

## Brief Communication

### Neonatal dexamethasone exposure in rats resulted in hippocampal learning and memory defects with decreased convulsion threshold later in adult life

*Shubbar N. Qaheri, MD, AB Psych, Aysba B. Ali, MD, Amal A. Alalwaan, MD, Fatma A. Ahmed, MD, Maryam M. Ahmed, MD, Amer H. Kamal, PhD.*

Premature infants may be born with lung disease and difficulty in breathing. Dexamethasone (DEX) is used widely for prevention and treatment of such cases. A previous study showed that later in adulthood, neonatal DEX treatment had a significant deteriorating effect on hippocampal synaptic plasticity in the form of defects in long-term potentiation (LTP), induction, and expression.<sup>1</sup> Therefore, our objectives in this study were to investigate the consequences and effects of neonatal administration of DEX on hippocampal functions in adult mice.

This study was carried out in the Physiology Department, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain between September 2011 and June 2012. All experiments were carried out when the animals were 3-4 months of age, and the Committee for Animal Experimentation of the Arabian Gulf University approved all experimental procedures. Pregnant Sprague-Dawley rats weighting 250-280 g were individually housed once they were 10 days pregnant. On the day of birth (day 0), pups were removed from their nests and 6 pups were randomly placed back with each dam. Newborn rats were intraperitoneally injected with dexamethasone-21-phosphate on neonatal day one (0.5 µg/g body wt), day 2 (0.3 µg/g), and day 3 (0.1 µg/g) or with equal volumes (10 µl/g) of sterile pyrogen free saline (SAL). Assessment of learning and memory was conducted using the Morris water maze. At 3 months after DEX (n=6) or SAL (n= 6) administration, animals were assessed daily for a total of 5 days in the Morris water maze. The water maze pool is 140 cm in diameter, and was filled with water to a depth of 30 cm. The water temperature was kept around 20-22°C. Each rat was given 5 acquisition trials/day for 5 consecutive days to learn the position of a hidden 'escape' platform

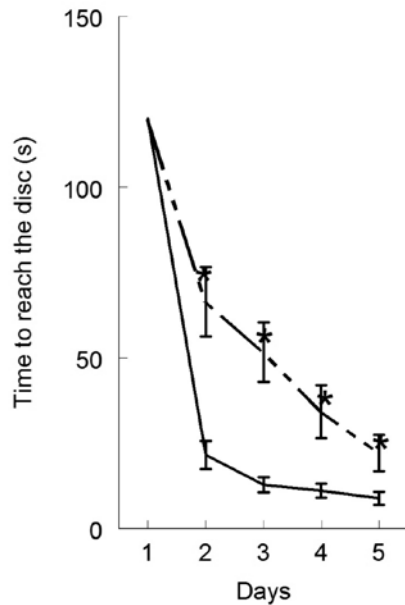
submerged 2 cm below the water surface at a fixed location inside the pool. The pool was divided into 4 quadrants. In each trial, the animals were released from each quadrant in a predetermined way. The starting position was varied on each trial in a quasi-random sequence. Animals were given a maximum of 2 minutes to find the platform, and were allowed to remain on the platform for 30 seconds. The experimenter put rats that failed to locate the disc onto it.

The position and movement of the animals, in the pool, were captured and analyzed every 0.2 seconds, using a video-camera computer system, and the ANY-maze video-tracking system (Stoelting Co, Wood Dale, IL, USA). Outcome measures were latency time, and distance swam to reach the platform. Performance in the 5 daily trials was averaged to yield one data point per rat per day. Animals were administered with doses of the epileptogenic pentylenetetrazole (PTZ) (Sigma P6500, Oakville, Ontario, Canada), 3 months following DEX or SAL administration to determine susceptibility for convulsions. Following PTZ administered (7-140 mg/kg intraperitoneally dissolved in saline) latency and probability of seizures were compared within (namely, across days) and between treatment groups.

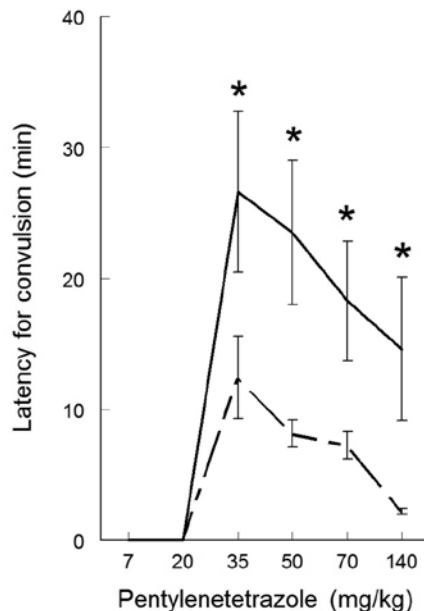
All statistical analyses were performed with Microsoft EXCEL (version 2010). Comparisons within- and between- treatments groups were conducted using analysis for repeated measures ANOVA test. For convulsion latency, between groups comparisons were made by student's unpaired t-test. Data were expressed as mean ± SEM. Statistical significance was set at a *p*-value of less than 0.05.

During the experiment, a weekly body weight measurement revealed slightly lower values recorded in the DEX compared with the control SAL treatment groups. During acquisition, the control, and DEX-treated animals showed improvements in their performance over the 5 days training period, as shown by the reductions in time (so called 'escape latency'), and distance swam to reach the platform (Figure 1). However, these improvements were greater in the control SAL group compared with the DEX-treated animals on days 2, 3, and 4 (escape latency, *p*=0.0215; distance, *p*=0.0096) of the trials. The swimming speeds were no different within each group or between the groups across all 5 days during the acquisition trials. Comparison of probability and latency of convulsions between the control (SAL) and DEX-treated animals were investigated by administration of PTZ. Convulsions occurred at a low threshold dose of PTZ in both DEX-and SAL-treated animals (35 mg/kg). In both groups, the greater the PTZ dose

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**Figure 1** - Learning curve in Morris water maze of DEX-treated (dashed line) and control (continuous line) neonatal rats. The control animals reached the hidden platform with significantly lower latency than the DEX-treated rats. DEX - dexamethasone



**Figure 2** - The convulsion threshold of DEX-treated (dashed line) and control (continuous line) neonatal rats was measured by pentylentetrazole injection using 6 different concentrations. The latency to the appearance of convulsion was significantly shorter in the DEX-treated animals when the injected pentylentetrazole concentrations were higher than 35 mg/kg body weight ( $p < 0.05$ , t-test). DEX - dexamethasone, \* - indicates significant difference at the 0.05 level between DEX-treated and the control group.

beyond threshold, the shorter the latency response. However, the latency responses were comparatively shorter in the DEX-treated animals compared with controls ( $p = 0.0004$ , Figure 2). All DEX-treated animals convulsed following administration of PTZ at doses of 50 mg/kg body weight and greater; for control animals the probability of convulsion induction showed that only approximately one third of the animals showed convulsion after streptozotocin injection of 50 mg/kg. All animals were convulsing at doses of 70 mg/kg and 140 mg/kg (data not shown).

In this study, we found that spatial learning is impaired in adult rats that had been treated with DEX as neonates. This cognitive deficit appeared to be associated with decreased threshold and increased probability of convulsion in response to increasing concentrations of PTZ injections. Our water maze findings agree with and extend those of others who reported similar acquisition deficits in the reference memory version of the water maze in adult rats treated with DEX in the first week of life. A previous study demonstrated defects in function and composition of the hippocampal N-Methyl-D-aspartate receptors in adult rats that were exposed to DEX in their neonatal life. The defect in these receptors compromised the hippocampal synaptic plasticity, and cognitive performance in these animals.<sup>1</sup> The animals showed decreased LTP and increased long term depression expression. The present study shows defects in hippocampal functions of learning and memory. With previously published data on the electrophysiology of the hippocampus in these animals, the present data strongly suggest that neonatal DEX affects the integrity and function of the hippocampus later in life. Pentylentetrazole is a GABA blocker and is known to cause epileptic seizures and brain damage. Pentylentetrazole was frequently used to study the seizure threshold in animals and to evaluate anticonvulsant therapies. The hippocampus is implicated in temporal lobe epilepsy. Since the hippocampal learning and memory functions were affected by neonatal DEX administration, it was expected that convulsion threshold in these animals would be affected as well. Research reports showed that treatment of epileptic animals with exogenous corticosterone increased the interictal epileptiform activities.<sup>2</sup> After prolonged exposure to stress, the hippocampus, which is implicated in temporal lobe epilepsy, showed alteration in dentate granule cell proliferation and changes in synaptic plasticity.<sup>3</sup> It is well known that the excitability of cortical and hippocampal neurons is regulated by the highly expressed cortisol receptors.<sup>4</sup> In normal, non-epileptic animals, corticosterone increased

the frequency of miniature excitatory postsynaptic currents and enhanced L-type calcium currents, depending on the timing of treatments and presence of other neuromodulators.<sup>5</sup> Most neural networks and brain maturation and myelination take place during the perinatal period. It is highly expected that during this period of brain development the effect of stress (or stress hormones) on brain function would be prominent.

The main conclusion of this study was that neonatal corticosterone administration could have a severe compromising effect on brain function, especially learning and memory, and also brain electrical excitability resulting in decreased threshold for convulsion. Therefore, it is suggested that corticosteroid hormones administration in neonatal life should not be used unless strongly advised.

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*From the Departments of Psychiatry (Qaheri), and Physiology (Ali, Alalwaan, Ahmed F, Ahmed M, Kamal), College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain.*

*Address correspondence and reprint requests to: Dr. Shubbar N. Qaheri, Department of Psychiatry, College of Medicine and Medical Sciences, Arabian Gulf University, PO Box 40309, Manama, Kingdom of Bahrain. Tel. +973 39633302. Fax. +973 17422828. E-mail: qaheri.clinic@gmail.com*

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