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Beta-endorphin expression and sex affect the ability of alcohol to alter tyrosine hydroxylase immunoreactivity in the reward pathway in mice

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Hannah Kelly-Quigley[1], Madison Waldron[2], Kiarah Leonard[1], Erin M. Rhinehart[1], Judy E. Grisel[2]
[1]Department of Biology, Susquehanna University, Selinsgrove, PA; [2]Department of Psychology, Bucknell University, Lewisburg, PA

Introduction

➢**Sex Differences**

- Females are more susceptible to the negative health consequences of alcohol, and escalate from alcohol use to alcohol use disorder (AUD) more rapidly than males.
- Females have a higher incidence of anxiety-related disorders.
- Alcohol is a potent anxiolytic.

➢ **β-Endorphin**

- Stress activates the hypothalamo-pituitary-adrenal (HPA) axis.
- β-endorphin (β-E), an endogenous opioid neuropeptide, provides negative feedback to turn off HPA axis activation.
- Low β-E enhances stress reactivity and is a risk factor for AUD.

➢**Mesolimbocortical Dopamine Reward Pathway**

- Reward → increased dopamine (DA) in ventral tegmental area (VTA)/release in the nucleus accumbens (NAc).
- Tyrosine hydroxylase (TH), the rate limiting enzyme for DA production, can be used as an indirect measure of DA production.
- Alcohol stimulates GABA signaling, facilitating increased DA signaling in the reward pathway via disinhibition.

➢Therefore, it is possible that females might find alcohol more rewarding or benefit more from its anxiolytic properties compared with males.

➢**We hypothesized that ethanol would affect DA production and neuronal activation in a site-specific, β-E dependent and sexually differentiated manner.**

➢**To test this hypothesis, we examined the effect of ethanol on the amount TH protein expression and c-fos immunoreactive (c-fos-ir) cells in reward, motivation and stress-related brain regions in the brain in both sexes of wildtype and β-E null mice.**

Methods

➢**Subjects**

- 24 male and 29 female WT (+/+) and β-E null (-/-) mice were injected i.p. with saline or 2 g/kg ethanol. All procedures were approved by Bucknell University IACUC .
- 90 minutes post-injection, mice were given pentobarbital (40mg/kg) and were perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). Brains were removed and post-fixed in 4% PFA followed by a 30% sucrose sink until sectioning.

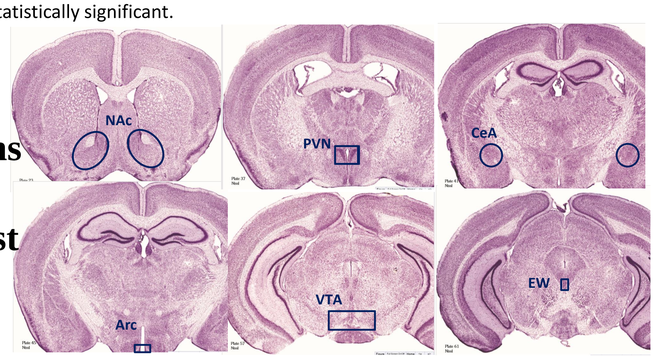
➢**Immunohistochemistry**

- 35µm thick brain sections were incubated in polyclonal rabbit anti-c-fos (1:5000; Synaptic Systems) for 48 hrs at 4˚C followed by goat biotinylated anti-rabbit IgG (1:600, Vector Laboratories) for 1 hr at RT, followed by Avidin-biotin complex solution (ABC, PK-7200, Vector Labs) for 1 hr. Afterward, sections were incubated in ImmPACT® SG Peroxidase Substrate (Vector Laboratories), according to manufacturer's instructions.
- Brain sections were then incubated in polyclonal rabbit anti-TH at 1:10,000 (EMD Millipore, Darmstadt, Germany) for 48 hrs. at 4˚C, followed by goat biotinylated anti-rabbit IgG (1:600, Vector Laboratories) for 1 hr at RT, followed by Avidin-biotin complex solution (ABC, PK-7200, Vector Labs) for 1 hr and finished with ImmPACT® NovaRED Substrate (Vector Laboratories,) according to manufacturer's instructions.

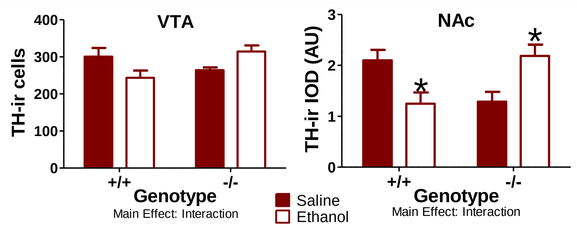
➢**Image Analysis**

- Brain section images at 20X (Evos XL light microscope, Life Technologies) were used to count the number of TH-immunoreactive (TH-ir) cells in the VTA, Arc and PVN, c-fos-ir cells in the EW, CeA, Arc, VTA, and PVN and to determine the integrated optical density (IOD) of TH-ir fibers in the NAc using ImagePro Plus (Media Cybernetics).
- All results are presented as mean ± SEM. Data were analyzed using a two-way ANOVA with Bonferroni post hoc comparisons using GraphPad Prism software with $p<0.05$ considered statistically significant.

Regions of Interest



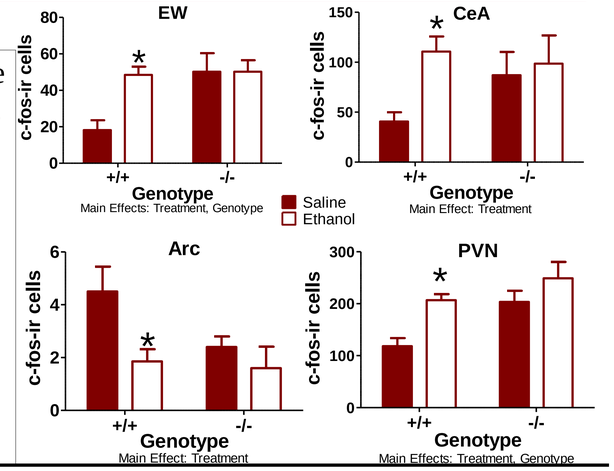
RESULTS

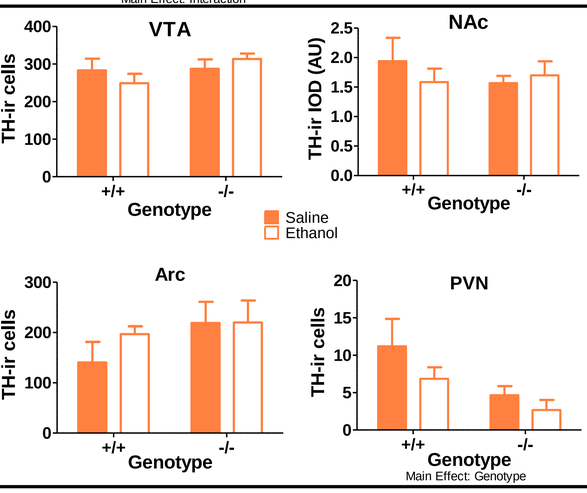


Females

Left: EtOH decreased TH-ir in +/+ mice in VTA/NAc and increased it in the Arc. EtOH in β-E null mice increased TH-ir in reward (VTA/NAc) but not hypothalamic areas (Arc/PVN).

Right: EtOH increased c-fos-ir in the EW, CeA, and PVN and decreased it in the Arc, but only in female +/+ mice. EtOH had no effect on c-fos expression in β-E null mice *=$p<0.05$

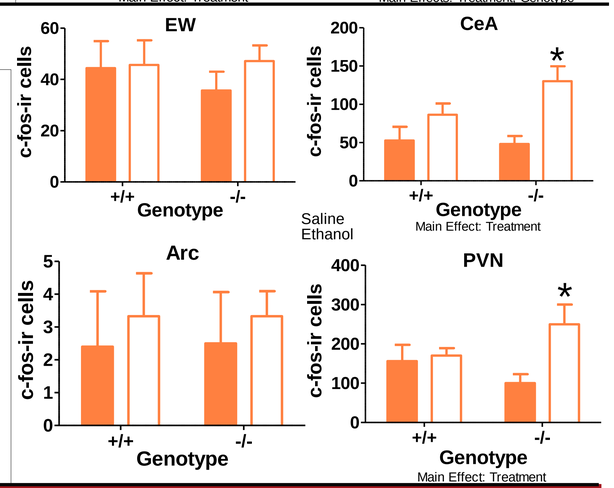




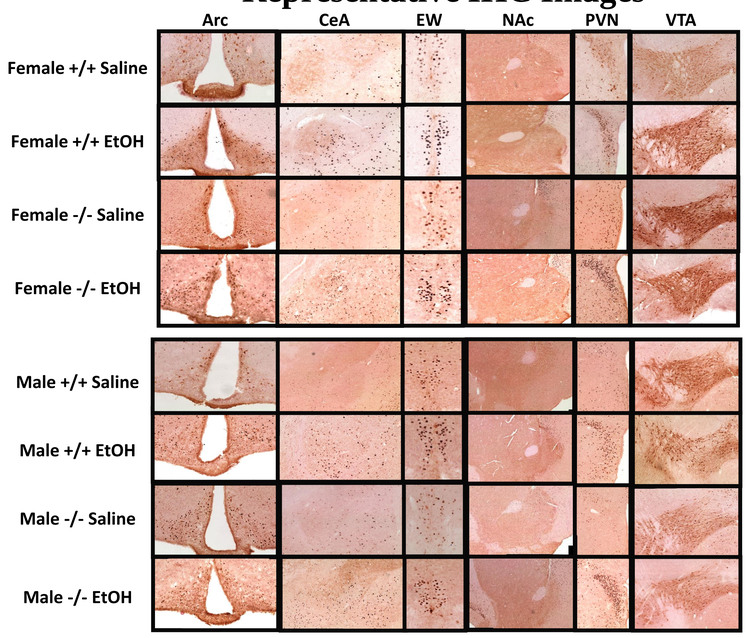
Males

Left: EtOH did not affect TH-ir in any brain region in males regardless of genotype. β-E null mice had less TH-ir in the PVN than WT mice independent of treatment.

Right: EtOH increased c-fos-ir in the PVN and CeA, but only in β-E null mice. EtOH had no effect on c-fos expression in +/+ male mice. *=$p<0.05$



Representative IHC Images



CONCLUSIONS

➢All males and female -/- mice appeared relatively insensitive EtOH-induced neuronal activation compared to +/+ females.

➢Female -/- mice, regardless of treatment, had comparable neuronal activation to EtOH treated +/+ mice, possibly indicating a ceiling effect in neuronal activation in these mice.

➢EtOH had opposite effects on TH in female +/+ vs. -/- mice, indicating that the effects of EtOH on TH expression are dependent on β-E expression.

➢In males, EtOH only affects c-fos in stress-related brain regions and only in mice not expressing β-E.

➢Sex differences in EtOH-induced effects on central stress and reward circuits are responsible for sexually dimorphic effects of EtOH in a β-E dependent manner.

Acknowledgements

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➢Bali, Randhawa, Jaggi, Stress and opioids: role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference. Neurosci. Biobehav. Rev. 51: 138-150 (2015).
➢Becker, Koob, Sex differences in Animal Models: Focus on Addiction. Pharmacol. Rev. 68(2): 242-263 (2016).
➢Barfield et.al, 2010. b-endorphin mediates behavioral despair and the effects of ethanol on the tail suspension test in mice. Alcohol Clin Exp. Res. 34, 1066-1072.
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