Role of Sex in the Dorsal Hippocampus Following Trace Fear Conditioning

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INTRODUCTION

- In trace fear conditioning, the conditioned stimulus (tone) and the unconditioned stimulus (shock), is separated by a stimulus-free trace interval (Detert et al. 2008).
- Immediate early genes (IEG) are genes, which are activated transiently and rapidly in response to a wide variety of cellular stimuli.
- IEG's including c-fos, and activity-regulated cytoskeletal-associated protein (Arc) are increased following fear learning (Minatohara et al., 2015).
- Sex differences have been reported in multiple classical conditioning paradigms including eyeblink conditioning and context fear conditioning. Adult female rodents perform better than adult males in both delay and trace eyeblink conditioning (Dalla & Shors, 2009). Additionally, females show stronger context fear conditioning and greater generalization of fear than males (Keiser et al., 2017).
- Very few studies have examined sex differences in classical fear conditioning paradigms.
  - Thus, we investigated:
    - ❑ Sex differences in trace fear conditioning, which requires the involvement of the dorsal hippocampus.
    - ❑ Following trace fear conditioning, we measured neuronal activity markers, called immediate early gene expression (IEG) in the DH.

METHODS

Trace Fear Conditioning

GROUPS	DAY ONE: TRACE FEAR CONDITIONING	DAY TWO: PROBE TEST	DAY TWO: TISSUE COLLECTION
NAÏVE	-	-	Brains were collected simultaneously with pseudo and trace
PSEDUO-CONDITIONG	6 CS and 6 US	2 CS alone	Brains were collected 60 minutes following probe test
TRACE FEAR CONDITIONNG	6 CS-US	2 CS alone	Brains were collected 60 minutes following probe test

Rats received one 6-trial session of auditory trace fear conditioning using a 15 s conditioned stimulus (CS; 80 dB of white noise) followed by a 30 s trace interval (stimulus-free period) and a 1s footshock unconditioned stimulus (US; 1 mA). Control rats were either pseudoconditioned (explicitly unpaired CS and US presentations), or experimentally naïve (never exposed to the training or testing chambers). A PC running FreezeFrame 2.04 (Actimetrics Software, Coulbourn Instruments, Whitehall, PA) controlled the delivery of all stimuli during training and testing.

Western Blots:
Following trace fear conditioning, brains were removed and frozen on dry ice at –80° F. The Dorsal Hippocampus was dissected and homogenized. The samples were then centrifuged and the supernatant was removed and measured using a Lowry protein assay (Bio-Rad). Protein samples were normalized and loaded for SDS-PAGE (10%). Proteins were transferred onto PVDF membranes using a semidry transfer apparatus (Bio-Rad). Membranes were probed using Arc and PKA antibodies. Membranes were then incubated in blocking buffer (2hr), primary antibody overnight at 4° C; 1:500 mouse Arc [Santa Cruz Biotechnology] and secondary antibody (90 min; 1:1000 goat anti-mouse [Santa Cruz Biotechnology]. Membranes were then washed, placed in a chemiluminescence solution (Santa Cruz Biotechnology), and exposed to autoradiographic film (Hyperfilm MP). Images were taken and densitometry was performed using Syngene Gbox. Images were taken with GeneSys software, and densitometry for each band was analyzed with Genetools software (Cambridge, England).

TRACE FEAR CONDITIONING

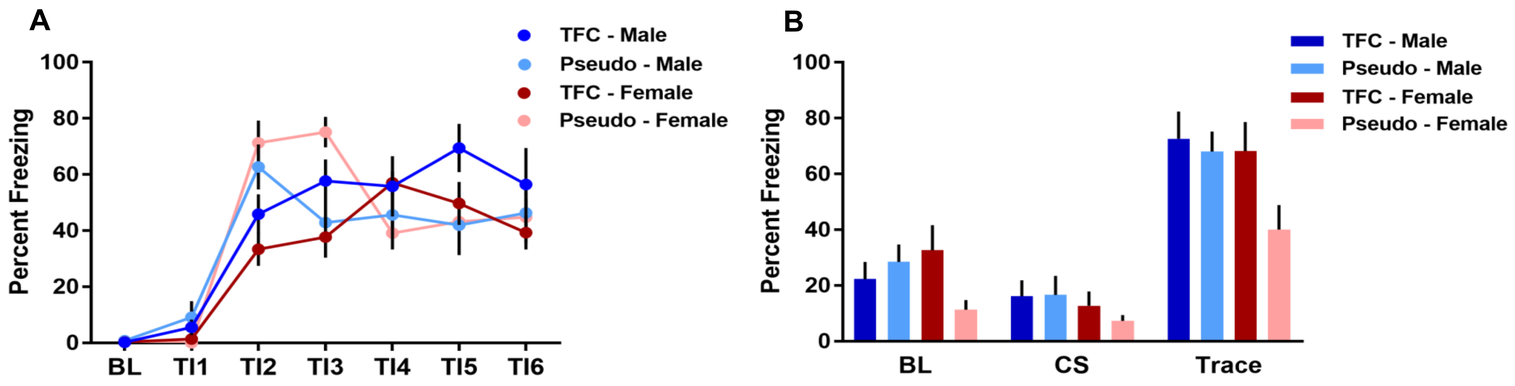

Figure 1A) Behavioral responses of trace fear-conditioned (TFC) males (n = 8), pseudo-conditioned males (n = 7), trace fear-conditioned females (n = 7), pseudo-conditioned females (n = 6). All four groups froze at comparable levels during the trace free ($P > 0.05$). 1B) During the probe test, all groups froze at similar levels during baseline, CS, and trace interval following CS offset ($P > 0.05$).

ARC EXPRESSION IN DORSAL HIPPOCAMPUS

PKA EXPRESSION IN DORSAL HIPPOCAMPUS

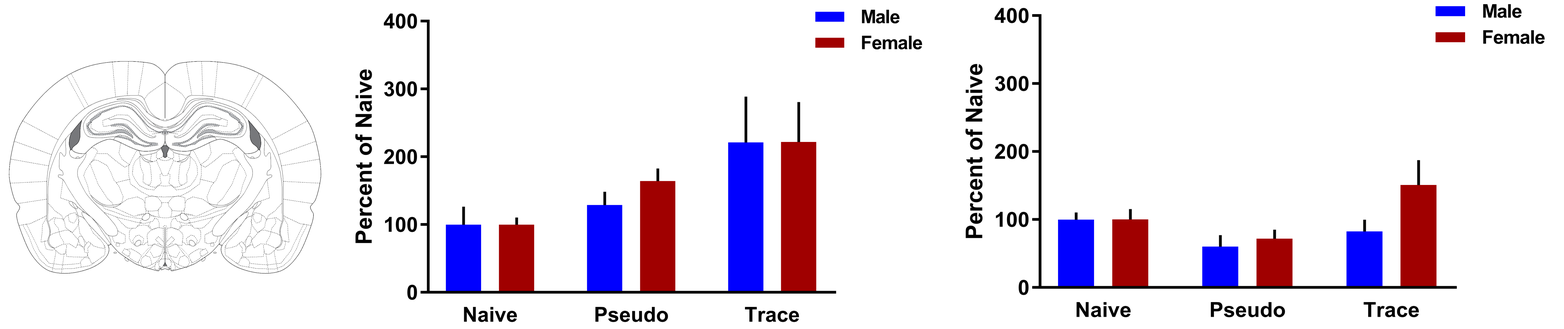

Figure 3) Arc, and PKA expression was fear-conditioned measured in the dorsal hippocampus of naïve males (n = 6), naïve females (n = 6), trace fear-conditioned males (n = 7), trace females (n = 6), pseudoconditioned males (n = 5), and pseudoconditioned females (n = 6).

OBSERVATIONS/CONCLUSIONS

- Trace fear conditioning is comparable in male and female rats
- A trend towards a learning-specific increase in dorsal hippocampal ARC expression in both female and male rats
- A trend towards a learning-specific increase in dorsal hippocampal PKA expression in female rats

LIMITATIONS & FUTURE WORK

- Explore adding additional control groups to tease out learning-specific change in behavior
- Add more subjects and repeat experiments to increase statistical power

DEFINITIONS

Arc is an activity regulated cytoskeletal-associated protein. Arc protein is considered to play a critical role in memory as a marker for plastic changes in the brain. Plastic changes refers to the brains ability to constantly change throughout life. (McIntyre, 2005).

PKA stands for protein kinase A. PKA regulates CREB, a cAMP response element-binding protein. Long term memory formation is dependent on CREB and PKA. (Horiuchi et al., 2008).

Dorsal Hippocampus is located on the upper portion of the rodent brain and is shaped like two bananas joined. The dorsal hippocampus has been found to be responsible for spatial memory, verbal memory, and learning of conceptual information. (Fanselow et al., 2010).

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