

Effects of REM Sleep Deprivation on CA1 Hippocampal Glucocorticoid Receptor Expression and Emotional and Cognitive Behavior in Rats

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Background:

Rapid eye movement (REM) sleep deprivation (SD) has been implicated in impairment of hippocampal-dependent memory and in increased emotionality possibly linked to HPA axis hyperactivity. Glucocorticoids represent an important regulator of allostasis in response to various stressors. Among brain regions, CA1 hippocampal pyramidal neurons are rich in glucocorticoid receptors (GR). Glucocorticoids plasma levels are elevated after SD but alterations in GR expression in the brain following SD remains to be characterized. Thus, the present study aims to investigate the impact of acute (ARSD) and chronic (CRSD) REM sleep deprivation on emotional arousal and memory performance of rats in an open field environment, the Y Maze and the Morris Water Maze, respectively. We also assessed expression of GR in the hippocampus by immunohistochemistry.

Method:

Animal groups and sleep deprivation procedure:

Male Long Evan rats (N =50) , weighing between 250–400g were separated into five groups. The acute REM sleep deprivation ARSD rats (n=5) underwent 4 hours of REM sleep deprivation for one day while the chronic REM sleep deprivation CRSD animals (n= 4) underwent 4 hours of REM sleep deprivation everyday for five days using the small platform sleep deprivation paradigm. The acute and chronic control groups, ACG (n= 6) and CCG (n= 4), respectively, were placed on a larger platform. An additional control group was formed of rats remaining undisturbed in their home cages (HC; n= 4) throughout the experiment.

Behavioral testing:

Open field test:

Rats were placed in a random corner of the open-field area (LWH: 75 cm × 75 cm × 30 cm), and locomotion and exploratory behavior examined for 10 minutes by quantifying the amount of locomotion (i.e., crossed squares) in the central and peripheral zones.

Aversive Learning in the Y Maze:

Rats learned to avoid an arm of the Y Maze that greeted them with an air-puff. Learning was assessed by measuring the latency to re-enter the branch in which the rat had received the aversive stimulus and the number of risk assessments performed.

Morris Water Maze

After the habituation trial, each rat underwent 12 consecutive trials in which the rat would attempt to find the platform with an interval of approximately ten minutes between trials. The platform alternated location between trials, by being placed on the left or the right from the starting quadrant where the rats initiate the task. Spatial learning was determined by measuring the distance traveled to find the platform.

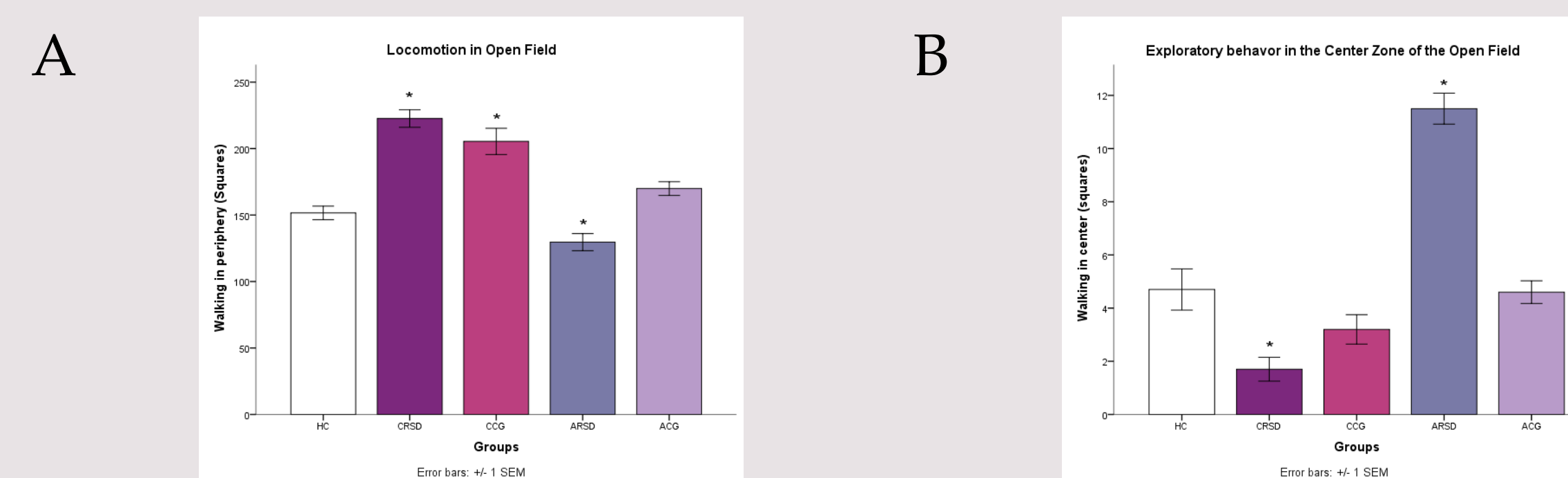
Immunohistochemistry:

Three days after the end of the behavioural testing, the rats received an euthanasol injection and were decapitated. The brains were retrieved, frozen in dry ice and stored at -80 C . 14  m brain slices were subsequently obtained using a cryostat and immunoreactivity for glucocorticoid receptors (GR-ir) was assessed. Brain sections were processed with a primary rabbit GR antibody (dilution of 1:500; Santa Cruz Biotechnology, Inc.) and a secondary Donkey anti-rabbit antibody (dilution 1:500; Invitrogen Corporation). Using a fluorescence equipped microscope, GR positive labeled cells were examined. GR immunoreactivity was quantified from digital images using Image J software (Image J, National institutes of health). Optical densities (Mean grey values), estimates of the staining intensity were obtained by using the threshold technique. This technique required to first subtract the background and then measure labelled areas. Data are presented as background corrected standardized image densities for each brain.

Results:

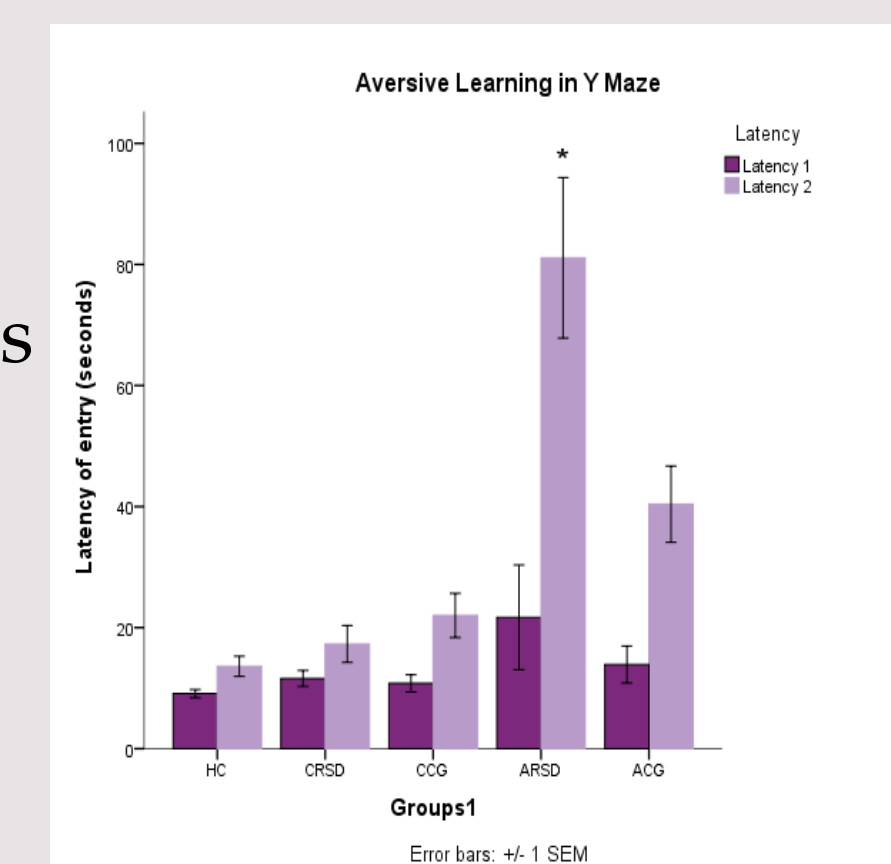
Open Field

Fig.1. Effects of REM sleep deprivation on locomotion and exploratory behavior of rats in the Open Field arena: (A) the CRSD and CCG showed increased locomotion in the peripheral zone as compared to other groups while ARSD showed decreased exploration. (B) In contrast, ARSD showed increased exploration of the anxiogenic center zone. Reported differences were significant at $p < .05$. Values are expressed as mean \pm S.E.M.



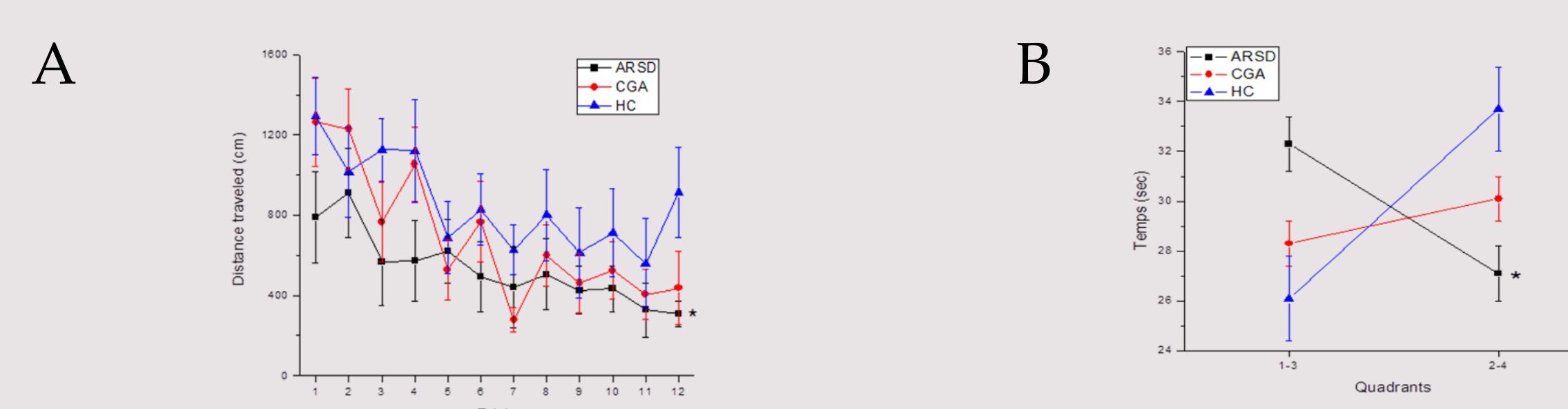
Aversive Learning in the Y Maze

Fig.2. Effects of REM sleep deprivation on aversive learning: the ARSD group showed a significant difference from all other groups characterized by increased latency to re-enter the aversively conditioned arm compared to all other groups. Values are expressed as mean \pm S.E.M. and differences significant at $p < .05$.



Spatial Learning in the Morris Water Maze

Fig.3. Effects of REM sleep deprivation on the distance traveled and the latency to reach the platform: (A) The ARSD group travelled less distance to reach the platform than all other groups. (B) The ARSD group spent less time in quadrant 2-4 where no platform was placed than all other groups. Values are expressed as mean \pm S.E.M and reported differences significant at $p < .05$.



GR-ir in the CA1 of the hippocampus:

Fig.4. Photomicrographs showing immunohistochemical staining of GR in the CA1 field of the hippocampus taken from chronic REM sleep deprived rats (CRSD), acute REM sleep deprived rats (ARSD), chronic control group rats (CCG), acute control group rats (ACG) and home cage rats (HC). There was a significant increase in the density of neurons for the CRSD group.

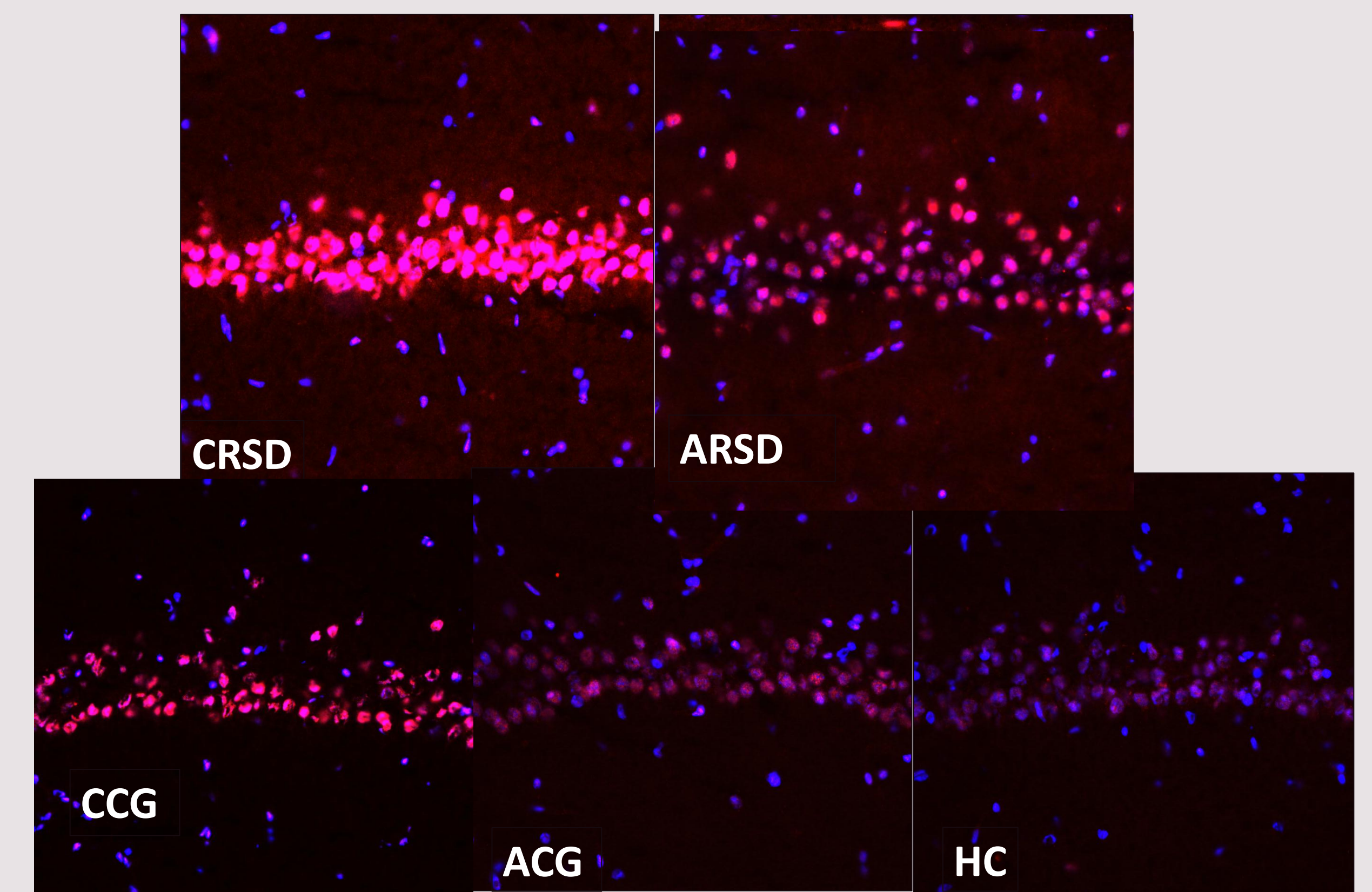
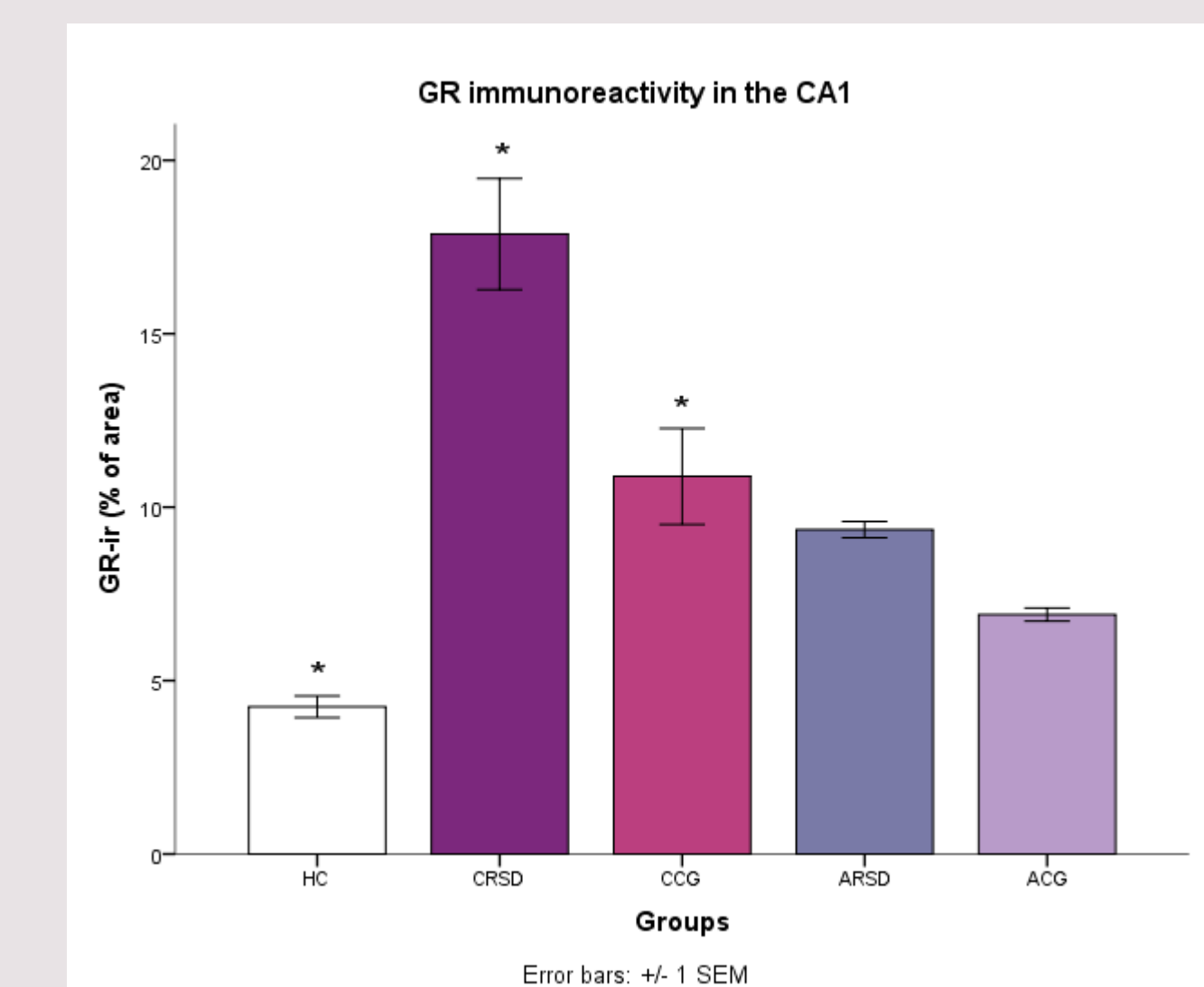


Fig.5. Average percent area \pm SEM for the chronic REM sleep deprived rats (CRSD), acute REM sleep deprived rats (ARSD), chronic control group rats (CCG), acute control group rats (ACG) and home cage rats (HC). The CRSD, CCG and the HC differentiated significantly. * $p < 0.05$



Conclusion:

In the current study, we were interested in changes in behavior and GR immunoreactivity in the hippocampus following REM sleep deprivation. At the behavioral level, our findings indicate that CRSD rats show increased locomotion and exploratory activity in the periphery of an open field arena, while opposite effects were found for the ARSD group, which spent increased time in the open area. These findings suggest differential arousal and/or anxiety level in these animals. Interestingly, the ARSD group showed enhanced memory to an aversive stimulus, as well as enhanced spatial memory in the water maze as compared to the CRSD and control groups. Immunohistochemical analysis showed that GR was markedly increased in CA1 neurons of the hippocampus of CRSD while mildly increased in ARSD as compared to controls. We conclude that acute and chronic RSD confer differential effects on emotional and spatial learning performance, which may be related to alterations in brain glucocorticoid receptors. Increased hippocampal vulnerability to stress may play important roles in the persistent emotional arousal induced by Sleep Deprivation.