Extended-release buprenorphine (XR), a new FDA-approved opioid, is indicated for pain management in rodents. However, little is known about its use in mice. This study aimed to investigate whether high-dose XR effectively attenuates post-operative hypersensitivity better than low dose XR in a model of incisional pain. Mice (n=44) were randomly assigned to 1 of 4 treatment groups: 1) Saline (1 mg/kg SC, once); 2) sustained-release buprenorphine (Bup-SR, 1 mg/kg SC, once); 3) low-dose extended-release buprenorphine (XR-Lo, 3.25 mg/kg SC, once); or 4) high-dose extended-release buprenorphine (XR-Hi, 6.5 mg/kg SC, once). On days -1, 4, 1, 2, and 3, mechanical and thermal hypersensitivity were evaluated, and plasma buprenorphine concentrations were measured. Mechanical (day 0-2) and thermal (day 0-1) hypersensitivity were observed in the saline group. Bup-SR, XR-Lo, and XR-Hi attenuated mechanical hypersensitivity on days 0-2. None of the treatment groups, except XR-Lo, attenuated thermal hypersensitivity on day 0 or 1. Plasma buprenorphine concentration peaked at day 0 (4 hrs) in all treatment groups and achieved greater than 1 ng/ml on days 0-2. No abnormal clinical observations or gross pathologic findings were seen in any groups. Results indicate XR-Hi did not effectively attenuate post-operative hypersensitivity, better than XR-Lo. Both 3.25 and 6.5 mg/kg XR are recommended to attenuate post-operative hypersensitivity for at least up to 48 hrs in this model.

**Methods**

**General Methods.** Adult male C57BL/6 mice weighing 27-30 g (The Jackson Laboratory, Bar Harbor, ME) were used.

**Drug Administration & Surgery.** Mice (n=44) were randomly assigned to 1 of 4 treatment groups: Saline (1 mg/kg SC), Sustained Release buprenorphine (Bup-SR 1 mg/kg SC), or extended-release buprenorphine (XR-Lo 3.25 mg/kg SC). Ethical, Fetal, North Brunswick, NJ, or high-dose extended-release buprenorphine (XR-Hi 6.5 mg/kg SC). Mice were induced and anesthetized with isoflurane. After induction, mice were transferred to the surgical field. Drugs were administered once prior to surgery. The plantar surface of the hindpaw and paw was then aseptically prepared. A 0.5 mm linear incision was made into the skin on the plantar surface, the underlying muscle bundle elevated, and a stab incision made into the muscle bundle to avoid muscle attachments or underlying structures. A pair of forceps is used to distort the muscle horizontally for 5 sec. The muscle was then replaced, and the skin closed with 4-0 silk sutures. Animals were recovered in a warm recovery cage monitored continuously until fully ambulatory.

**Part 1: Hypersensitivity Assessment.** Baseline data for assessment was obtained on day -1 (D1), D0 (D1), D2, D1, and D2. Mechanical hypersensitivity: Mice were placed on an elevated mesh platform (Fig 3A) within clear plastic chambers and were acclimated to the testing environment for 15 min prior to testing. Response thresholds were assessed with calibrated von Frey monofilaments (5.88 mN). The von Frey monofilaments were applied to the plantar surface of each hindpaw, from below the mesial forefoot. The contralateral hindpaw served as control. Mechanical hypersensitivity was defined as a significant increase in paw withdrawal of ipsilateral hindpaw.

**Thermal hypersensitivity.** Radiant heat was applied to the plantar surface of each hindpaw and thermal response latencies were determined according to a method adapted from Harpes et al. [15]. Prior to testing, mice were acclimated to the testing environment for 15 min within clear plastic chambers on top of a clear 3-mm-thick glass elevated to allow exposure to controlled radiant heat source beneath (Fig 5B). Heat stimuli were produced by a 50-W light bulb with a beam intensity of 20%. The radiant heat beam was applied to the plant surface of the hindpaw taking care to avoid the incision site, first pads, and sides of the paw. The time it took for an animal to withdraw a paw from the heat stimulus was defined as the thermal latency (20 sec cutoff). The contralateral hindpaw served as control. Thermal hypersensitivity was defined as a significant decrease in withdrawal latency of ipsilateral hindpaw to heat stimuli.

**Part 2: Plasma Concentration.** Animals were randomly assigned to the same treatment groups as utilized in the surgery portion of this experiment. Animals were induced with isoflurane and injected with either Saline (n=12), Bup-SR (n=12), XR-Lo (n=16), XR-Hi (n=16) and recovered in a warm recovery cage. Animals were euthanized under isoflurane with exsanguination at D0 (4 hrs), D1, D2, and D3 for whole blood collection followed by cardiac dislocation. Samples were analyzed by HPLC-MS/MS (Santifled University, Birmingham, AL).

**Body weight & Gross Pathology.** Animals were weighed daily during the experiment prior to testing or surgery. At the end of the experiment, animals were euthanized, and a gross necropsy was performed to assess for any gross pathologies.

**Statistical Analysis:** To assess significance of differences in withdrawal responses by group and over time, repeated-measures ANOVA with Bonferroni correction for multiple comparisons (R Development Core Team, 2015) was performed. Data were expressed as mean ± SEM. Weight between D1 and D3 were compared using paired t-test with one-tailed test. The value of less than 0.05 was considered significant.

**Results**

**Fig 1:** Harris-IX & Fomulation of XR used in this experiment (1.3mg/Kg).

**Fig 2:** Behavioral assessment environments. A) Testing environment for mechanical hypersensitivity testing. B) Testing environment & equipment for thermal hypersensitivity testing.

**Fig 3:** Mechanical Hypersensitivity. Measured in number of paw withdrawals (mean ± SEM) of ipsilateral hindpaw after sham surgery on D0. * indicates p < 0.05 from baseline (D0) with the same treatment group. Saline treated animals had significantly higher number of paw withdrawals on D0 (4 hrs). D1, D3, D2, and D3 contralateral thermal hypersensitivity. None of the animals treated with opioid experienced increased number of hindpaw withdrawals.

**Fig 4:** Thermal hypersensitivity. Measured in seconds until paw withdrawal from heat stimulus (mean ± SEM) of ipsilateral hindpaw. Arrow indicates surgery on D0. * indicates value is significantly different (p < 0.05) from baseline (D1-1) within the same treatment group. Saline treated animals had significantly decreased thermal latencies on D0 (4 hrs) & D1. On D0 (4 hrs), only XR-Lo treated animals did not experience a significant decrease in thermal latency. On D1, all opioid treated animals experienced decreased thermal latencies.

**Fig 5:** Buprenorphine plasma concentrations. Concentrations of buprenorphine in Bup-SR, XR-Lo, and XR-Hi treated mice (ng/ml, mean ± SEM), n = the number of animals sampled (3 or 4) at each time point. Samples were analyzed by HPLC-MS/MS. Administration * indicates values are significantly (p < 0.05) different from that on D0 within the same treatment group.

**Body Weight & Gross Pathology:** The weights of animals in Bup-SR and XR-Hi groups were significantly lower on D3 than D1 and D2 (data not shown). There were no abnormal clinical observations or gross pathologic findings seen in any groups at the end of the experiment (data not shown).

**Conclusions**

- XR-Hi (6.5 mg/kg SC) did not attenuate post-operative mechanical & thermal hypersensitivity more effectively than XR-Lo (3.25 mg/kg SC) in an incisional pain model in mice. Both dosages were as effective as Bup-SR (1 mg/kg SC) in attenuating post-operative mechanical hypersensitivity.
- Plasma concentration of buprenorphine were achieved higher than 1 ng/ml in all treated groups for 2 days.
- Both XR-Lo (3.25 mg/kg) & XR-Hi (6.5 mg/kg) are recommended to attenuate post-operative hypersensitivity in an incisional pain model in mice for 2 days.