Effect of Dehydroepiandrosterone Sulfate on the Modulation of Memory and Learning in Sprague-Dawley Rat Hippocampus

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Abstract. Dehydroepiandrosterone sulphate (DHEAS) is a neurosteroid that is found in greater concentration within the brain rather than in any other body organ (Corpechot et al., 1981) and studies have shown that in the brain, DHEAS has a role in enhancing both learning and memory (Markowski et.al., 2001). This present study investigated the relationship between DHEAS and spatial learning and memory in the rat hippocampus. Male Sprague-Dawley rats were divided into two groups and their spatial learning behaviour was evaluated with the Morris Water Maze. The intensity of DHEAS was simultaneously recorded in real time via the Fiber Fluorescence Microscopy (FFM) S-650 probe of the Cellvisio system. There were significant changes in the swimming pattern of the experimental groups obtained via the Morris Water Maze from day 1 until day 5 and day 6 for the probe test. Meanwhile, it was also seen that the intensity of DHEAS fluorescence increased in parallel to the swimming pattern of the experimental rats in comparison to the control group. The findings suggest that the changes in DHEAS fluorescence has a strong link to both spatial learning and memory.

Keywords: DHEAS, Imaging, Learning and memory, Morris water maze

INTRODUCTION

Baulieu (1991) was the first demonstrate neurosteroids that are synthesized *de novo* in the rat brain. Dehydroepiandrosterone sulfate (DHEAS) is one of vital neurosteroids, which decreases with age (Sorwell & Urbanski 2010). Previous studies have been shown that DHEAS declined during brain aging in humans, animals, and cellular model; paralleled by a loss of memory (Racchi et al., 2003; Caruso et al. 2013; Grimm & Eckert 2017). The result showed that the concentration of DHEAS was higher in the brain than in the plasma (Corpèchot et al. 1981; Dong & Zheng 2011). However, due to low levels of steroidogenic proteins in the brain and lack of sensitivity of the existing technique, neurosteroidogenesis has not been well elucidated (Kawato et al. 2003).

DHEAS act as modulator of N-methyl-d-aspartate (NMDA) receptors in the brain. The finding reported that the enzymes P450scc and P450c17 for conversion neurosteroids from cholesterol were stimulate of NMDA receptor (Kimoto et al. 2001; Maninger et al. 2009). Several studies suggest that DHEAS can be effective in memory and learning enhancement in animals as well in humans. DHEAS levels correlate with cognitive impairments such as Alzheimer's disease and characterized by age and stress-related cognitive (Corrêa et al. 2016). Some studies showed that DHEAS as a neuroprotective against of cognitive disorders such as Alzheimer's disease (Aly et al. 2011). These results suggested DHEAS play a therapeutic role in the cognitive impairments such as Alzheimer's disease.

DHEAS have been reported that effect memory enhancement in humans and rodents. First reported that

DHEAS significantly enhanced retention of footshock active avoidance training (Flood et al. 1988). In animal models, the performance significantly decreased of latencies and distances. The findings may prove to be relevant a role of DHEAS in memory enhancement. Administration of DHEAS showed that aged animals took longer time to reach the platform but the finding did not improve spatial cognition either young or aged animals (Bodensteiner et al. 2008). A review of the literature found a positive correlation of relationship between DHEAS and global cognition in women and men, and positive correlations with working memory, attention and verbal fluency in women only. These findings demonstrate that DHEAS may affect the cognitive function (De Menezes et al. 2016).

Specifically, we have investigated the effects of DHEAS on the modulation of learning and memory in Sprague-Dawley rat hippocampus.

MATERIALS AND METHODS

Subjects

Adult male Sprague-Dawley rats of 8 weeks of age and weighing 250 - 280 g were procured from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia, Kelantan, Malaysia. They were maintained under controlled conditions $(27 \pm 2^{\circ}C)$ temperature; $40 \pm 5\%$ relative humidity). Animals were given standard rat pelleted feed and water *ad libitum* with a 12 h light-dark cycle with lights going on at 08 00h. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Surgery

For implantation guide cannula, animals were anaesthetised with isoflurane gas (3-5% to induce, 1.5% maintenance) and positioned in stereotaxis frame. A midline skull incision was made. Using motorised stereotaxis, guide cannula was implanted into the CA1 region of the hippocampus (anterioposterior, -3.00 mm; mediolateral, 1.0 mm; dorsoventral, 1.25 mm from bregma) (Paxinos & Watson 2004). Guide cannula were permanently mounted to the skull with three screws and dental cement. Animals were allowed one week to recover from surgery.

Fluorescence Labeling

After recovery from surgery the day of behavioural task, freely moving rats were injected with DHEAS-Alexa Fluor 488 dyes. Injection needles were connected with polyethylene tubing to a microsyringe (10 μ L) driven by the infusion pump of motorised stereotaxis (brand). Solutions were infused during 1 min at a constant rate of 0.5 μ L/min. Control rats received the same volume (0.5 μ L) of the same dye at the same rate of infusion. Injection needles were removed from the guide cannula 10 min after infusions in order to prevent drug reflux.

Morris Water Maze (MWM) Task

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Spatial learning and memory function in rats was assessed in the MWM task by conducting hidden platform acquisition and probe test (Vorhees & Williams 2006; Ashraf & Ismail 2015). Rats were placed in the water at point A. On each trial, if rats did not find the platform within 60 s, the experimenter guided them to it. Rats remained on the platform for 10 s to allow orientation to visual cues. Rats were allowed to rest for 2 min between trials. Rats were tested in four trials a day for 5 consecutive days. After training acquisition, a probe test was conducted by removing the platform. The rats were allowed to swim and recorded the percentage of time that they spent in the target quadrant and non-target quadrant.

Imaging with Fiber Fluorescence Microscopy (FFM)

The S-650 FFM probe with a holder was placed into the mount of the stereotaxis device. The probe was fixed on top of the implantation and set the bregma point. Fluorescence labeling were injected before and after behavioral task. The expression of DHEAS were calculated of the intensity of DHEAS in the CA1 region of the hippocampus. The FFM probe was connected to Cell visio Lab system, and the excitation and emission of cells were measured at 488 nm and 594 nm, respectively.

Statistical Analysis

Data were represented as mean \pm SEM. Analyses involving two data sets were performed with a two-way ANOVA for Morris water maze task and analysis paired t-test for quantifying the DHEAS of modulation learning and memory. Significance was set as a *p*-value of less than 0.05. We performed statistical analysis using graph pad Prism software.

RESULTS AND DISCUSSION

During training, learning process occurred by the measurement of escape latency by time elapsed the rats found the platform. Figure 1 illustrates the swimming pattern during training indicating spatial learning occurred.





Figure 2. A) Escape latency, and B) Distance length during the training days. Data are presented as the mean \pm SEM (n = 8 animals). The graph shows statistical significance, p < 0.05 (A) and (B) as determined using two-way ANOVA with Greenhouse-Geisser correction for LSD post-hoc tests.

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Escape latency decreased over the 5 days training period to reach the platform (Figure 2). Analysis twoway ANOVA identified a significant reduction in escape latency over training days (main effect of day, F (4, 60) = 34.32 p < 0.0001). There was a significant interaction effect between days and latencies to reach the platform (interaction effect: F (4, 60) = 2.694 p = 0.0392). For (B), the animals also had significantly decreased of distance length (F (4, 60) = 37.21 p < 0.0001) comparison to control. There was a significant interaction effect between days and distance length to reach the platform (interaction effect: F (4, 60) = 6.036 p = 0.0004).



way ANOVA revealed that a significantly increased percentage of time *F* (3, 48) = 23.28 p < 0.0001) in the target quadrant (zone 1) relative to the non-target quadrants (zone 2, 3, and 4). There was a significant of interaction effect (*F* (3, 48) = 3.535 p = 0.0215). The means of sample in zone 1 (22.49% ± 1.6) spending more time than the other zone.



Figure 3. Probe test administered as an assessment of memory on day 6. A) The swimming pattern while doing the probe test with four trials, B) The graph shows the target quadrant (zone 1) relative to non-target quadrant (zone 2, 3 and 4). Data are presented as the mean \pm SEM (n = 8 animals). Significant different from target quadrant at p < 0.05 as determined by two-way ANOVA with the Greenhouse-Geisser correction for LSD *post hoc* tests.

The probe test was administered on day 6 to assess memory. Data were normally distributed. Analysis two-

Figure 4. Fibered fluorescence microscopy visualise and evaluate the changes of DHEAS level in the CA1 hippocampus. A) The kinetic graph indicates the region of interest of DHEAS signal after behavioural task. B) The expression of DHEAS fluorescence over time, and C) The graph shows the condition on spatial learning and spatial memory. Data are presented as the mean \pm SEM (n = 8 animals). Significant different at p < 0.05 as determined by paired *t*-test with LSD post hoc *t*-test.

To examine the effect of DHEAS on the modulation of learning and memory, we quantified the changes of DHEAS levels occurred during training and probe test. Data were normally distributed. Analysis paired *t*-test were performed (df = 6, t = 36.07, p < 0.0001) and found the correlation between two group (r = 0.63, p < 0.05).

Discussion

The result showed that swimming pattern had significantly decreased in escape latency and distances. The performance of Sprague-Dawley rats in the Morris water maze exhibited significant changes in spatial learning. Mean latencies to find the hidden platform indicating that all animals have learned the location of the platform over 5 days training. Similarly, the finding of rats required less time to find the platform and increased the time and path length in the target quadrant (Galea et al. 2000; D'Hooge & De Deyn 2001; Barnhart et al. 2015). Recently reported that demonstrated consistent decreased the escape latency over a 5 days training period (Frye & Lacey 1999). A few factors when evaluating and comparing the effectiveness of DHEAS on learning and memory are time, dosage, route of administration, and testing paradigm (Bodensteiner et al. 2008). Researchers have reported that no effect on the Morris water maze performance but has effects on the Y maze performance with injection (s.c.) of DHEAS into ovariectomized rats (Frye & Sturgis, 1995).

Sample that we did the behavioural task during training days took a longer time and distance length on day 1 until day 3. Then, the animals were made learning process and become faster to reach the platform on training day 4 and day 5. This finding suggest that the animals having stress and hard to learn on day 1 until day 3. The implantation of the skull and the surgery process may influence learning process. Some studies have shown DHEAS level associated with depressed mood and anxiety disorders. Association was independent of age, lifestyle, and weight change (Walton et al. 1995; Elizabeth et al., 2015).

Data was collected during the probe test and significantly increased the percentage of time in the target quadrant by given meaning of time within 1 minute. The rats have learned the location of platform, and tried to recognise and remember the location. Therefore, the rats swim and spend more time in the target quadrant (Yin et al. 2013). Similar findings, the weanling mice had significantly increased the percentage of time at the target quadrant (Barnhart et al. 2015). Some studies observed that significantly increased the performance in working memory (Aasebø et al. 2018). This study suggested memory enhancement in rats (Galea et al. 1999; Aasebø et al. 2018).

By using FFM probe connected to Cell visio Lab System, the changes of DHEA level can be imaged, recorded and quantified. Several studies have previously investigated this approach enable high speed, high resolution, minimal invasiveness while insert the probe, and allowed the researchers to monitor the signal of fluorescence in real-time imaging (Davenne et al. 2005; Vincent et al. 2006; Chen et al. 2015). The result of the present investigation are consistent increases in probe test over the time of the DHEAS fluorescence intensity in parallel to the swimming pattern of the experimental rats indicated that the changes of DHEAS level in the rat hippocampus obtained. In this study, the evidence that DHEAS effect the modulation of spatial learning and memory enhancement during behavioural test. Researchers have been reported that fluorescence increase in the region of interest while adding bicuculline in response to calcium activity (Vincent et al. 2006). On the other hand, GCaMP3 to detect the calcium signals in dopamine neuron demonstrated that the changes of fluorescence intensity (Gore et al. 2016).

Collectively, the correlation of DHEAS level in the rat hippocampus with the behavioural task for learning and memory can be determined. This finding is the first report of DHEAS fluorescence in the rat hippocampus due to modulation of learning and memory. In addition, the hippocampus, an important location in learning and memory and cognitive impairments in Alzheimer's disease. Cell visio can be one of the new insights to understanding the role of DHEAS in improving learning and memory impairments associated with diseases such as Alzheimer's disease.

CONCLUSIONS

The intensity of DHEAS was simultaneously recorded in real time via the Fiber Fluorescence Microscopy (FFM) S-650 probe of the Cell visio system. There were significant changes in the swimming pattern of the experimental groups obtained via the Morris Water Maze from day 1 until day 5 and day 6 for the probe test. Meanwhile, it was also seen that the intensity of DHEAS fluorescence increased in parallel to the swimming pattern of the experimental rats in comparison to the control group. The findings suggest that the changes in DHEAS fluorescence has a strong link to both spatial learning and memory. Cell visio can be one of the new insights to understanding the role of DHEAS in improving learning and memory impairments associated with diseases such as Alzheimer's disease

ACKNOWLEDGEMENTS

This study was supported by Fundamental Research Grant Scheme; FRGS (203/PPSK/6171153) and P3Neuro (304/PPSK/652204/K134).

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