Abnormal post-stress anxiety response related to locus coeruleus dysfunction in Prader-Willi syndrome mice

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Abstract:

Introduction: Prader–Willi syndrome (PWS) is a multisystemic disorder. Notably, many characteristic symptoms of PWS are correlated with locus coeruleus norepinephrine system (LC-NE) dysfunction, including impairment in arousal, learning, pain modulation, and stress-induced negative affective states. Although electrophysiological experiments in necdin-deficient mice, an established PWS animal model, have revealed decreased spontaneous neuronal firing activity in the LC and impaired excitability, the behavioral phenotypes related to LC-NE dysfunction remain unexplored.

Methods: In this study, heterozygous necdin-deficient mice (B6.Cg-*Ndn*^{tm1ky}) were bred from wild-type (WT) females to generate WT (+m/+p) and heterozygous (+m/−p) animals.

Results: Compared to WT mice, *Ndn* +m/-p mice demonstrated impaired visual-spatial memory in the Y-maze test, reduced social interaction, impaired sexual recognition, and shorter falling latency on the Rotarod. Using the open field test (OFT) and elevated plus maze (EPM), we observed similar locomotion activity of Ndn +m/-p and WT mice, but *Ndn* +m/-p mice were less anxious. After acute restraint, *Ndn* +m/-p mice exhibited significant impairment in stress-induced anxiety. Additionally, the plasma norepinephrine surge following exposure to acute restraint stress was also impaired. Pretreatment with atomoxetine, a norepinephrine reuptake inhibitor aimed to enhance LC function, restored Ndn +m/-p mice to exhibit a normal response to acute restraint stress. Furthermore, by employing chemogenetic approaches to facilitate LC neuronal firing, post-stress anxious responses were also partially rescued in Ndn +m/-p mice.

Conclusion: These data strongly suggest that LC dysfunction is implicated in the pathogenesis of stress-related neuropsychiatric symptoms in PWS. Manipulation of LC activity may hold therapeutic potential for patients with PWS.

RESULTS

I. Behavior phenotypes of *Ndn* +m/-p mice

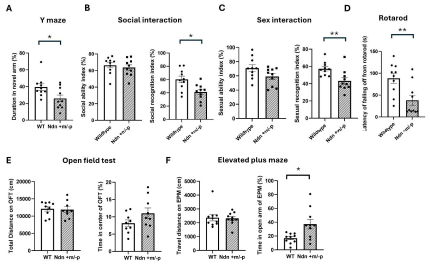

Figure 1. Behavioral phenotypes of Ndn +m/-p mice (n = 10 in each group). (A) Compared to wildtype (WT) mice, Ndn +m/-p mice spent less time in the novel arm of the Y-maze, indicating impairment of visual-spatial memory. (B, C) While Ndn +m/-p mice exhibited similar social and sexual abilities compared to their WT siblings, their social and sexual recognition were impaired. (D) Ndn +m/-p mice had a shorter latency to fall off from the Rotarod. (E, F) Moving distance on the Open Field Test (OFT) and Elevated Plus Maze (EPM) were similar between WT and Ndn +m/-p mice. However, Ndn +m/-p mice tended to travel toward the center of the OFT and spent a significantly increased amount of time in the open arm of the EPM. Data are presented as mean ± standard error of the mean. *P<0.05, **P < 0.01.

II. *Ndn* +m/-p mice had impaired stress-induced anxiety and abnormal stress-induced norepinephrine release

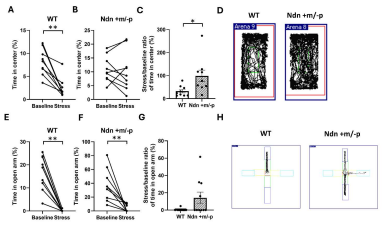

Figure 2. Post-restraint open field test (OFT, n = 9 in each group) and elevated plus maze (EPM, n = 10 in each group) of WT and Ndn +m/-p mice. (A, B) Percentage of time in center of OFT at baseline and post-stress stage of WT(A) and Ndn +m/-p (B) mice. (C) Significantly increased stress/baseline ratio of time in center in Ndn +m/-p mice. (D) Representative tracings of post-restraint OFT of WT and Ndn +m/-p mice. (E, F) Percentage of time in open arm of EPM at baseline and post-stress stage of WT(E) and Ndn +m/-p (F) mice. (G, H) Stress/baseline ratio of time in open arm of EPM for WT and Ndn +m/-p mice (G) and representative post-stress tracings (H). Data are presented as mean ± standard error of the mean. *P<0.05, **P < 0.01.

RESULTS

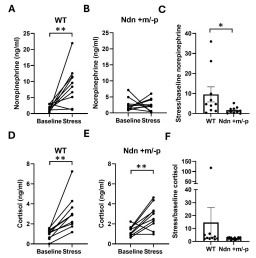

Figure 3. Baseline and post-stress plasma norepinephrine (NE, n = 10 in each group) and cortisol levels (n = 10 in each group) in WT and Ndn +m/−p mice. (A, B) Plasma NE levels were significantly elevated after exposure to restraint stress in WT mice (A), but did not change in Ndn +m/−p mice (B). (C) The stress/baseline norepinephrine was significantly higher in WT mice. (D, E) Plasma cortisol levels were significantly elevated after exposure to restraint stress in both WT (D) and Ndn +m/−p mice (E). (F) The stress/baseline cortisol concentration revealed no significant difference in WT and Ndn +m/-p mice. Data are presented as mean ± standard error of the mean. *P<0.05, **P < 0.01.

III. Atomoxetine but not hydrocortisone restored impaired stress response in *Ndn* +m/-p mice

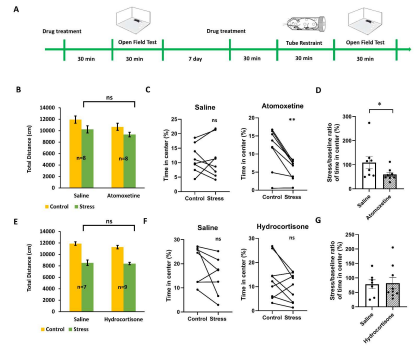

Figure 4. The effects of atomoxetine (ATM) and hydrocortisone on stress-induced anxiety in Ndn +m/−p mice. (A) Schematic illustration of the experimental paradigm. (B-D) ATM did not affect moving distance but significantly decreased the time-in-center percentage of Ndn +m/−p mice after exposure to restraint stress (C, n = 8 in each group). Stress/baseline ratio of time in center of OFT is significantly decreased for Ndn +m/-p mice pretreated with ATM (D). (E-G) Hydrocortisone did not affect moving distance or the time-in-center percentage of Ndn +m/−p mice after exposure to restraint stress (n = 7 in saline group, n = 9 in hydrocortisone group). Data are presented as means ± standard error of the mean. *P < 0.05, **P < 0.01.

RESULTS

IV. Chemogenetic excitation of LC neurons partially rescued the abnormal post-stress anxiety of *Ndn* +m/-p mice

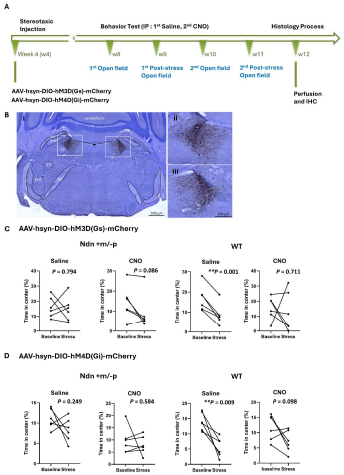

Figure 5. Chemogenetic excitation and inhibition of LC-NE neurons. (A) Schematic illustration of the experimental paradigm. (B) Representative DAB staining of mCherry in brain stem. (C) Excitation of LC-NE neurons in Ndn +m/-p mice partially rescued their abnormal post-stress anxiety, but impaired post-stress anxiety in WT mice (n = 6 in each group). (D) Inhibition of LC-NE neurons in Ndn +m/-p mice and WT mice both resulted in abnormal post-stress anxiety (n = 6 in each group). *P < 0.05, **P < 0.01.

CONCLUSION

This study demonstrated that necdin-deficient mice, serving as an animal model of PWS, exhibited several phenotypes similar to human patients with PWS, including impaired visuospatial memory, reduced social and sexual recognition, and coordination deficits. Furthermore, impaired stress-induced responses were observed from both biochemical and behavioral perspectives. The abnormal stress-induced anxiety in necdin-deficient mice was restored by pretreatment with ATM but not hydrocortisone, suggesting that LC-NE dysfunction plays a major role in the defective stress response.