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GABA-A Delta Subunit Expression in the Hippocampus of High and Low Licking/Grooming Rats

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BACKGROUND

Adverse early life environments characterized by maternal neglect is **A** associated with alcohol use disorder and anxiety in humans.¹ Similarly, in rodents, maternal care is hypothesized to influence the development of stress reactivity and GABA(A) receptor expression in brain regions that deal with stress responses.² We can investigate maternal care by looking at the frequency of licking/grooming (LG) behavior. ²⁻⁴

High and low LG female offspring Long Evan rats were used in a study investigating the effect of maternal care on the expression of the delta subunit of the GABA(A) receptor in High and Low (LG) groups based on relative frequencies of LG behaviors exhibited in the first week postnatal. ³ We expect to see that Low LG groups will have greater GABA(A) delta receptor expression density in the hippocampus relative to High LG groups.

METHODS

Maternal Observations: Dams were observed and characterized by their frequency of licking/grooming (LG) behavior between postnatal day (PND) 1-6, during 5 daily sessions (every 3 minutes) for 75 minutes, and a total of 750 observations. Female offspring of High (n=8) and Low (n=11) LG mothers were selected for this experiment. Estrous cycle: Female estrous cycle were followed for approximately 8-10 days using vaginal lavage and cell histology.

Tissue Collection: Animals were sacrificed at metestrus with Fatal Plus (Covetrus) and perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Whole brains were dissected and postfixed in 4% PFA and cryoprotected. Brains were flash frozen in isopentane on dry ice and stored at -20 °C until sectioning. Forty-mm coronal sections will be collected at -15 °C using a Leica cryostat. Immunofluorescence Staining: Ventral (VHPC) and dorsal hippocampal (DHPC) tissue was washed in PBS and then permeabilized in blocking buffer (5% normal donkey serum, 1% bovine serum albumin, 22.52 mg/mL glycine, 0.2% Triton X-100, 1X PBS). Slices were incubated in anti-GABRD antibody (PhosphoSolutions, #868A-GDN) and Alexa Fluor 488-conjugated FluoroPan Neuronal Marker (Millipore Sigma, #MAB2300X) diluted in blocking buffer. Slices were incubated in Alexa Fluor 555 donkey anti-rabbit secondary antibody and diluted in blocking buffer. Slices were washed with PBS and incubated in diamidino-2-phenylindole for one minute and mounted. Images were captured at 40X magnification using a Keyence BZ-X800 fluorescence microscope.

Statistic analysis: An ANOVA was used to investigate the effect of maternal care.

GABA(A) DELTA SUBUNIT EXPRESSION IN THE HIPPOCAMPUS OF HIGH AND LOW LICKING/GROOMING RATS

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4. Psychoneuroendocrinology, 76, 29-37.





Figure 1 A-B.

Average cell population density in the dorsal (A) and ventral (B) hippocampus of high and low licking/grooming female rats. No significant effect of phenotype was found.

Figure 1 C-D.

Average density of GABA(A) Delta subunit expression in the dorsal (C) and ventral (D) hippocampus of high and low licking/grooming female rats. No significant effect of phenotype was found.

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