Schafer, & Rafols, 2010), an alternative model of stroke in future investigations may help to confirm the results of the present study.

Overall, our preliminary results in this study may open more windows into the better understanding of stroke, stress and stress-relevant conditions. However, many other questions regarding the brain function under stress conditions and post-ischemic alterations by stressful experiences still remain to be answered.

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