An investigation of the reinforcing potential of PN6047 by conditioned place preference testing in rats

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INTRODUCTION

PN6047, which is a novel, selective δ-opioid agonist, is being developed to treat persistent chronic pain. PN6047's reinforcing potential has been evaluated in conditioned place preference (CPP) using rats as the experimental subjects. Heroin was used as the positive control and hydroxy-propyl-methylcellulose (HPMC) vehicle as the negative control.

METHOD

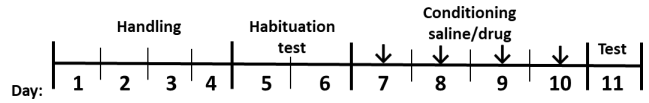
Male Wistar rats (6 - 7 weeks) were obtained from the University of Bath breeding colony. All animals were housed in groups of 4 in a behavioural holding room with controlled temperature (24 ± 2°C), humidity (50 - 60%), and a 12:12h light-dark cycle (lights on 07.00h – 19.00h). Food (standard rat chow) and water were available *ad libitum*. Rats were allowed to habituate to laboratory conditions for 4 days before experiments began during which time they were handled daily in the experimental room. A total of 60 rats were used in this study, 12 rats/ treatment group.

CPP was conducted in a 3-compartment apparatus (MED Associates, UK) consisting of a two-compartment box (40 x 40 cm each), one with vertical and one with horizontal black and white stripes, separated by removable guillotine doors and a central neutral zone measuring (10 x 20 cm). The floors of each chamber were also different; one with 2 cm round holes and the other with 1 x 1 cm square holes, respectively. Experiments were performed in dim light, and each conditioned place preference apparatus was covered with sound-attenuating material.

After 4 days of handling, male, Wistar rats were allowed to move freely between the compartments for 15 min while cameras tracked their movements (Habituation). On Days 7-10 (Conditioning), rats received saline (1mL/kg, s.c.), heroin (1 mg/kg, s.c.), PN6047 (3 mg/kg, i.p.), PN6047 (9 mg/kg, i.p.) or vehicle (HPMC; 1.5 mL/kg, i.p.) and were restricted to their corresponding compartment for 40min. The following day, those that received heroin or PN6047 received saline or vehicle and were restricted to the opposite compartment. This procedure was repeated over 4 days. CPP was performed 24hr after the last conditioning day. The guillotine doors were removed and rats were allowed to roam freely for 15 min. The schedule of procedures is illustrated in Figure 1.

Results are reported as mean±SEM, n=12/group. Data were collected via a camera and analysed through a PC equipped with an auto-monitoring system (Ethovision XT version 8.0). Data throughout are presented after multiplying by a correction factor. The correction factor was calculated by dividing duration of test (900 sec) by total time spent in the two large compartments. This factor is used to proportionally divide the time spent in the neutral central compartment between the 2 conditioning compartments. Preference scores (time spent in sec in the drug-paired compartment minus 450 sec [50% of the test session]).Statistical outliers in each stage of conditioned place preference were determined using Grubbs' test for outliers (no data-points were deemed to be statistically-significant outliers). All statistical analyses were performed with GraphPad Prism 5 using paired Student's t test.

Figure 1: Experimental protocol



RESULTS

- All of the groups of rats showed normal rates of growth (Figure 2)
- Saline did not produce any preference between the compartments (Post-conditioning vs Habituation: Saline = 6.92 ± 51.78 sec vs 8.09 ± 23.4 sec; Figure 3A).
- Heroin produced robust preference for the drug-paired compartment (99.54 ± 41.26 sec vs 5.61 ± 23.7 sec, p<0.05; Figure 3B).
- The PN6047 vehicle did not produce any preference between the compartments (Post-conditioning vs Habituation: Vehicle = -9.61 ± 34.35 sec vs 4.36 ± 23.16 sec; Figure 4A).
- Neither dose of PN6047 induced place preference (PN6047 [3mg/kg] = -27.66 ± 40.32 sec vs 4.69 ± 27.78 sec; PN6047 [9mg/kg] = 64.86 ± 51.57 sec vs 5.07 ± 20.18 sec).

Figure 2: Mean bodyweights of the treatment groups

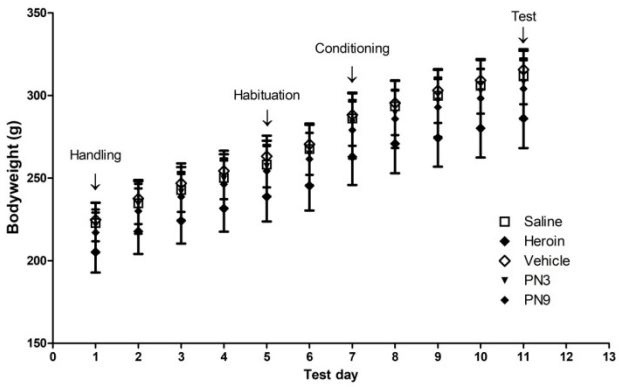
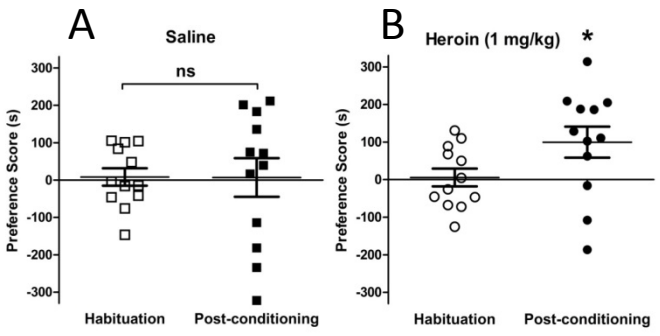
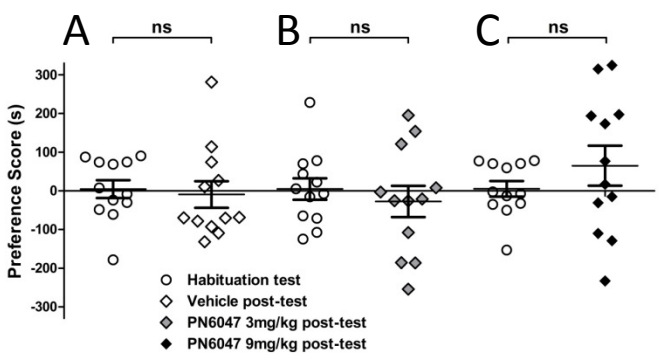


Figure 3: Preference scores for rats conditioned with saline (s.c.) or heroin (1 mg/kg s.c.)



Data points are individual rat responses with mean ± overlaid. Paired Student's t-test post-conditioning vs habituation scores *p<0.05, n=12 rats/group.

Figure 4: Preference scores for rats conditioned with PN6047 (3 or 9 mg/kg i.p.) or HPMC vehicle (i.p.)



Preference scores for rats conditioned with HPMC vehicle (open diamonds), PN-6047 3 mg/kg (grey diamonds) or PN-6047 9 mg/kg (black diamonds). Data points are individual rat responses with mean ± SEM overlaid. Paired Student's t-test post-conditioning vs habituation scores (ns: not significant). n=12 rats/group.

CONCLUSIONS

The μ-opioid agonist, heroin, produced significant CPP. However, CPP was not elicited by the δ-opioid receptor agonist, PN6047. Therefore, PN6047 does not produce reinforcing effects that induce CPP in rats. If these results translate to humans they predict PN6047 will not produce rewarding effects that could lead to abuse.

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