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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.



## 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).