Impact of Social Stress on Brain Vasopressin Expression in Mice

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Introduction

Chronic social stress is associated with a higher risk of mental health disorder, including major depression, PTSD, intermittent explosive disorder, and other forms of anxiety. In the U.S. alone, almost 50 million people suffer from these conditions each year, with staggering economic and social costs.

Our interest is in changes in neurochemical function that accompany stress and may contribute to these disorders. While there is widespread recognition that changes in "stress axis" function are a major sign of these disorders, recent studies point increasingly to the role of peptide hormone neurotransmitters in the altered processing of emotional stimuli that defines these diseases.

In the current study, we focused on brain changes in Arginine vasopressin (AVP), a peptide neurotransmitter involved in regulating social and emotional responses. We used an animal model of stress, individual housing, to determine if 1) altered AVP expression was observed in the brain and 2) any changes in AVP were associated with alterations in plasma corticosterone, a steroid hormone that is part of the stress response. AVP levels in the paraventricular (PVN) nucleus and blood levels of corticosterone were determined using established assay protocols. The PVN was chosen because it is one of the two major sites of AVP synthesis in the brain.

Hypothesis

The experiment tested whether individual housing produced a difference in brain AVP expression and corticosterone levels in blood compared to group housed control animals.

Methods

Animal care and housing paradigms: All protocols were approved by the Lehigh University IACUC and were conducted in accord with Public Health Service Guidelines for the Care & Use of Animals. Male and female C57BL/6 mice (Charles River) that were at least 60 days old were used in the study. Male mice were distributed into isolation and group housing situations for 14-days. Group housing included one male and two ovariectomized female mice, which was designed to eliminate the stress associated with the high levels of aggression that are typically observed among males. At the end of the 14-day housing period, the males were sacrificed and brain and blood tissue samples were collected. The experiment with females was conducted in the same manner with the exception of that groups were all female.

Brain tissue processing: Brains were fixed in 3.7% formaldehyde/PBS for 48 hours at 4˚C followed by another 48 hr in 25% sucrose/PBS at 4˚C. Brains were dried, wrapped in aluminum foil, and stored at -80˚C until sectioning. Thirty microns were cut and stored in PBS at -20˚C. Sections from the PVN were used for AVP immunohistochemical staining. Sections were treated with a Triton X-100 (TX-100) blocking solution to reduce nonspecific binding for 30 min. After three PBS/0.3% TX-100 washes, sections incubated for 48 hours at 4˚C in primary antibody solution with guinea pig anti-AVP antibody. Following three PBS/0.3% TX-100 washes, sections incubated for 1 hour in secondary antibody solution with goat Biotinylated anti-guinea pig IgG antibody. After three PBS/0.3% TX-100 washes, sections were incubated for 30 min in a tertiary solution containing Avidin/Biotin Complex (ABC). Avidin binds to Biotin with extremely high specificity. The ABC kit used is specifically designed to create complexes for immunoperoxidase staining. After three PBS/0.3% TX-100 washes were completed, the sections were incubated in a DAB staining solution for two minutes. Stained sections were washed in PBS and mounted on gelatin-coated microscope slides for analysis. Plasma corticosteroid levels were tested following manufacturer methodology according to Corticosteroid EIA kit.

Image analysis: Sections were examined and photographed under a microscope (4x or 10x magnification) and then analyzed using Scion Image by the NIH. Signals were measured by Integrated Particle Density (IPD), which is a product of staining area multiplied by staining density.

Results

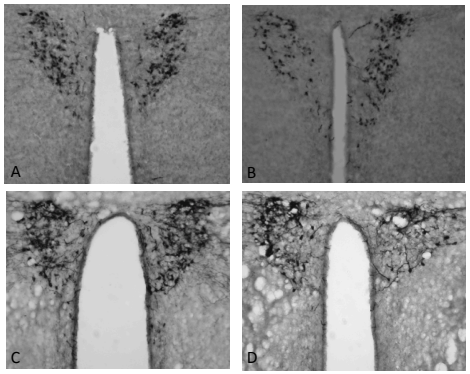

Figure 1A-D. Representative immunochemically stained sections showing the effect of housing condition on AVP expression in the PVN of male and female mice. A. MALE CONTROL (4x) B. MALE ISOLATION (4x) C. FEMALE CONTROL (10x) D. FEMALE ISOLATION (10x)

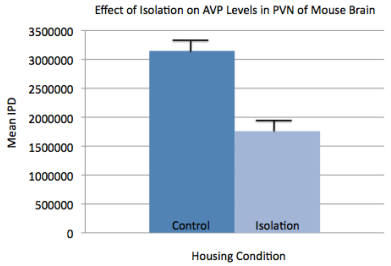

Figure 2. Levels of AVP expression in the PVN of mouse brain was measured using Integrated Particle Densities (IPD) in Scion Image by the NIH. The group housing condition had higher levels of AVP expression than the isolation housing condition.

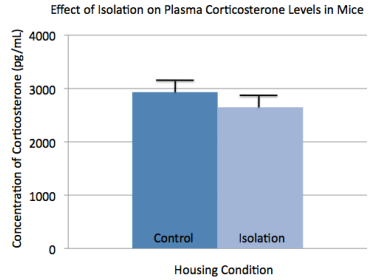

Figure 3. Concentration of corticosterone found in blood plasma of mice.

Discussion

Group housed females and males had higher expression of AVP in the PVN than the isolated animals. This is interesting because isolation is considered more stressful and it is generally thought that higher stress levels result in elevated brain AVP. If subsequent studies confirmed this finding, it would raise a number of issues concerning social structure and associated stress. However, an alternative explanation should be considered. Under stressful conditions, AVP is released from the PVN into the portal blood system via neurons that synapse in the posterior pituitary. Isolated animals thus may have released greater levels of AVP into the blood and lowered detectable stores in the PVN. This possibility would explain the decreased levels of AVP expression in the PVN in isolated compared to the group housed animals. To test this hypothesis, levels of AVP in the blood should be tested combined with in vivo microdialysis in the PVN, which would provide a determination of release rates.

No significant difference was observed in the plasma levels of corticosterone between the housing conditions. Interpretation of these data is limited by the absence of repeated sampling of blood corticosterone over time, which is not possible in mice.

Future research opportunities include testing AVP release and turnover rates in the PVN and other major brain regions as well as determination of plasma AVP levels in both male and female mice housed in group and isolation housing conditions.

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