Blockade of glutamate NMDA receptors facilitates the effect of chronic stress

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Introduction

Research has shown that chronic stress can be associated with altered hypothalamic-pituitary-adrenal (HPA) axis activation, and elevated release of glutamate in the mesolimbic brain reward and motivation pathway, including the ventral tegmental area (VTA) and nucleus accumbens (NAc).

The principal objective of this study is to assess the role of a selective NMDA receptor antagonist (D-AP5) in regulating effects of chronic restraint stress on body weight, and on the expression of type 2 vesicular glutamate transporter (vGluT2). We hypothesize that the blockade of NMDA receptors with D-AP5 prior to the stressor will attenuate HPA axis activation and serve to reduce glutamate excitotoxicity in the mesolimbic system. The current study examined changes of glutamate uptake in stressed rats and the impact of blockade of NMDA receptor activation prior to stress on such variable.

The findings of the current study will help determine the role of NMDA receptor activation in regulating glutamate uptake and neurotoxic effects of stress in important mesolimbic brain regions, such as the VTA and Nac, critical for reward and motivational processes.

Methods

Subjects

Experimentally naïve adult male Wistar rats, weighing between 275 and 325g, were randomly assigned to one of four groups: saline - no stress, D-AP5 1.0nmol - no stress, D-AP5 1.0nmol - stress and D-AP5 2.5nmol - stress.

Surgical procedure

Stainless steel 22-gauge guide cannula was implanted 0.7mm above the VTA on the right hemisphere, according to the Paxinos and Watson (1998) rat brain atlas. Stereotaxic coordinates are AP, -5.60mm from Bregma; ML, -2.1mm (angled at 10° toward the midline to avoid the sagittal sinus); and DV, 7.6mm below dura. The internal injectors used for drug infusions extended 0.7mm past the end of the guide cannula, for a final depth below dura of 8.3mm for intra-VTA injections.

Drug and stress

7 days post-surgery, the animals were infused 0.5µl of either saline or D-AP5 (Glutamate NMDA receptor antagonist; 2.5 nmol or 1.0 nmol) 30 minutes prior to being subjected to restraint stress (30 minutes/day) or no stress. Drug/saline infusion rate was 0.5µl/min.

Histology

On the day following the last stress or no stress experimental protocol, animals were killed and brain tissue collected for immunohistochemical detection. Expression of the vesicular glutamate transporter 2 (vGluT2), a marker for the presynaptic glutamatergic terminals, was used in various mesolimbic brain regions including the hippocampal cell layers CA1, CA3, and dentate gyrus (DG), the basolateral amygdala (BLA), the VTA and the NAc. Immunohistochemical staining was analyzed using a fluorescence microscope and optical density determination using Image J. Groups differences in vGluT2 expression was performed in the aforementioned brain areas.

Results

Rat 15	Saline	No stress
Rat 16	D-AP5 1.0nmol	No stress
Rat 21	D-AP5 1.0nmol	Stress
Rat 11	D-AP5 2.5nmol	Stress

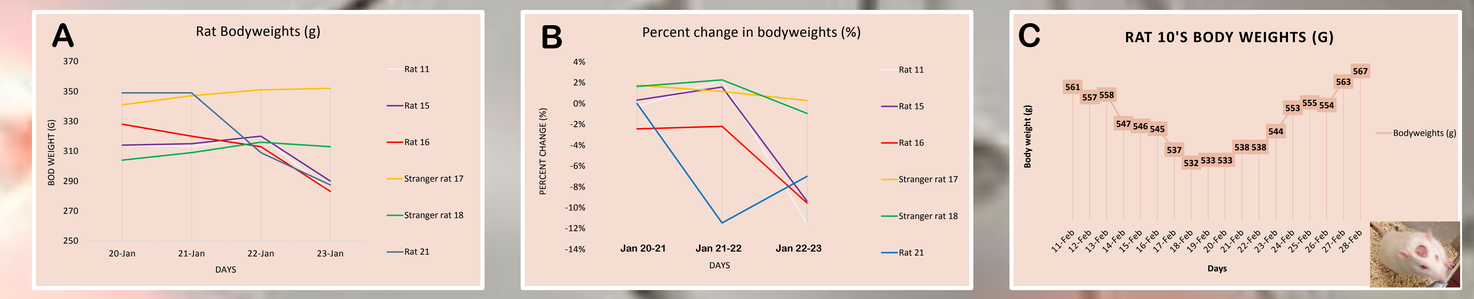

Figure 1: (A) Rats' body weight from January 20th to January 23rd . Rats 11, 15, 16, and 21 underwent DAP5 or saline infusion paired with stress or no stress. Rats 17 and 18 did not receive infusion or stress. (B) Percent change in bodyweights for rats 11, 15, 16, 17, 18, and 21 from January 20th to January 23rd . (C) Rat 10's bodyweight. The rat underwent daily infusion of D-AP5 0.41nmol (or 0.08 µg) and 30 min restraint stress from Feb 11 to Feb 18. The remaining 10 days (Feb 19 to Feb 28) were rest days where no manipulations were done.

Immunohistochemistry

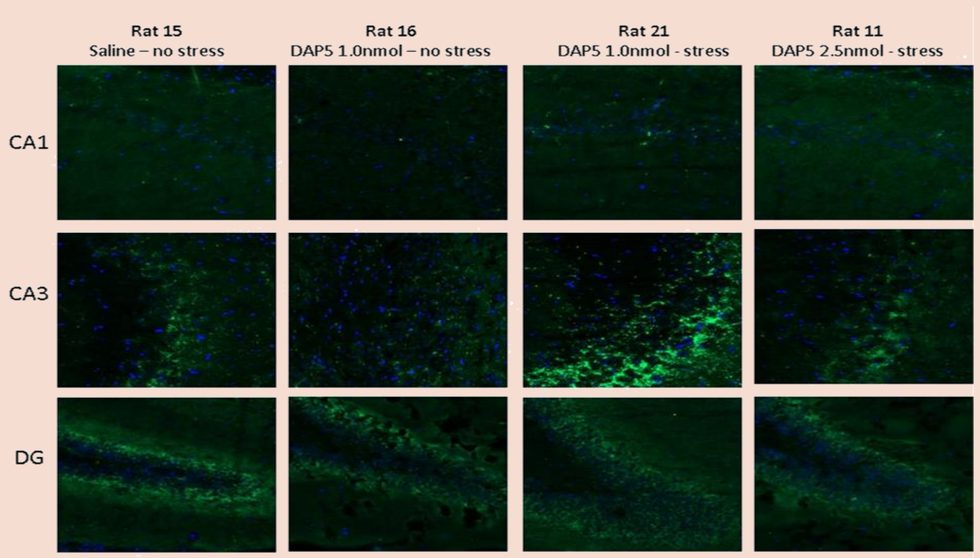

Figure 2: Photomicrographs (200X magnification) of vGluT2 markings in the CA1, CA3, and DG. Differences are mainly seen within the CA3. Rat conditions: Rat 15 – Saline + no stress; Rat 16 - DAP5 1.0nmol + no stress; Rat 21 – DAP5 1.0nmol + stress; Rat 11 – DAP5 2.5nmol + stress.

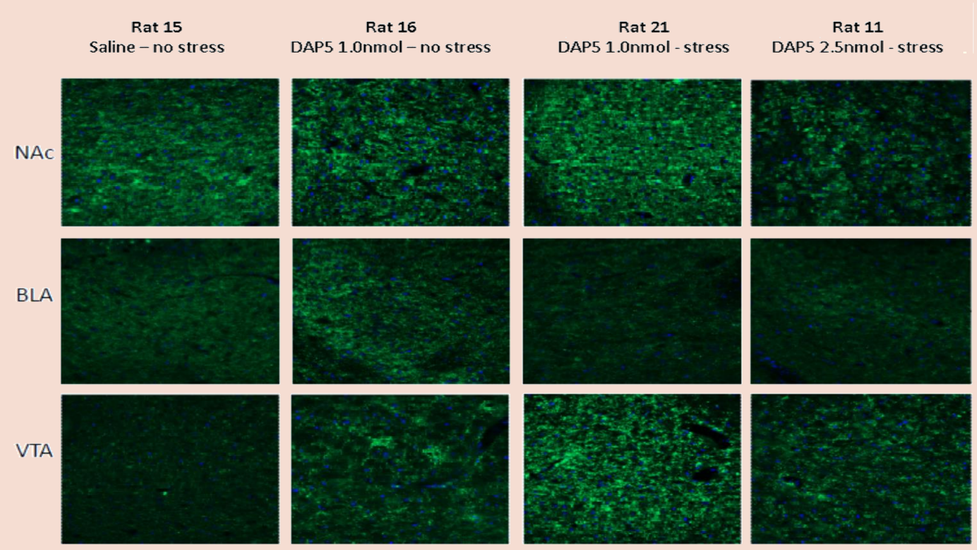

Figure 3: Photomicrographs (200X magnification) of vGluT2 markings in the NAc, BLA, and VTA. Between group differences are present in the VTA and NAc. The 1.0 nmol dose of DAP5 appears to upregulate vGluT2 in the NAc and VTA, which could attenuate the excitotoxic damage induced by repeated stress in these regions and better preserve neurons against excitotoxic damage.

Conclusion

This project is part of a pilot study in which we investigated the role of NMDA receptor activation in the effects of stress on body weight regulation and on the expression of regulators of glutamate uptake, via changes in vGluT2 expression.

Our findings indicated stress-induced reduction in body weight. Similar effects are commonly reported in the literature. Thus, body weight of stressed mice decreases rapidly after initiating daily restraint stress and remain significantly lower than that of controls after the stress (Jeong et al, 2013). NMDA receptor blockade did not prevent reduced body weight related to stress exposure. However, DAP5 was associated with a fast return to pre-stress body weight during the 10 day post stress recovery period (rat 10). This suggests that NMDA receptor activation may contribute to prolonged body weight loss post-stress.

Notably, region-specific changes in vesicular glutamate transporter 2 (vGluT2) expression were noted.

At the hippocampus, low-dose D-AP5 treatment prior to stress led to increased vGluT2 expression in the CA3 layer of the hippocampus. This effect was dose-related and not observed in animals treated with the higher 2.5 nmol dose. This is an interesting finding as it could promote better reuptake of glutamate and attenuate excitotoxic effects associated with chronic exposure to stress fostering protection of the hippocampus against stress-induced toxic effects and stress-induced cognitive impairments.

In the mesolimbic system, a similar dose-related profile of DAP5 action was present at the nucleus accumbens (Nac) and ventral tegmental (VTA) regions. Low dose of the drug prior to stress elevated vGluT2, a phenomenon which could compensate for elevated glutamate transmission post stress and reduce excitotoxic damage from excess glutamate release post stress. Such changes could be related to dose-related effects of D-AP5 on neuroprotection.

This pilot study indicates dose-related effects of NMDA receptor antagonism in regulating glutamate uptake in the hippocampus, Nac and VTA. These findings are likely to underlay neuronal and functional changes following exposure to chronic stress. Further study will clarify how NMDA receptor activation affects viability of neurons, HPA activation and emotional and cognitive behavior post stress, as well as characterize effects of DAP5 on *in vivo* glutamate release and/or expression in distinct brain regions.

References

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