Dipsogenic Role of Angiotensin II during Fear Memory Retrieval

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Background

Previous studies (Marren 1994, Frenkel 2005, Shi 2011, Sierra 2013) have shown the influence of endogenous processes like water and sleep deprivation on behavioral responses and their impact on consolidation of fear memory in various species

Renin angiotensin system (RAS) and its components have been implicated in fear memory, learning and behavior in both rodents and human.

Brain angiotensin II (AngII) is a potent thirst producing peptide that can also modulate threat learning and memory by impacting the physiological internal state.

We hypothesized that acute water deprivation (WD), with subsequent increases in endogenous AngII, contributes to fear memory retrieval plasticity.

Methods

Animals: Adult male (3–4 months old) C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME, United States) were used for all experiments.

Auditory Cue-dependent Fear Conditioning: Fear conditioning consisted of five trials of a conditioned stimulus (CS) tone (30s, 6 kHz, 75 dB) co-terminating with an unconditioned stimulus (US) foot shock (0.5mA, 0.5s,) spaced by 3min 30s inter-trial intervals.

Contextual Fear Conditioning: Fear conditioning consisted of three trials of a unconditioned stimulus (US) foot shock (0.7mA, 1s,) spaced by 30s inter-trial intervals. The retrieval and memory tests were performed in the same chamber

Retrieval Protocol: Twenty-four hours after fear conditioning, animals were water restricted for 18hrs and then re-exposed to the CS to reactivate the memory and initiate reconsolidation. The fear conditioning chamber was modified by (only for auditory FC) replacing the shock grid with a clear Plexiglas floor clear walls of the chamber with patterned construction paper and the chamber was scented with peppermint oil. The mice was given a peripheral injection of either saline (0.2ml ip) or losartan (10mg/kg ip) 10 minutes after retrieval. Water deprivation was maintained 6hrs after memory reactivation to a total of 24hrs. Long-term memory (LTM) was assessed 24hr after memory retrieval and at 1wk in water replete conditions. Animals were returned to the retrieval context and exposed to 4 CS presentations.

Tissue Collection, RNA Extraction and RT-PCR: Bilateral tissue punches were collected from mice and Trizol was used to extract RNA. Gene expression changes were detected from 0.5mm brain punches using Taqman primers.

Summary and Conclusions

- ❑ Water deprivation during ***auditory cue-dependent*** memory retrieval updates the memory and decreases the freezing response. Peripheral administration of losartan, however, blocked this effect.
- ❑ Water deprivation during contextual fear memory retrieval had no effect on the freezing behavior
- ❑ Increased level of Angiotensin II and Agt mRNA expression in SFO are observed after water deprivation. Plasticity marker Bdnf is also modified after water restriction in SFO.

Our findings indicate that, elevated angiotensin II as a result of WD, reduced LTM while altering the expression of specific RAS genes in SFO.

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Results

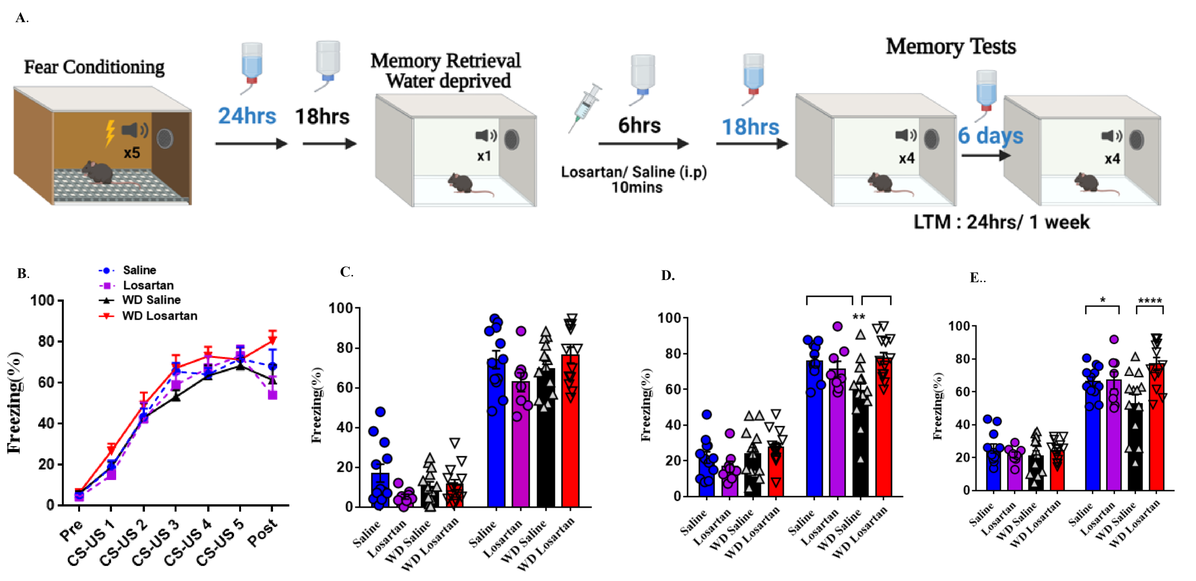

Figure 1: Water deprivation during fear memory retrieval of Auditory Cue-dependent Fear Conditioning reduces Freezing Behavior. (A) Schematic of the conditioning, waster deprivation, retrieval, and testing protocol. **(B)** Average freezing during 5CS-US presentation of fear conditioning. **(C)** Freezing behavior before and during the 1CS retrieval cue in water deprived (WD) mice and non-water deprived controls. The controls were not deprived of water for any time period. Freezing response to 4 CS presentations during the 24hr short-term memory (LTM) test **(D)** and 1wk long-term memory (LTM) **(E)** test in retrieval groups. (n = 11-13/group, #saline vs. losartan); Error bars are ± SEM. *p < 0.05, **p <0.01, ***p<0.001 , *Bonferroni post hoc p<0.05).

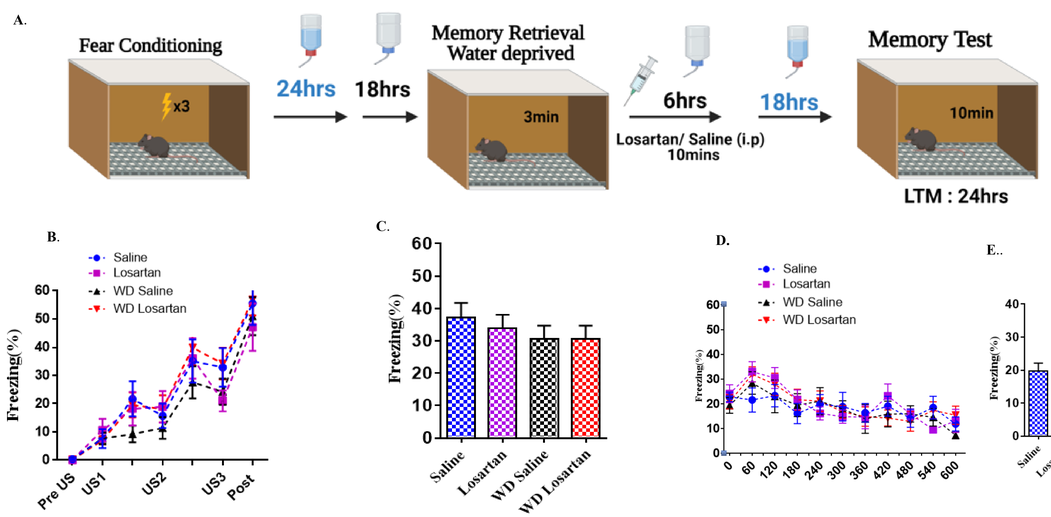

Figure 2: Water deprivation during fear memory retrieval of Contextual Fear Conditioning does not affect Freezing Behavior. (A) Schematic of the conditioning, waster deprivation, retrieval, and testing protocol. **(B)** Average freezing during 3US presentation of fear conditioning. **(C)** Freezing behavior during the 3min retrieval in context for water deprived (WD) mice and non-water deprived controls. The controls were not deprived of water for any time period. **(D)** Freezing response to the memory test 24hr later in water replete condition (LTM) test and **(E)** average freezing for 10mins (LTM) test in retrieval groups. (n = 10/group, #saline vs. losartan); Error bars are ± SEM.

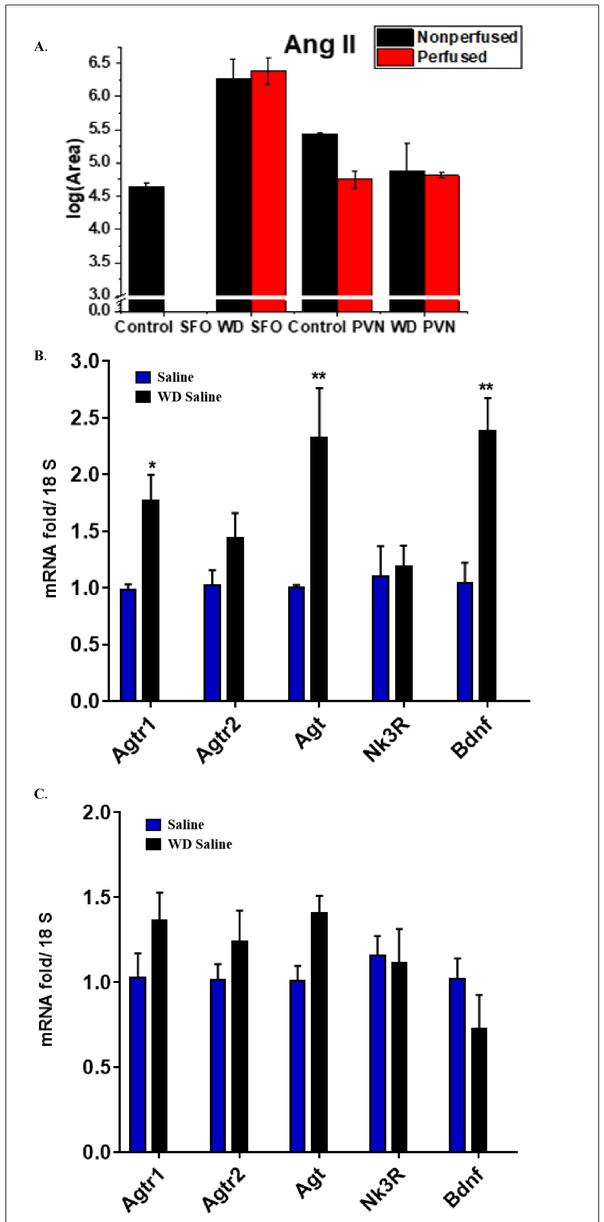

Figure 3: Quantitative Analysis of Angiotensin II and RAS and Plasticity genes in SFO and PVN by Mass Spectroscopy and RT-PCR. (A) Robust detection of all Ang peptides in the SFO and PVN of perfused/non –perfused control and water-deprived (WD) mice. Each of the three biological replicates were analyzed in technical triplicates. Error bars show SD based on technical duplicate–triplicate**. (B)** RT-PCR showing the increased expression of RAS genes –Agtr1, Agt and Bdnf in SFO of WD mice at 18hr time point. (C) No significant changes are observed in the mRNA levels of RAS and plasticity genes in OVN region after 18hr . (n=6; Error bars are ± SEM. *p < 0.05, ***p <0.001 by One way ANOVA – Tukey's test