Altered Phosphorylation Status of CNS Signaling Proteins By Drugs of Abuse: Utility of Peggy Sue™

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1 Background

Alcohol abuse, altered dopamine signaling and motivated behavior

Activation of dopamine signaling proteins AKT & ERK via phosphorylation has been implicated in the acute behavioral effects of alcohol and other abused drugs; and may also represent a central mechanism in the pathogenesis of addiction.

Our Aim: Using a phosphoprotein analysis approach, we compared the phosphorylation status of ERK (p44/p42) and AKT1-3 isoforms in brains of individual animals which showed a varying behavioral sensitivity to the dopaminergic agonist drug quinpirole, based on their past alcohol exposure history.

2 Methods

Model of Early-Life Binge Exposure to Alcohol using CIE[1]

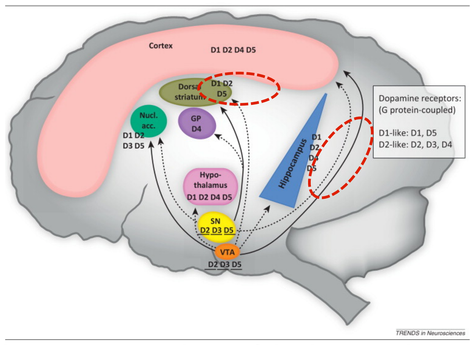
Adolescent male Wistar rats ~28 days old were exposed for 14 days to ethanol vapors in inhalation chambers using a chronic intermittent ethanol **(CIE)** schedule (14 h on/10 h off) shown to induce alcohol dependence and synaptic plasticity changes (Sabeti 2011). Mean peak BALs 250 ± 10 mg/dL. Age-matched control rats **(Ctr)** were exposed to ambient air in adjacent chambers during this period.

Dopamine Induced Behavioral Sensitization[2]

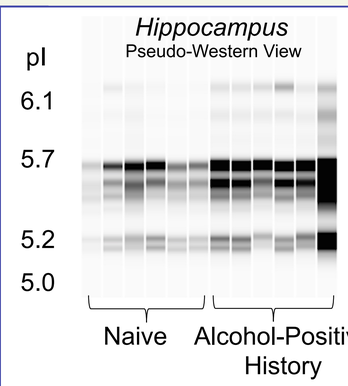
As matured adults (PND 92-100), rats were administered saline or the DA agonist quinpirole QP (0.25 mg/kg/ s.c.) twice a week for a total of 10 injections to induce locomotor sensitization accompanied by a compulsive-like checking behavior[2].

IEF Based Assay: Peggy Sue™ Simple Western System[3]

Dopamine D2 receptor expressing brain regions implicated in the learning or unlearning of behaviors



Hippocampus, striatum & brain stem tissue were collected & analyzed from alcohol abstinent adult animals, which were previously exposed or not as adolescents to episodes of binge alcohol intoxication.



Electropherogram peaks detected by a phospho-site specific pERK1/2 antibody. Tissue samples from rats with or without a positive history of prior adolescent alcohol exposure.

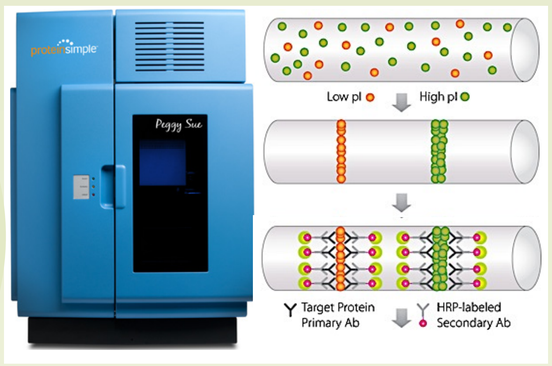
Drug Exposure Timeline

Early life exposure to alcohol vapor or ambient air (14 days) → **Prolonged abstinence during maturation to adulthood (52 ± 4 days)** → **Quinpirole injections & Behavioral monitoring (5 weeks)** → **Brain tissues collected at 100 min post 10th injection**

* References

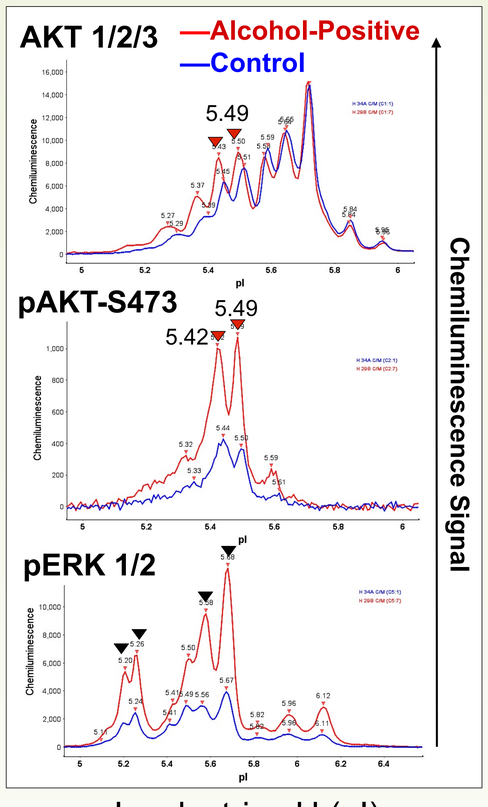
1. Sabeti Alcoholism: Clinical and Experimental Res 2011; 35 (5) 885-904
2. Szechtman et al Behav Neurosci 1998; 112 (6) 1475-85
3. Gentalin, White and Proctor. Nature Methods 2013 June
4. Pittenger et al Pharmacology and Therapeutics 2011; 132 (3) 314-332
5. Salles et al. J Neurochemistry 2013;125:532-544

How Peggy Sue™ Works[3]



Peggy Sue is an automated nanocapillary platform that uses isoelectric focusing (IEF) technology & immunoassay methods to resolve unphosphorylated and phosphorylated protein isoforms in nanogram amounts of tissue[3]. Cytosolic proteins were extracted from frozen brain tissue samples of alcohol-exposed or control rats. Extracted samples are run during the same cycle against selected monoclonal antibodies (Cell Signaling) to compare isoform peak differences.

3 Sample Data



Electropherograms show non-phosphorylated & phosphorylated isoforms of AKT or ERK after Day 10 injection with quinpirole. Total AKT antibody detected several phosphorylated & non-phosphorylated isoforms. Phosphorylation states affect pI values with an expected shift of 0.04-0.07 pH units per event. Peaks are consistent with theoretical pI calculations and reflect a complex pattern of phosphorylation.

4 Results

FIGURE 1 ERK & AKT activation by quinpirole

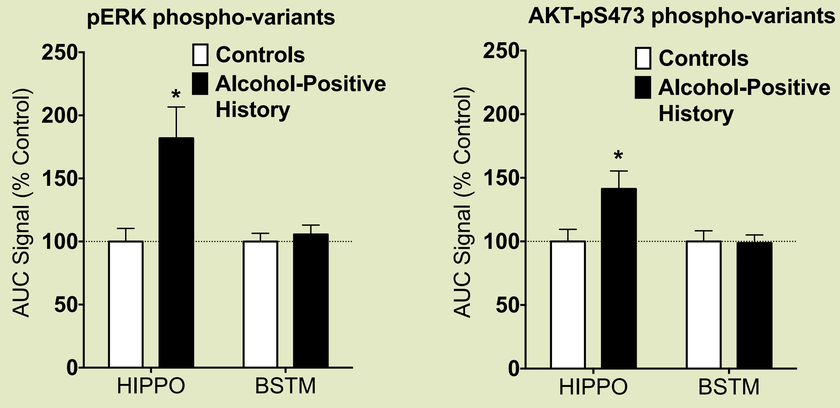
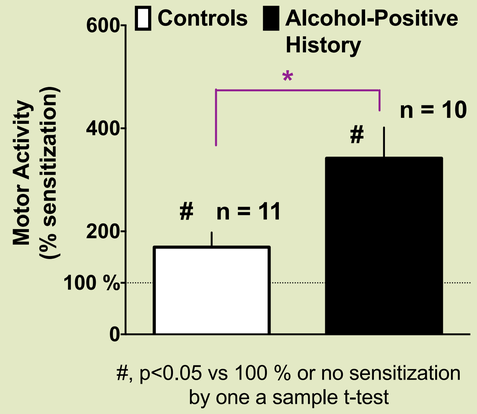


Fig. 1 Quantitation of pERK-1/2 & AKT-pS473 signal responses. Phosphorylated AKT-Ser473 & ERK1/2 levels in hippocampus were 40-80% higher in adult rats exposed as adolescents to alcohol, indicating lasting effects of alcohol on dopamine D2 sensitivity & modifications in discrete phospho-isoform signaling molecules.

FIGURE 2



#, p<0.05 vs 100 % or no sensitization by one a sample t-test

Fig. 2 Behavioral responding to repeated administration of the dopamine D2/D3 agonist quinpirole. Alcohol exposure in adolescence has a robust potentiating effect on later adult expression of quinpirole-induced locomotor sensitization. Total motor activity measured by photo-beam breaks in an open field 50-70 min post drug.

5 Conclusions

- **Peggy Sue™ offers a sensitive platform for detecting** & quantifying a complex mixture of multiple protein phospho-isoforms in brain tissue lysates.
- **Animals with a positive history of adolescent alcohol** exposure show brain region-specific elevations in the phosphorylation status of pAKT-S473 & pERK-1/2, in parallel with their behavioral super-sensitivity to the dopamine D2 agonist quinpirole.
- **Elevated phosphorylation of AKT-S473 & ERK-1/2** are features of lasting shifts in dopamine signaling after only a brief encounter with alcohol at an early adolescent age.
- Phosphorylation modifications of specific ERK/AKT isoforms can be potentially utilized as therapeutic targets **to aid in 'unlearning' addictive behaviors**.

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