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Molecular and behavioral mechanisms mediating paclitaxel-induced changes in affect-like behavior in mice

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Abstract

The antineoplastic paclitaxel is associated with negative affective outcomes, such as depression, anxiety, and decreased quality of life during treatment and convalescence. With the Baby Boomer population approaching peak cancer age, it is dire that the mechanisms behind paclitaxel-induced changes in mood are uncovered. Cancer-free male and female C57BL/6J mice were treated with one set of four injections of vehicle or paclitaxel (32mg/kg cumulative), or two sets of four injections of vehicle or paclitaxel (64mg/kg cumulative), and periodically assessed for depression-like behaviors. Paclitaxel caused significant, time-dependent deficits in sucrose preference and operant responding for palatable food. Because there is growing evidence to support the role of kappa opioid receptors (KORs) in stress-mediated depression and reward dysfunction, we investigated KOR signaling as a putative mechanism of paclitaxel-induced depression-like behaviors. The selective KOR antagonist norbinaltorphimine (norBNI) reversed paclitaxel-induced attenuation of sucrose preference. At the molecular level, paclitaxel time-dependently induced an increase in the expression of *Prodynorphin* mRNA, the precursor for endogenous KOR agonists, in the nucleus accumbens (NAc). Using the [^{35}S]GTPγS assay, we discovered that a history of paclitaxel time-dependently attenuated activation of dopamine D_2 receptors (D_2R) and KORs in the NAc but not caudate putamen. These data suggest that paclitaxel-induced changes in affect-like behavior may be due to time- and region-dependent dysregulation of KOR and D_2R signaling. These observations help to establish the roles of KOR and D_2R systems in paclitaxel-induced disruption of behavioral reward, thus revealing potential neurochemical targets for therapeutic intervention in cancer survivors with treatment-resistant depression.

Introduction

- No efficacious treatment for paclitaxel-induced depression in cancer survivors.[1]
- PAC-induced depression can last for up to 5 years or longer following cessation of treatment.[2]
- We previously characterized the effects of a clinically relevant dosing schedule of PAC on behavior in male C57BL/6J mice over the course of 0-4 months.[3]

Behavior	Assay	Weeks Post-PAC Injection					
		1	2–3	4–5	6–7	8–9	10–11
Nociceptive	Mechanical Allodynia	+	+	+	+	+	+
	Cold Allodynia	+	+	ND	ND	ND	ND
Natural	Nesting	–	–	ND	ND	–	–
Anxiety-like	NSF	ND	+	ND	ND	+	–
	LDB	ND	+	ND	+	+	ND
Depression-like	FST	–	+	–	ND	ND	ND
	Sucrose Preference	+	–	ND	ND	ND	ND

(Toma et. al, 2017)

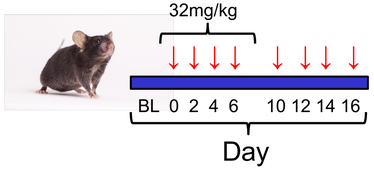
- Here, we further characterize PAC-induced changes in affect-like behavior over the course of 6 months, and we investigate putative molecular mechanisms.

Hypothesis: Negative affective state produced by PAC is mediated by kappa opioid receptor signaling in the nucleus accumbens.

Mouse Model of Paclitaxel-Induced Neuropathy

To further characterize the model used by Toma (2017), adult (8-10 wks) C57BL/6J mice received one set of four injections of Vehicle (1:1:18 [ethanol:kolliphor:saline]) or PAC (8mg/kg x 4 = 32mg/kg cumulative). Injections were every other day.

For select behavior experiments, mice received two sets of four injections of Veh or PAC (8mg/kg x 8 = 64mg/kg cumulative). Sets were separated by 3 days.



Role of Kappa Opioid Receptors

1: *PAC decreases sucrose preference via KOR.*

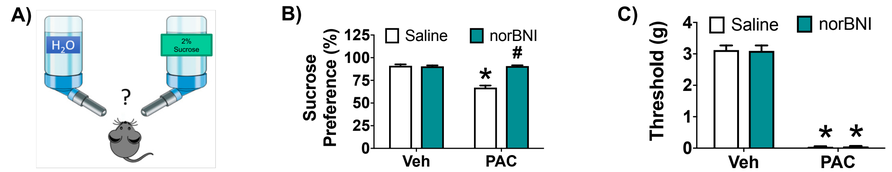
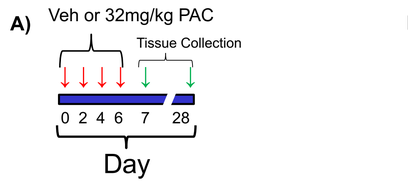
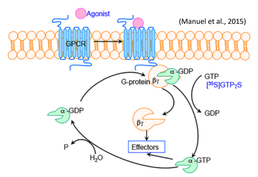


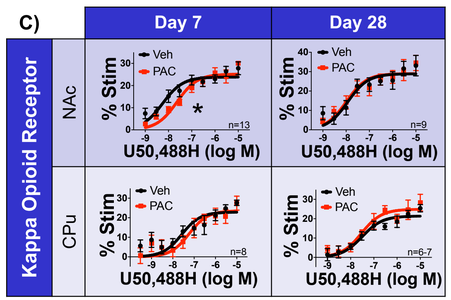
Fig. 1: 32mg/kg PAC and Veh mice were given a single injection of saline or the selective, long-lasting KOR antagonist norBNI (10 mg/kg, s.c.) on Day 6, and 2% sucrose preference in the 2-bottle choice test **(A)** was measured 24 hours later (Day 7). **B)** PAC induced deficit for sucrose preference (Tukey's multiple comparisons test, *p <0.0001 PAC/Saline vs. Veh/Saline). norBNI fully reversed sucrose preference deficit (#p <0.0001 PAC/norBNI vs. PAC/Saline. **C)** Consistent with our previous reports, 32mg/kg PAC induced mechanical hypersensitivity in the von Frey test (Two-way ANOVA: $F_{(1,44)}$ = 722.2, *p <0.0001 PAC vs. Veh). norBNI did not reverse PAC-induced mechanical hypersensitivity. Data are expressed as Mean±SEM, n=8/group. These findings support our hypothesis that altered KOR signaling contributes directly to PAC-induced reward deficit. Importantly, we demonstrate that nociception and anhedonia-like behavior are separable facets of chemotherapy-induced neuropathic pain states.

2: *PAC has temporal-, region-, and receptor-selective effects in the brain.*





[^{35}S]guanosine-5'-O-(γ-thio)-triphosphate ([^{35}S]GTPγS) assay. Agonist-stimulated guanine nucleotide exchange was measured in NAc and CPu. Data are expressed as Mean±SEM % Stimulation (net stimulated [^{35}S]GTPγS binding [fmol/mg] / basal [^{35}S]GTPγS binding [fmol/mg] x 100%).



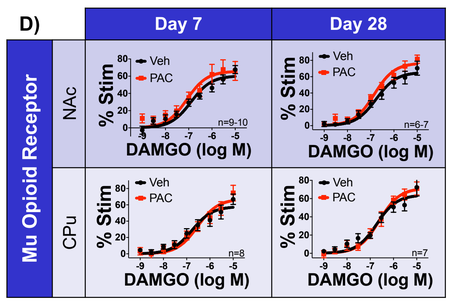
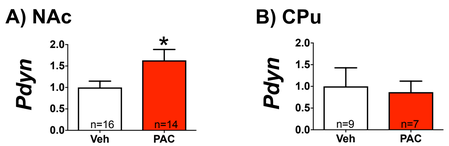


Fig. 2: In order to determine if PAC time-dependently modulates KOR or other opioid receptor activation, Veh and 32mg/kg PAC mice were sacrificed at Day 7 and Day 28 **(A)**. **B)** Schematic of the [^{35}S]GTPγS assay. **C)** 32mg/kg PAC attenuated U50,488H (KOR agonist)-stimulated [^{35}S]GTPγS binding in NAc homogenates at Day 7 (Two-way ANOVA: $F_{(1,181)}$ = 4.180, *p<0.05) and decreased U50,488H potency in PAC-treated mice (EC_{50} = 23.3±5.4 nM) as compared to vehicle-treated mice (EC_{50} = 5.6±1.6 nM); *t*-test *p<0.01. This shift resolved by Day 28. No shift was observed in CPu at either Day 7 or Day 28. **D)** PAC had no effect on DAMGO (MOR agonist)-stimulated [^{35}S]GTPγS binding in NAc or CPu at either time point. PAC had no effect on basal activity in either tissue at either time point. These results suggest that PAC has time- (Day 7), region- (NAc), and receptor- (KOR) specific effects.

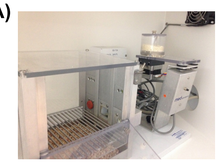
3: *PAC induces time- and region-dependent endogenous KOR agonists.*

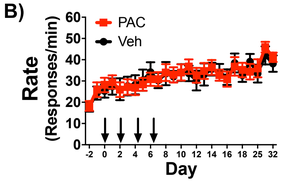
Fig. 4: The results of the norBNI experiment suggest that increased endogenous KOR agonists cause PAC-induced reward deficit. In order to test this hypothesis, Veh or 32mg/kg PAC mice were sacrificed at Day 7 or Day 70. Total RNA was extracted with TRIzol®, followed by reverse transcription with iScript™ cDNA Synthesis Kit (BIO-RAD). Real-time PCR was performed by reacting cDNA with TaqMan® Universal PCR Master Mix and mouse Prodynorphin or β-Actin FAM-MGB probes. **A)** Systemic PAC increased *Pdyn* mRNA, the precursor for endogenous KOR agonists, in the NAc at Day 7. **B)** There was no change in *Pdyn* levels in CPu at Day 7. Further, history of PAC had no effect at Day 70 in either tissue. Data are expressed as relative to *Actb* mRNA using the $2^{-\Delta\Delta Ct}$ method; t-test, *p <0.05.



Characterization of Motivation Deficit Behavior

5: *PAC has work-, time-, and sex-dependent effects on operant responding.*





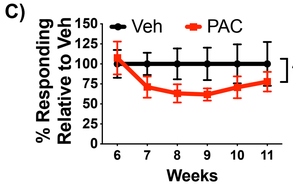
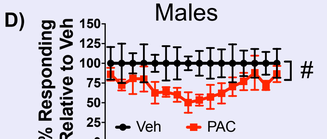
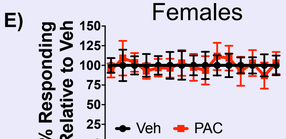
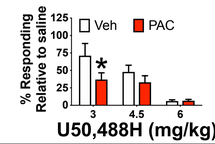


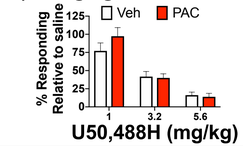
Fig. 5: A) Apparatus: operant chamber outfitted with nose-pokes and a dispenser of Purified Rodent Diet, lightly sweetened 14mg pellets. The inactive nose-poke is plugged. **B)** Food-regulated male mice responded on a fixed-ratio 10 schedule of reinforcement. Acute 32mg/kg PAC treatment did not change rate of responding during 15 min sessions. Arrows indicate injections. Data expressed as Mean±SEM, n=7-9. **C)** The subjects from **B** were presented with the Richardson & Roberts[4] progressive ratio ([5*e (R*0.2)] – 5, where R is the # of food reinforcers earned + 1) during 1 hr sessions. A true break point was not reached when food-regulated or on free-feed, tested in sessions up to 4 hrs long. However, PAC-treated mice had a drop in total number of nose-pokes starting at Week 7, suggesting motivation deficit; two-way ANOVA: $F_{(1,73)}$ = 6.570, *p <0.05, n=7-9. Data expressed as total nose-pokes per session, normalized to Veh.





D) A new cohort of male mice was challenged with a variety of progressive ratios of increasing steepness in order to determine the true break point. Of note, the ratios of 2^x (1, 2, 4, 8, 16, 32...) and 10^x (1, 10, 100, 1000...) produced break points in a quarter of the mice during 1 hr sessions. Data were collected weekly using both steep ratios. The 10^x ratio was not sensitive to changes in motivation, whereas the 2^x ratio revealed ~50% decrease in operant responding, which first emerged 4 weeks after completion of 64mg/kg PAC treatment and began to resolve after 12 weeks; two-way ANOVA: $F_{(1,191)}$ = 24.00, #p <0.0001, n=6-8. **E)** 64mg/kg PAC had no effect on free-feeding females performing 2^x progressive ratio, n=10.





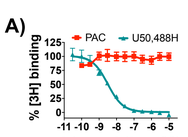
Pilot Data: F) 32mg/kg PAC exacerbated U50,488H-induced depression of operant responding for sucrose during week 11, Richardson & Roberts ratio. Two-way ANOVA: $F_{(1,38)}$ = 4.6, p <0.05, n=7-8. Sidak's multiple comparisons test identified 3mg/kg U50,488H to have a greater impact on PAC mice, *p <0.05. **G)** 64mg/kg PAC did not interact with U50,488H-induced depression of operant responding when tested at week 19, 2^x ratio, n=6-8.

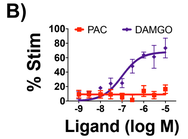
4: *PAC is not a KOR agonist.*

Fig. 4: Because systemic PAC has well-documented effects on mood in human patients, we investigated if PAC is a KOR ligand.

A) PAC did not displace [^{3}H]Diprenorphine binding in mKOR-expressing CHO cells at concentrations up to 10 μM (data expressed as Mean±SEM [^{3}H]Diprenorphine binding, n=4-5).

B) PAC did not stimulate guanine nucleotide exchange in NAc from naïve mice at similar concentrations (data expressed as Mean±SEM % basal stimulation, n=3-5). Further, primary binding assays performed by the Psychoactive Drug Screening Program did not identify likely targets. These data suggest that PAC is not a direct KOR or GPCR $G_{(i/o)}$ agonist.





Summary & Conclusions

- Mice had temporal-, region-, and receptor-selective changes in their brains.
- PAC is not a direct KOR agonist, but may activate KOR indirectly via upregulating *prodynorphin* mRNA.
- Mice show work-, time-, and sex-dependent effects on operant responding for mildly sweetened food pellets.
- Preliminary data suggest that onset, duration, and magnitude of deficit in operant responding may be PAC dose-dependent.
- Future studies will further investigate the effects of KOR modulators on PAC-induced changes in affect-like behavior.

Acknowledgements & Works Cited

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Cartoon of 2-bottle choice test modified from: https://bio-protocol.org/e3061