Establishment of an antinociceptive dose of oxycodone

Initial studies were done to establish effective antinociceptive doses of oxycodone using oral gavage in male and female rats using the hot plate procedure. We initially used a dose range of 1 – 25 mg/kg with a single time point assessment of 30 min. We then further characterized a time course response at 5 and 15 mg/kg oral oxycodone in males and females (n=8 per sex). Latency to lick the hind paws when the rat was placed on a hotplate (52°C; Ugo Basile, Varese, Italy) was recorded. The maximum latency was set at 30 sec in order to avoid any tissue damage. Rats were subjected to three days of baseline testing wherein one trial per day was performed. On the fourth day, rats received water vehicle only followed by testing conducted at 15 min intervals for 3 hr. On the next day they received 5-mg/kg oxycodone. After a three-day clearance break, they received 15 mg/kg.

As can be seen in Supplemental Figure 1, 5 mg/kg led to a mild and transient degree of antinociception, while 15 mg/kg caused a more robust and sustained response, especially in the females. For females, there was a Treatment effect (F 2, 21 = 14.68, p <0.0001) and a marginal Treatment x Time interaction (F 24, 252 = 2.33, p < 0.0007). For males, there was a Treatment effect (F 2, 21 = 3.34, p = 0.05) and a marginal Treatment x Time interaction (F 24, 252 = 1.51, p = 0.06). A priori comparisons were done between the two doses and the water vehicle treated groups. For females,
both doses induced antinociception ($F_{1, 14} = 7.92$ and $23.34$, $p's <0.02$ and $0.0004$ for $5\text{ mg/kg}$ and $15\text{ mg/kg}$ respectively). While for males, only the $15\text{-mg/kg}$ dose was significantly different than controls ($F_{1, 14} = 4.82$, $p = 0.05$). Thus for the subsequent studies examining the pro-emetic pica response, the $15\text{ mg/kg}$ dose was used.

**Supplemental Figure 1:**

**Females**

![Graph showing the effect of Oxycodone 5 mg/kg and Oxycodone 15 mg/kg on females over time.]

**Males**

![Graph showing the effect of Oxycodone 5 mg/kg and Oxycodone 15 mg/kg on males over time.]

**Oxycodone administration i.p. induces the pica response**

We had concerns that pica response was a consequence of the oral route of administration, so an additional experiment was conducted using an i.p. route of administration in female rats. We used two doses of oxycodone, 10 mg/kg and 15 mg/kg, and compared the pica response to female rats injected with saline (n=3-4 subjects per group). Pica was assessed as described in the Methods of the main paper. As shown in supplemental data Figure 2, the pica response was found after i.p. injection of oxycodone (Treatment effect: F_{2, 7} = 174.0, p <0.0001; Treatment x Time interaction: F_{4, 14} = 4.97, p <0.02). Saline-treated subjects did not exhibit the expression of pica behavior. Both doses of oxycodone i.p. induced the pica response across the 3 hr time frame (* p <0.05 vs. respective saline control; Tukey’s post-hoc test).

**Supplemental Figure 2:**

![Supplemental Figure 2](image)

Naloxone attenuates the antinociceptive response to oxycodone

Before determining that naloxone could block the oxycodone-pica response, we determined the doses of naloxone that were effective in attenuating the antinociceptive response to oxycodone. Antinociception was assessed by the hotplate test as described...
above. Female rats (n=6 per group) were subjected to three days of baseline testing wherein one trial per day was performed. On the fourth day (water test day), rats received vehicle only followed by testing conducted at 15 min intervals for 2 hr. On the test day (24 hr later), rats were pretreated with either vehicle or different doses of naloxone (0.5, 1.0 and 2.0 mg/kg s.c.) followed 10 min later by oxycodone (15 mg/kg oral). Following drug treatment, rats were tested on the hotplate every 15 min during a 2 hr session. Data were analyzed by a repeated measures ANOVA.

There was an overall effect of Treatment (F_{3, 20} = 4.02 p <0.03) and a (Treatment x Time interaction: F_{15,100} = 3.65, p <0.0001). As can be seen in supplemental Figure 3, each of the naloxone doses was effective in reducing the efficacy of oxycodone in the hotplate antinociceptive test (p <0.05 or lower for each post-hoc comparison). Thus, the dose range of 0.5 – 2.0 mg/kg was used in to determine the involvement of opioid receptors in the oxycodone-induced pica response.

Supplemental Figure 3: