Effects of selective TrkB inhibition by ANA-12 on the expression of CRH and vGluT2 following a repeated stress paradigm in rats

Student: Nivedh Patro, Faculty of Science
Supervisors: Dr. Hélène Plamondon and Idu Azogu, School of Psychology, Faculty of Social Sciences
University of Ottawa

uOttawa

Cerebro-Vascular Accidents and Behavioural Recovery Laboratory

Introduction

Repeated stress induces elevated levels of brain-derived neurotrophic factor (BDNF) and its primary receptor, tyrosine-related kinase B (TrkB), in the nucleus accumbens (Nac) shell of the basal forebrain. Elevated BDNF-TrkB signalling has been implicated in the pathophysiology of mood disorders [1], and increased levels within the Nac shell can result in anxiogenic and pro-depressive mood over time. Recently, the selective TrkB antagonist, ANA-12, was developed which prevents the binding activation of TrkB by BDNF, thus inhibiting processes downstream of TrkB signalling.

In the present study, we examined the effects of inhibiting TrkB signalling with ANA-12 on levels of the stress hormone corticotropin-releasing hormone (CRH) and expression of vesicular glutamate transporter 2 (vGLuT2), in the Paraventricular nucleus (PVN) and the Basolateral amygdala (BLA), following repeated stress in male Wistar rats. **We hypothesize that the inhibition of TrkB in the NAc shell may attenuate depression- and anxiety-like response induced by stress, and modulate the levels CRH and vGluT2 in these brain regions.**

Methodology

Animals

40 male Wistar rats underwent intra-NAc shell guide cannula implantation surgery. Following recovery, the rats were randomly separated into four groups (n=10 per group): (1) ANA-12 + stress, (2) ANA-12 + no stress, (3) Vehicle + stress, and (4) Vehicle + no stress. Groups 1 and 3 were subsequently subjected to a ten-day stress paradigm alternating between restraint (30 minutes) and forced-swim stress (15 minutes). Over the course of the ten days, all rats were infused with either ANA-12 or a vehicle at three-day intervals, according to their assigned group. Rats were scored on a variety of behavioural tests (e.g. open field test and elevated plus maze) prior to euthanasia. Their brains were subsequently collected and samples were sliced at 14 µm for immunohistochemical analysis.

Immunohistochemistry

Corticotropin-releasing hormone (CRH) and vesicular glutamate transporter 2 (vGluT2) were double-labeled within the paraventricular nucleus and basolateral amygdala. To stain CRH, brain sections were processed with a primary polyclonal rabbit anti-CRH antibody (1:400, ImmunoStar) and a secondary donkey anti-rabbit antibody. For vGluT2, a primary polyclonal guinea pig anti-vGluT2 antibody (1:5000, Santa Cruz) and a secondary donkey anti-guinea pig antibody were used. Staining was observed using a fluorescence microscope at 20X magnification, and optical density analyses for the intensity of the staining in regions of interest were conducted with ImageJ. Group differences were then determined based on the statistical significance of the observed fluorescence patterns.

Results

Figure 1: (A) Photomicrographs (20x magnification) showing vGluT2-ir (green), CRH-ir (red), and Hoescht (blue) in the PVN of the hypothalamus in VNS, ANS, VS and AS rats. **(B)** Graph represents vGluT2-ir levels in each group. No significant differences were found. **(C)** Graph represents CRH-ir levels in each group. The ANS group showed a significant increase in CRH-ir levels only when compared to the VNS group (p = 0.04). The vertical bars represent Mean ± SEM. *$p < 0.05$.

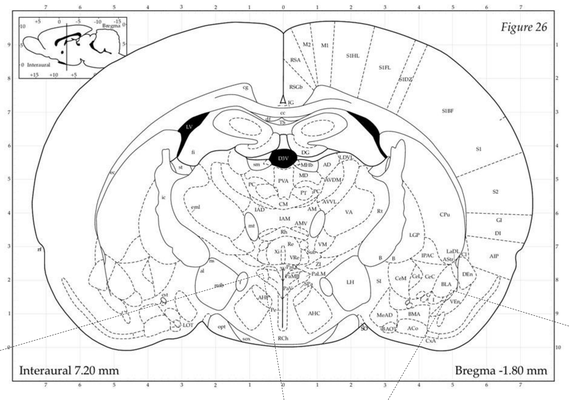
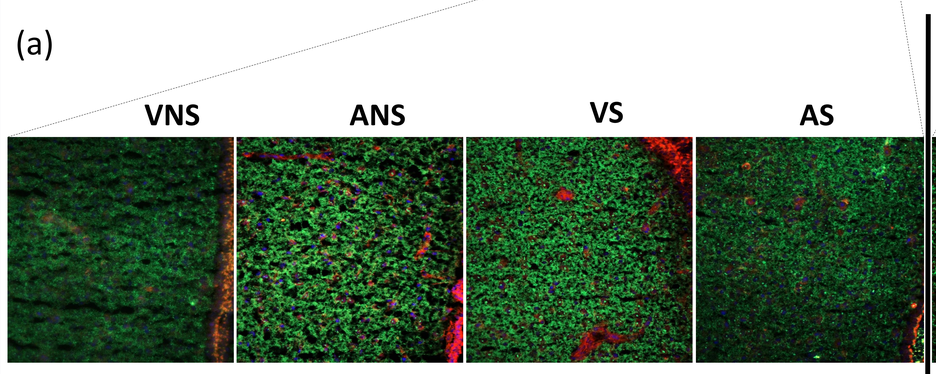
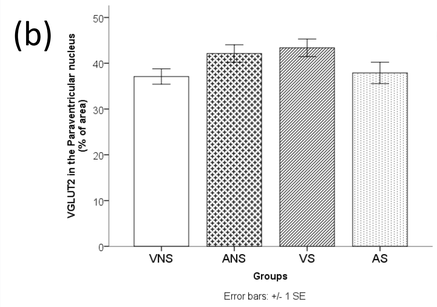
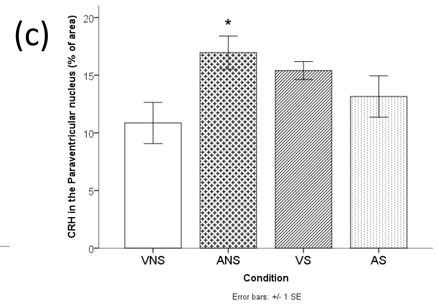
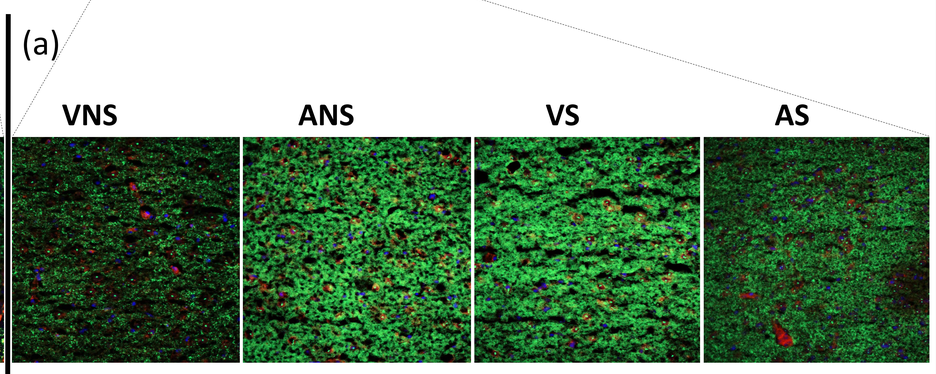


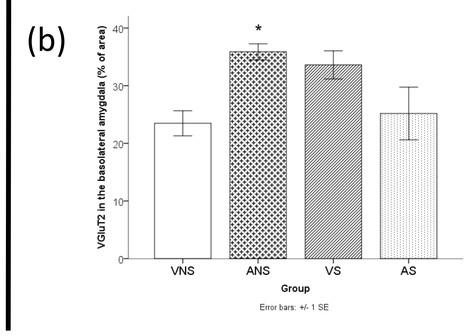
Figure 2: (A) Photomicrographs (20x magnification) showing vGluT2-ir (green), CRH-ir (red), and Hoescht (blue) in the Basolateral amygdala in VNS, ANS, VS and AS rats. **(B)** Graph represents vGluT2-ir levels in each group. The ANS group showed a significant increase in vGluT2-ir levels only when compared to the VNS group (p = 0.028). **(C)** Graph represents CRH-ir levels in each group. The ANS group showed a significant increase in CRH-ir levels only when compared to the VNS group (p = 0.027). The vertical bars represent Mean ± SEM. *$p < 0.05$.

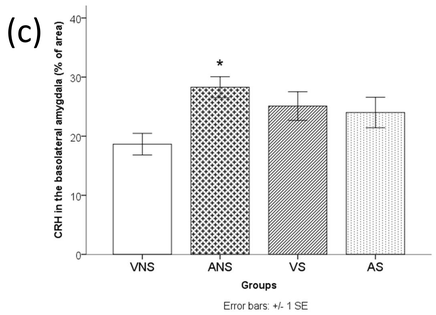












Schematic of Methodology

Conclusions

The results show that ANA-12, when delivered intra-Nac shell, was able to cause a decrease in the expression of CRH and vGluT2 following repeated stress in the parvocellular portion of the PVN and BLA brain areas of rats. This was in comparison to the VS and ANS groups, but not the VNS group. In contrast, the ANS group showed significant increases in the expression of CRH in both the PVN and BLA, and in the expression of vGluT2 in the BLA only when compared to the VNS group. Such an outcome may suggest that in the absence of stress, inhibition of TrkB by ANA-12 may trigger or disinhibit a downstream mechanism(s), allowing for increased release of CRH and activity of vGluT2. Further characterization of TrkB signaling in the Nac is needed.

Acknowledgements and References

I would like to thank my supervisors, Idu Azogu and Dr. Hélène Plamondon, for their constant support and mentoring, and UROP for granting me the opportunity to participate in this project. Special thanks to NSERC for their aid in funding the project.

Reference

1. Russo SJ and Nestler EJ (2013). The brain reward circuitry in mood disorders. *Nat. Rev. Neurosci.* **14:** 609-625

For image references and additional questions/comments, please contact Nivedh Patro at npatr057@uottawa.ca.