Investigating the role of *Casein kinase 1 epsilon* in Addiction Liability

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ABSTRACT Genes encoding *Casein kinase 1(Csnk1)* have recently been implicated in regulating sensitivity to drugs of abuse (1). Opioids such as fentanyl and heroin stimulate dopamine release in the striatum of the brain's reward pathway and increase locomotor activity (**LA**) (2) . Inhibition of isoform Casein Kinase 1 Epsilon (**Csnk1e**) increases sensitivity to locomotor activation by psychostimulants and opioids in mice, suggesting a role in addiction liability in humans (1). We investigated whether Csnk1e inhibits sensitivity to drugs by examining opioid-induced LA including distance traveled, spins, and rotations of *Csnk1e* knockout (**KO**) and wild-type **(WT**) mice injected with fentanyl (0.2 mg/kg, i.p). Not only did the fentanyl-treated *Csnk1e* KO mice travel a greater distance than WT mice, but they also traveled at a greater velocity as seen in number of spins and rotations, thus displaying increased sensitivity to the drug in multiple phenotypes.

To examine the dopaminergic signaling mechanisms of these behaviors (2), brain dissections of the striatum were completed immediately after the LA portion for a side-by-side comparison of behavior and protein signaling. Immunoblotting results of phosphorylation of **DARPP-32** (a key dopaminergic signaling molecule) (3) showed enhanced phosphorylation at **Serine 130** (a CK1 phosphorylation site) in Csnk1e KO fentanyl-treated mice. This supports the hypothesis that Csnk1e inhibits dopamine release in the reward pathway, while other isoforms facilitate it. By knocking out this gene the activity of other isoforms is unmasked, and dopaminergic signaling is stimulated, leading to increased sensitivity to opioid drug.

METHODS Protein expression of drug- versus saline-treated WT and KO mice was analyzed in conjunction with locomotor activity data for a side-by-side behavior and signaling experiment. The LA experiment was 3 days long (fig. 1) and measures such as total distance traveled, number of spins, and rotations were investigated to measure sensitivity to drug between genotypes. On days 1 and 2, the habituation days, all mice – KO and WT – were given saline then placed into a box for 30 minutes during which their activity was recorded on video. On day 3, half of the KO and WT were treated with fentanyl and the other half with saline again as a control and were again placed into the box. We dissected the striatum and frontal cortex – brain areas which have shown significant involvement in the neurocircuitry of addiction (2) – from mice immediately after for a side-by-side behavior and signaling analysis. A third of the protein samples of the striatum were prepared, and the rest will be prepared in the fall to run more immunoblot experiments. We began to run western blots to study expression and phosphorylation of DARPP-32. In particular, we wanted to investigate the sites of T-34, T-75, and Ser-130 (Ser-137 antibody was used, which is the rat nomenclature for the CK1 phosphorylation site), which are all involved on drug-induced dopaminergic signaling; we hypothesized we would see a difference between knockouts and wild-types.

FUTURE DIRECTIONS The T-34, T-75, and Ser-130 sites will continue to be analyzed via immunoblotting; we are currently running additional samples to increase our sample size. We will also complete an identical experiment looking at the effects of methamphetamine on locomotor & signaling in KO mice.

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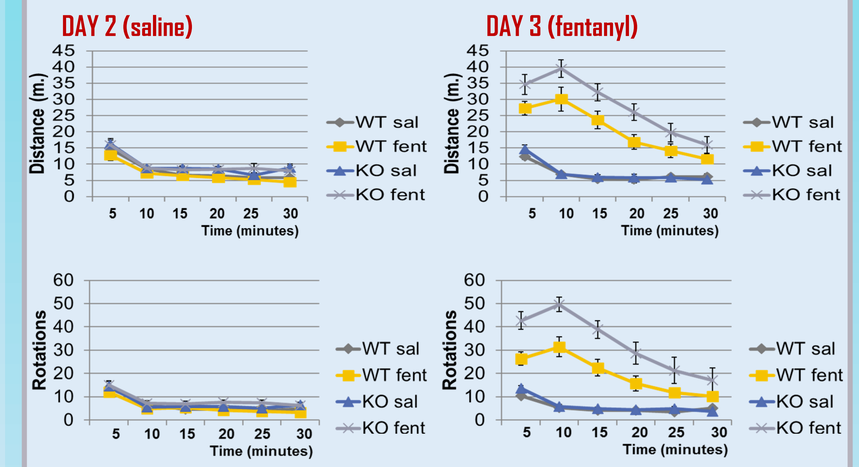
Fig. 1: Locomotor Activity Paradigm

D1	D2	D3
saline	saline	fentanyl

Fig. 2: Total Distance Traveled and # of Rotations



RESULTS The knockout mice displayed more fentanyl-induced locomotor activity than the wild-type mice not only by distance traveled and rotations (fig. 2a), but by spinning more (fig. 3a) and displaying preference for the anti-clockwise direction.

In the signaling portion of the experiment, we found a significant difference in the phosphorylation of site Ser-130 on DARPP-32 between saline-treated and fentanyl-treated KO mice, while no such difference was observed between the saline- versus fentanyl- treated WT mice.

CONCLUSIONS

- Mice lacking Csnk1e (KO mice) are more sensitive to locomotor stimulant effects of opioids than WT mice.
- KO mice display increased phosphorylation of site Ser-130 on DARPP-32 when treated with fentanyl, linking a lack of CK1 to an increased dopaminergic response to opioid drug, therefore, leading to increased drug sensitivity and perhaps addiction liability.

Sources:

1. Bryant CD, Parker CC, Zhou L, Olker C, Chandrasekaran RY, Wager TT, Bolivar VJ, Loudon AS, Vitaterna MH, Turek FW, Palmer AA. Csnk1e is a genetic regulator of sensitivity to psychostimulants and opioids. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2012;37(4):1026-35. doi: 10.1038/npp.2011.287. PubMed PMID: 22089318; PubMed Central PMCID: PMC3280656.3.
2. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2010;35(1):217-38. doi: 10.1038/npp.2009.110. PubMed PMID: 19710631; PubMed Central PMCID: PMC2805560.5.
3. Svenningsson, Per, Angus C. Nairn, and Paul Greengard. "DARPP-32 Mediates the Actions of Multiple Drugs of Abuse." The AAPS Journal 7.2 (2005): 353-60.

Fig. 3: Number of Spins in five minute bins

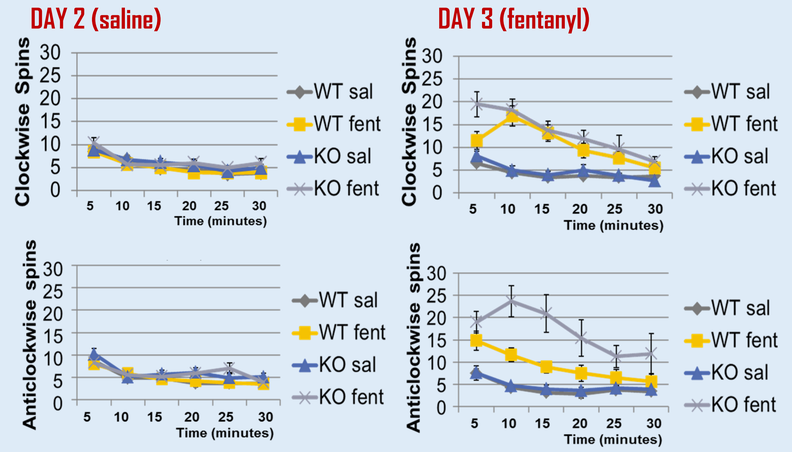
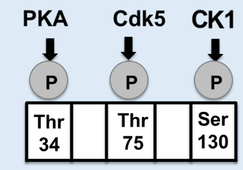
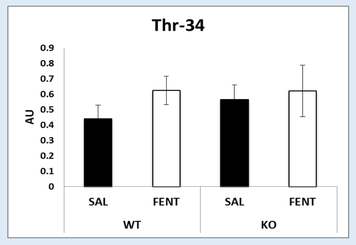


Fig. 4: DARPP-32 Phosphorylation Sites and Western Blots

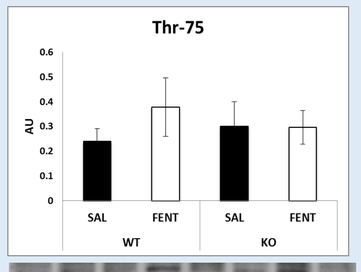


KO sal | KO fent | WT fent | WT sal | WT fent | WT fent | WT sal | KO sal

Thr-34



Thr-75



Ser-137