Published in final edited form as:

Neuropharmacology. 2017 May 01; 117: 401–407. doi:10.1016/j.neuropharm.2017.02.005.

Genetic Variation in the Behavioral Effects of Buprenorphine in Female Mice Derived from a Murine Model of the *OPRM1* A118G Polymorphism

Caroline A. Browne^{1,*}, Rebecca L. Erickson², Julie A. Blendy³, and Irwin Lucki^{1,3}

¹Department of Psychiatry, University of Pennsylvania, Philadelphia, PA

²Department of Pathobiology, University of Pennsylvania, Philadelphia, PA

³Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA

Abstract

Pharmacogenetic studies have identified the non-synonymous single nucleotide polymorphism (A118G) in the human mu opioid receptor (MOR) gene (*OPRM1*) as a critical genetic variant capable of altering the efficacy of opioid therapeutics. To date few studies have explored the potential impact of the *OPRM1* A118G polymorphism on the pharmacological effects of buprenorphine (BPN), a potent MOR partial agonist and kappa opioid receptor antagonist, which is approved by the FDA for the treatment of opioid addiction and chronic pain. The goal of these studies was to determine whether the MOR-mediated behavioral effects of BPN were altered in the *Oprm1* A112G mouse model of the human *OPRM1* A118G SNP. All studies were conducted in female, AA, AG and GG mice. BPN's maximal analgesic effect in the hot plate test was significantly blunted in AG and GG mice compared to wild type AA mice. Similarly, the BPN-induced reduction of latency to consume food in the novelty induced hypophagia test was blocked entirely in AG and GG mice compared to their AA littermates. In addition, GG mice exhibited marked reductions in psychostimulant hyperlocomotor activity compared to the AA group. In contrast, reduced immobility in the forced swim test, an effect of BPN mediated by kappa opioid receptors, was not affected by genotype. These studies demonstrate the ability of the *Oprm1* A112G SNP to attenuate the analgesic, anxiolytic and hyperlocomotor effects of BPN. Overall, these data suggest that the *OPRM1* A118G SNP will significantly impact the clinical efficacy of BPN in its therapeutic applications.

Keywords

mu opioid receptor; *Oprm1* A112G; analgesia; anxiety; Buprenorphine hydrochloride (PubChem CID: 441364); Chlordiazepoxide (PubChem CID: 2712)

*Corresponding Author: Caroline A. Browne, Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of Health Sciences, 4371 Jones Bridge Road, Bethesda, MD 20814, caroline.browne.ctr@usuhs.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 INTRODUCTION

Pharmacogenetic studies have highlighted the ability of specific genetic variants to alter the effectiveness of opioid therapeutics (Crist and Berrettini, 2014; Reed et al., 2014). One of these variants, a non-synonymous single nucleotide polymorphism (A118G) in the human mu opioid receptor (MOR) gene (*OPRM1*) (Bergen et al., 1997; Bond et al., 1998), is distributed widely among various populations. It is most frequently observed in individuals of Asian (49–60%) and European ancestry (15–30%) (Gelernter et al., 1999; Mura et al., 2013). The A118G SNP corresponds to an amino acid substitution of asparagine (N) to aspartic acid (D) at position 40 in the N-glycosylation site of the receptor, resulting in protein instability and reduced expression of MORs at the cell surface (Huang et al., 2012; Mague et al., 2009; Oertel et al., 2012; Zhang et al., 2015). Subjective pain scores are noticeably higher in carriers of the G allele and they require significantly larger doses of opioid analgesics to alleviate their pain (Baber et al., 2016; Bonenberger et al., 2015; Chou et al., 2006b; Lopez Soto and Catanesi, 2015; Sia et al., 2008). In addition, the G allele is associated with enhanced rewarding effects of alcohol and nicotine and marked deficits in fronto-striatal inhibitory control (Bach et al., 2015; Courtney et al., 2013; Ray et al., 2006), indicating that this SNP may be associated with vulnerability to substance abuse.

Murine models of the A118G SNP have been generated. A knock-in mouse was developed that possesses the mouse-equivalent SNP of the human A118G SNP in the murine *Oprm1* gene (Mague et al., 2009). In a complimentary approach, a different mouse line was generated that express humanized receptors with and without the A118G variant (Ramchandani et al., 2011). The effects of morphine but not fentanyl on intracranial self-stimulation and dopamine release in the nucleus accumbens were blunted in the humanized mouse model of the *OPRM1* A118G polymorphism (Robinson et al., 2015). Using the same model, trigeminal ganglion neurons isolated from mice with the G allele exhibited blunted inhibition of Ca^{2+} channels following morphine but not fentanyl administration *ex vivo* (Mahmoud et al., 2011). These ligand-specific effects of the *OPRM1* A118G are also evident in humans, where hydromorphone failed to adequately alleviate pain in carriers of the G allele following caesarian section (Boswell et al., 2013) whereas the G allele was associated with better epidural analgesia with fentanyl during labor compared to AA women (Song et al., 2013). Buprenorphine (BPN) is a potent partial agonist at MORs and a kappa opioid receptor (KOR) antagonist (Cowan et al., 1977) that is commonly used for the treatment of opioid addiction and chronic pain. However, few studies have evaluated the consequences of the A118G SNP in relation to responses to BPN. A small clinical study identified a genotype dependent effect of BPN on HPA axis reactivity in heroin addicts, where BPN-maintained subjects with the AG/GG genotype exhibited greater attenuation of metyrapone provoked ACTH release compared to AA carriers (Kakko et al., 2008).

In addition to the treatment of opioid addiction and chronic pain, compelling clinical (Karp et al., 2014; Yovell et al., 2016) and preclinical evidence (Falcon et al., 2016) have recently highlighted the potential antidepressant effects of BPN. As such, BPN is likely to be repurposed for the treatment of major depressive disorder (MDD) in the near future. Considering the increasing number of individuals prescribed BPN for treating pain,

substance abuse and depression, it would be informative to evaluate the influence of the A118G SNP on a range of behavioral phenotypes produced by BPN mediated by different mechanisms. Gender specific determinants of neuropsychiatric disorders have not been adequately reflected in preclinical or clinical evaluation of novel antidepressant therapeutics. Per world health organization figures, MDD comprises 49% of all neuropsychiatric disorders reported in females and only 28% in males (Marcus et al., 2005; Weller et al., 2006). To date, all preclinical work evaluating BPN in the context of MDD has been reported in males and clinical studies have not evaluated potential sex differences. Moving forward, the need to screen potential therapeutic effects in both sexes is critical. This view is emphasized by previous pharmacological findings relating to the A118G SNP. Sex-specific associations of the AG and GG allele were associated with reduced reinforcing value of nicotine (Ray et al., 2006), blunted stress reactivity (Lovallo et al., 2015), development of persistent pain (Hasvik et al., 2014; Olsen et al., 2012) and higher incidences of alcohol dependence in female carriers compared to their male contemporaries. Similarly, in the OPRM1 A118G mouse model, *Oprm1* A112G, decreased morphine-mediated antinociception, ablated morphine-place preference and lower levels of DAMGO binding were observed in female but not male GG carriers (Mague et al., 2009; Wang et al., 2014). Therefore, the goal of these studies was twofold, to determine whether female mice were sensitive to the antidepressant and anxiolytic action of BPN and to assess the impact of the A118G SNP using a murine model of this SNP, *Oprm1* A112G, on BPN's effects in murine behavioral tests known to be mediated by MORs and KORs.

2 MATERIALS AND METHODS

2.1 Animals

Female C57BL/6J mice, 8 weeks of age when obtained from The Jackson Laboratory (Bar Harbor, MA), were used to evaluate the dose-response curve for BPN in the novelty-induced hypophagia test. For all other tests female *Oprm1*-A112G mice, with the following genotypes, A/A, A/G and G/G were used. These mice were generated on a C57BL/6 genetic background, and bred using a heterozygous breeding scheme as described previously (Mague et al., 2009). Briefly site-directed mutagenesis introduced the polymorphism into exon 1 of *Oprm1* gene, where an adenine (A) nucleotide at position 112 was changed to a guanine (G). This substitution is equivalent to that in the human *OPRM1* gene at position 118. All mice (8–19 weeks, 18–26 g) were housed in cages of two and maintained on a 12-h/12-h light/dark cycle with food and water available *ad libitum*. All studies were approved by Institutional Animal Care and Use Committee of the University of Pennsylvania and conducted in accordance to PHS policy.

2.2 Drugs

Buprenorphine hydrochloride (BPN) was provided by the Research Technology branch of the National Institute on Drug Addiction (NIDA). For all studies BPN (0.25, 0.5 and 1 mg/kg) was dissolved in vehicle (sterile HPLC grade water, MilliQ system, Millipore), and sonicated for 15 minutes. Control mice were administered vehicle. Chlordiazepoxide (CDP, Sigma-Aldrich) was dissolved in sterile 0.9% saline (Hospira Inc.). Control mice received

sterile saline. All compounds were prepared freshly on each experimental day and were administered using a 10 ml/kg injection volume.

2.3 Analgesia

The nociceptive threshold for analgesia was examined with a hot-plate analgesia meter (Columbus Instruments), set at a constant surface temperature of 55 °C. This temperature was sufficient to cause thermal nociception without harming the sensitive tissue on the paws. A small plastic cage surrounding the hot-plate prevented the animal from leaving the plate surface. Cumulative dosing was conducted as follows. Baseline values were recorded from mice on the first exposure to the hot plate, where the latency to lick the hind-paw or jump was recorded. As soon as the mice displayed either of these behaviors, or reached the predetermined cut-off time of 60 s, mice were immediately removed from the hot-plate. Mice were then administered the first dose of BPN, returned to their home cage and retested on the hot plate 30 min later. This process was repeated for all mice, which received doses of 0, 0.1, 0.2, 0.7, and 2 mg/kg, tested at 30 min intervals. The cumulative dose of the final injection of BPN was 3 mg/kg. Data is calculated $((\text{observed response} - \text{baseline response}) / (60 - \text{observed response}) * 100)$ and presented as percentage Maximum Possible Effect (%MPE)

2.4 Novelty induced hypophagia (NIH)

Animals were trained to consume a palatable food (peanut butter chips, Nestle) in the home cage for 12 days to establish stable latencies to consume the chips (Balu et al., 2009). Mice were housed in cages of two and had free access to chow up until training or testing session. On each training day, mice were separated with an opaque Plexiglas divider in the home cage 60 min prior to presentation of the food in a designated testing room. Each mouse was individually presented with chips (1.4 g) for 15 min, following which the divider was removed and mice returned to the colony room. Inclusion in the study required mice to exhibit latencies of ≥ 30 s over three consecutive days. BPN was shown to be effective in the NIH test 24 h post administration (Falcon et al., 2015); mice were injected with saline immediately following the last training exposure, 24 h prior to testing. As CDP is effective acutely, a separate cohort of mice was injected 30 minutes prior to testing. On the test day, testing was conducted in a different room to training. Each mouse was placed in a novel environment, consisting of a polycarbonate cage (48L $\times$ 26W $\times$ 20H cm) with no bedding, brightly lit (800 Lux) in a room illuminated by low light (400 Lux). A novel smell was introduced to the cage by cleaning it with diluted dish soap (Lemon Joy). Latency to begin eating the familiar palatable food in the center of the novel environment was then measured. The duration of the test was 15 min. If the mouse failed to approach and consume the food within this time, a latency value of 900 s was assigned to the mouse for data analysis.

2.5 Locomotor activity (LMA)

Activity was measured 30 min after BPN treatment in a cage identical to the dimensions of the home cage (28.9 $\times$ 17.8 $\times$ 12 cm) for 1 h identical to the dimensions of the home cage. The cage was placed inside an infrared sensitive motion detection system (30 $\times$ 24 $\times$ 8 cm) with an 8-beam photocell array strip (Med Associates Inc, St. Albans, VT). The number of crosses and rearing activity were measured for all mice. MOR agonists including BPN produce

robust hyperactivity in this test immediately following administration (Crawley et al., 1997; Falcon et al., 2015).

2.6 Forced Swim Test (FST)

Swim sessions were conducted 24 h after treatment with BPN (0.25 mg/kg). Briefly, mice were placed in individual polycarbonate cylinders (25.3 cm tall × 22.2 cm in diameter filled to a depth of 15 cm with water (25±1 °C) that was changed between each animal. Test sessions were 6 minutes in duration, recorded from an overhead view and scored by a blinded observer. Immobility, defined as making only the movements necessary to maintain the head above the water, was scored in the last four minutes of the 6 minute test (Lucki et al., 2001).

2.7 Statistical analysis

All data are expressed as mean ± SEM and analyzed using Graph Pad Prism 7.0 (La Jolla, CA). Two-way repeated measures ANOVA were used test for significant interactions of genotype × cumulative BPN dose on antinociception in the hot plate test and genotype × training day on dependent variables in the NIH training phase. A one-way ANOVA with Dunnett's multiple comparisons tests was used to determine statistical significance in the BPN dose-response experiment in the NIH test conducted using WT female mice. For all other dependent variables, Two-Way ANOVA were used to test for genotype × treatment interactions. Where appropriate, Tukey's multiple comparison tests were used for post hoc analysis. A p value of <0.05 was deemed to be statistically significant.

3 RESULTS

3.1 BPN-induced analgesia is blunted by the *Oprm1* A112G polymorphism

Baseline levels of analgesia did not differ between groups, AA: 11.4±1.2 s, AG 14.1±1.2 s and GG 11.2±1.1 s. However, a significant genotype × treatment interaction was observed on %MPE of BPN measured in the hot plate test ($F_{8, 195}=3.072$, $p=0.0028$), as shown in Figure 1. Multiple comparisons revealed that the analgesic response to BPN was dramatically blunted in GG mice, which differed significantly from AA mice at cumulative doses of 1 mg/kg ($p<0.01$) and 3 mg/kg ($p<0.001$). Similarly the analgesic effect of BPN was diminished in AG mice compared to AA mice at cumulative doses of 1 and 3 mg/kg ($p<0.01$).

3.2 Genotype differences observed during the early training phase of the NIH test

As mice are neophobic, the NIH test requires prior exposure to a period of training. Typically mice will approach and consume a palatable food in less than 30 s after several days of training. Mice were included in the study when they demonstrated three consecutive days of latencies < 30 s. During the initial stages of training, a significant genotype × training day interaction was observed in latency to consume the peanut butter chips ($F_{22, 957}=1.691$, $p=0.024$, data not shown). AA mice trained significantly faster than AG and GG mice. Tukey's multiple comparison test showed a significant difference in the latencies of AA and GG mice on day 3 ($p<0.01$), day 4 ($p<0.05$) and day 6 ($p<0.05$). During the following 6 days of training no genotype differences were observed. All but 2 mice reached

criterion by day 12 (one AG and one AA were excluded from the study) and similar approach latency baselines were evident for all groups prior to testing (AA 10.50 ± 1.86 s; AG 14.70 ± 1.62 s; GG 12.46 ± 1.31). All WT mice reached criterion by day 10 of training (14.94 ± 1.6 s)

3.3 BPN's effects in the NIH test are genotype dependent

Three doses of BPN were selected based on previous behavioral studies conducted in male C57BL/6J mice (Falcon et al., 2015), 0.25, 0.5 and 1 mg/kg. Mice were exposed to a novel environment and the latency to consume food was measured 24 hours following a single injection of BPN (see Figure 2). A significant main effect of treatment on latency to eat was observed in WT mice ($F_{3, 27}=3.085$, $p=0.044$, Figure 2A). Dunnett's multiple comparison tests revealed that BPN effectively reduced the latency to consume food at doses of 0.25 mg/kg ($p<0.05$) and 0.5 mg/kg ($p<0.05$). No effect was observed with the highest dose of 1 mg/kg. For subsequent experiments, 0.25 mg/kg of BPN was used. Next female mice derived from the *Oprm1* A112G mouse model were studied in the NIH test 24 h post BPN administration (Figure 2B). A significant genotype $\times$ treatment interaction was detected for the latency to consume food in the novel cage ($F_{2, 40}=3.394$, $p=0.0435$). Tukey's multiple comparisons revealed that BPN (0.25 mg/kg) produced a robust reduction of latencies in AA mice ($p<0.05$). BPN failed to reduce latencies in AG and GG mice. In a separate cohort of mice, the effects of the anxiolytic CDP were tested 30 minutes post injection (Figure 2C). CDP produced a significant reduction of approach latency across all three groups ($F_{1, 37}=74.92$, $p<0.001$) with no significant genotype $\times$ treatment interaction, indicating that the effects of the genotype in this test are specific to MOR binding ligands.

3.4 The motoric effects of BPN are diminished in mice with the GG genotype

Mice were placed in the arena 30 minutes following BPN administration and locomotor activity was evaluated for 1 hour. Significant genotype $\times$ treatment interactions were detected for crosses ($F_{2, 72}=4.165$, $p=0.019$, Figure 3A) and rearing ($F_{2, 72}=3.29$, $p=0.043$, Figure 3B) during the testing period. BPN significantly increased the number of crosses in AA ($p<0.001$) and AG ($p<0.001$) mice compared to vehicle treated controls. This effect was significantly blunted in GG carriers compared to AA mice ($p<0.05$). Interestingly, both AG and GG mice failed to exhibit a significant increase in rearing behavior post-BPN administration compared to their AA littermates ($p<0.01$).

3.5 BPN's behavioral effects in the FST are unaffected by *Oprm1* A112G polymorphism

The effects of BPN (0.25 mg/kg) in the FST were measured 24 hours post injection, (see Figure 4). There was a significant main effect of treatment on immobility scores in the FST ($F_{2, 56}=5.913$, $p=0.018$), with no significant genotype $\times$ treatment interaction. BPN decreased immobility scores in all three groups.

4 DISCUSSION

Buprenorphine, the mixed partial agonist of opioid receptors, is most widely associated with the treatment of opioid addiction. However, at lower doses it is also used to control moderate acute and chronic pain. Despite the large body of work examining the *OPRM1* A118G SNP

in pain and analgesia, no clinical data is available describing the interaction between this SNP and BPN treatment. Therefore, the goal of these studies was to evaluate the impact of the A112G SNP on the behavioral effects of BPN measured using murine models of pain sensitivity, locomotor hyperactivity, anxiety and antidepressant effects. The results demonstrate that MOR-dependent behavioral effects of BPN including pain sensitivity, locomotor hyperactivity and approach behavior in the NIH test, are modified by genetic variation in the A112G SNP with the A carrier being most responsive to drug effects. In contrast, the effects of BPN in the FST, mediated by the blockade of KORs, was not altered by genetic variation. These results suggest that the A118G SNP in humans is likely to contribute significant genetic variation in the pharmacological effects of BPN that can be attributed to MORs.

Although the analgesic action of BPN is mediated via MOR agonism and modulated by KOR, delta opioid receptors and nociceptin/orphanin FQ (N/OFQ) receptors (Lutfy et al., 2003), pharmacological and genetic blockade of opioid receptors confirmed that MORs alone are necessary for the analgesic properties of BPN (Grinnell et al., 2016; Ide et al., 2004). In the present studies, we demonstrate that the antinociceptive effects of BPN are dependent on the *Oprm1* A112G SNP. BPN-mediated antinociception was significantly reduced in AG mice and almost entirely absent in GG mice compared to their AA littermates. Although no genotype differences in baseline analgesia were observed in this study, it is probable that genotype differences in baseline analgesia would be more apparent with prior exposure to pain or indeed in a different behavioral test of pain. The data presented here agrees with prior observations in male and female GG mice, which exhibited blunted morphine-mediated antinociception compared to controls (Mague et al., 2009). Furthermore, studies conducted in humans report that G carriers consume greater quantities of morphine without comparable pain relief (Chou et al., 2006a; Chou et al., 2006b). This is of particular concern given the current opioid epidemic, with persistent use of higher doses of pain medications these patients are at increased risk to develop substance abuse disorders. Our data supports the hypothesis that the therapeutic benefits of BPN in the treatment of pain will be significantly limited in humans with the *OPRM1* A118G SNP. Most pharmacogenetic studies to date have examined the effects of *OPRM1* A118G in the context of post-operative pain relief; however, future studies should evaluate clinical outcomes of BPN in chronic pain treatment.

C57BL/6J mice typically display robust increases in locomotor activity following acute administration of morphine (Crawley et al., 1997) and BPN (Falcon et al., 2015), that are known to be mediated by MORs (Hall et al., 2004). In the present study, the magnitude of hyperactivity induced by BPN was significantly diminished in GG mice. GG mice exhibited significant attenuation of horizontal activity (the number of crosses) and no vertical activity (rearing behavior) following BPN administration compared to AA mice. AG mice showed an intermediate effect of BPN, exhibiting similar horizontal activity to those of their AA littermates but a marked reduction in vertical activity in response. These findings in *Oprm1* A112G females are consistent with previous studies where both male and female GG mice failed to exhibit behavioral sensitization to repeated administration of morphine (Mague et al., 2009). These data highlight the powerful impact of the genotype on BPN's behavioral effects.

As BPN is now being considered as a potential therapeutic for MDD, we evaluated the impact of the *Oprm1* A112G SNP on two distinct rodent behavioral tests relevant to anxiety and depression, the NIH test and FST respectively. Hyponeophagia tasks are sensitive to the acute effects of anxiolytics and antidepressants only after chronic administration (Dulawa and Hen, 2005). In agreement with previous findings in males treated with buprenorphine or a combination of buprenorphine/naltrexone (Almatroudi et al., 2015; Falcon et al., 2015), we now confirm that BPN is also efficacious in females. BPN (0.25 and 0.5 mg/kg) reduced the latency to consume palatable food in the NIH test 24 h post treatment in WT female C57BL/6J mice. It is proposed that blockade of MOR mediates this anxiolytic action, as MOR agonism is prevented by this dose of BPN 24 h post treatment (Robinson et al., 2016). In the *Oprm1* A112G mouse model, BPN produced a robust effect in the AA mice but failed to diminish the anxiogenic effects of the novel environment in AG and GG mice, suggesting that altered genetic signaling at MORs mediated these behavioral differences. In agreement with this characterization, approach behavior in the NIH test was blocked by the selective MOR antagonist cyprodime and in male *Oprm1*^{-/-} mice (Robinson et al., 2016). Conversely, we showed that the prototypical benzodiazepine CDP was highly efficacious across all genotypes, demonstrating the specific ability of *Oprm1* A112G SNP to attenuate the efficacy of MOR compounds. These data highlight the potential pharmacogenetic implications of the *OPRM1* A118G SNP for the efficacy of BPN in the treatment of mood disorders, particularly with in relation to comorbid anxiety.

Previously, we showed that BPN's antidepressant action in the FST is mediated via KORs (Falcon et al., 2016). In accordance with these findings, BPN's activity in the FST was not altered by A112G genotype, as immobility scores in all three genotypes were significantly decreased compared to their respective controls. There is considerable evidence that KOR antagonists produce antidepressant-like behavioral effects in the FST (Carr et al., 2010; Mague et al., 2003; McLaughlin et al., 2003), but all of these studies involved male rodents. There has been limited information regarding the effects of KOR ligands in females in behavioral tests for antidepressant and anxiolytic drugs. Previously it was reported that the irreversible KOR binding ligand 2-(3,4-dichlorophenyl)-N-methyl-N-((1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl)acetamide hydrochloride (DIPPA, 1 mg/kg) failed to reduce immobility in female Swiss mice (Kaster et al., 2007), but the dose used in this study was probably too low (Carr et al., 2010; Carr and Lucki, 2010). Overall our data suggest that the *Oprm1* A112G selectively diminished the efficacy of BPN in MOR but not KOR mediated behaviors.

The molecular basis of MOR activation and blockade is associated with specific signal transduction pathways which may interact with the *OPRM1* A118G (Knapman and Connor, 2015). *In vitro* assessment of BPN using CHO and AtT-20 cells expressing the *OPRM1* A118G variant (N40D) illustrate the significant impact of the A118G SNP on BPN signaling (Knapman et al., 2014). The N40D variant demonstrated a 50% reduction in BPN efficacy. Moreover, the ability of BPN to induced phosphorylation of ERK1/2 was reduced by 60%.

In contrast activation of inwardly rectifying potassium channels (GIRKs) was unaltered by genotype. Conversely, in HEK239 cells, no effect of genotype was observed on suppression of Ca²⁺ channel inhibition following BPN administration (Robinson et al., 2015). These

contradictory findings may be a result of cell specific differences in $G\alpha$ and $\beta\gamma$ subunits, that mask genotype dependent changes in BPN mediated second messenger coupling. Furthermore, Melief and colleagues proposed that the analgesic tolerance to acute morphine and buprenorphine is JNK-mediated, and that this is associated with a significant lack of receptor internalization. In contrast, under the same conditions fentanyl, methadone and oxycodone induce robust GRK/ β -arrestin-dependent MOR internalization (Melief et al., 2010). It is possible that differences such as those in β -arrestin-dependent internalization which is associated with rapid development of tolerance (Dang and Christie, 2012; Raehal and Bohn, 2011), may alter the sensitivity of compounds to the effects of *OPRM1* A118G polymorphism.

These studies show that the *Oprm1* A112G SNP altered the behavioral effects of BPN in behavioral tests relevant to pain sensitivity, locomotor activity and anxiety in female mice. Although male mice with the *Oprm1* A112G SNP were not evaluated with BPN here, male *Oprm1* A112G mice have demonstrated similar genotype differences to morphine-mediated antinociception and locomotor activity (Mague et al., 2009). However, consideration of females is important because sex-specific associations of the G allele have been reported for conditioned place preference and morphine withdrawal (Mague et al., 2009). Furthermore, females are at increased risk for the development of chronic pain than males (Greenspan et al., 2007). In addition, clinical studies revealed that compared to their male contemporaries, female carriers of the G allele exhibited blunted stress reactivity (Lovallo et al., 2015), elevated levels of persistent pain (Hasvik et al., 2014; Olsen et al., 2012; Sia et al., 2008) and reductions in the reinforcing value of nicotine (Ray et al., 2006). Overall the data presented here provide a compelling rationale to conduct clinical evaluation of the consequences of the *OPRM1* A118G polymorphism in relation to the therapeutic effects of BPN.

Acknowledgments

This research was supported by USPHS grant RO1MH092412 awarded to IL and R01DA033646 awarded to JAB

References

- Almatroudi A, Husbands SM, Bailey CP, Bailey SJ. Combined administration of buprenorphine and naltrexone produces antidepressant-like effects in mice. *J Psychopharmacol*. 2015; 29:812–821. [PubMed: 26045511]
- Baber M, Bapat P, Nichol G, Koren G. The pharmacogenetics of opioid therapy in the management of postpartum pain: a systematic review. *Pharmacogenomics*. 2016; 17:75–93. [PubMed: 26652709]
- Bach P, Vollsta Dt-Klein S, Kirsch M, Hoffmann S, Jorde A, Frank J, Charlet K, Beck A, Heinz A, Walter H, Sommer WH, Spanagel R, Rietschel M, Kiefer F. Increased mesolimbic cue-reactivity in carriers of the mu-opioid-receptor gene *OPRM1* A118G polymorphism predicts drinking outcome: a functional imaging study in alcohol dependent subjects. *Eur Neuropsychopharmacol*. 2015; 25:1128–1135. [PubMed: 25937240]
- Balu DT, Hodes GE, Anderson BT, Lucki I. Enhanced sensitivity of the MRL/MpJ mouse to the neuroplastic and behavioral effects of chronic antidepressant treatments. *Neuropsychopharmacology*. 2009; 34:1764–1773. [PubMed: 19177066]
- Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, Goldman D. Mu opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry*. 1997; 2:490–494. [PubMed: 9399694]

- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci U S A*. 1998; 95:9608–9613. [PubMed: 9689128]
- Bonenberger M, Plener PL, Groschwitz RC, Gron G, Abler B. Polymorphism in the micro-opioid receptor gene (OPRM1) modulates neural processing of physical pain, social rejection and error processing. *Exp Brain Res*. 2015; 233:2517–2526. [PubMed: 26019010]
- Boswell MV, Stauble ME, Loyd GE, Langman L, Ramey-Hartung B, Baumgartner RN, Tucker WW, Jortani SA. The role of hydromorphone and OPRM1 in postoperative pain relief with hydrocodone. *Pain Physician*. 2013; 16:E227–235. [PubMed: 23703421]
- Carr GV, Bangasser DA, Bethea T, Young M, Valentino RJ, Lucki I. Antidepressant-like effects of kappa-opioid receptor antagonists in Wistar Kyoto rats. *Neuropsychopharmacology*. 2010; 35:752–763. [PubMed: 19924112]
- Carr GV, Lucki I. Comparison of the kappa-opioid receptor antagonist DIPPA in tests of anxiety-like behavior between Wistar Kyoto and Sprague Dawley rats. *Psychopharmacology (Berl)*. 2010; 210:295–302. [PubMed: 20369354]
- Chou WY, Wang CH, Liu PH, Liu CC, Tseng CC, Jawan B. Human opioid receptor A118G polymorphism affects intravenous patient-controlled analgesia morphine consumption after total abdominal hysterectomy. *Anesthesiology*. 2006a; 105:334–337. [PubMed: 16871067]
- Chou WY, Yang LC, Lu HF, Ko JY, Wang CH, Lin SH, Lee TH, Concejero A, Hsu CJ. Association of mu-opioid receptor gene polymorphism (A118G) with variations in morphine consumption for analgesia after total knee arthroplasty. *Acta Anaesthesiol Scand*. 2006b; 50:787–792. [PubMed: 16879459]
- Courtney KE, Ghahremani DG, Ray LA. Fronto-striatal functional connectivity during response inhibition in alcohol dependence. *Addict Biol*. 2013; 18:593–604. [PubMed: 23240858]
- Cowan A, Lewis JW, Macfarlane IR. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br J Pharmacol*. 1977; 60:537–545. [PubMed: 409448]
- Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, Hitzemann RJ, Maxson SC, Miner LL, Silva AJ, Wehner JM, Wynshaw-Boris A, Paylor R. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)*. 1997; 132:107–124. [PubMed: 9266608]
- Crist RC, Berrettini WH. Pharmacogenetics of OPRM1. *Pharmacol Biochem Behav*. 2014; 123:25–33. [PubMed: 24201053]
- Dang VC, Christie MJ. Mechanisms of rapid opioid receptor desensitization, resensitization and tolerance in brain neurons. *Br J Pharmacol*. 2012; 165:1704–1716. [PubMed: 21564086]
- Dulawa SC, Hen R. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev*. 2005; 29:771–783. [PubMed: 15890403]
- Falcon E, Browne CA, Leon RM, Fleites VC, Sweeney R, Kirby LG, Lucki I. Antidepressant-like Effects of Buprenorphine are Mediated by Kappa Opioid Receptors. *Neuropsychopharmacology*. 2016
- Falcon E, Maier K, Robinson SA, Hill-Smith TE, Lucki I. Effects of buprenorphine on behavioral tests for antidepressant and anxiolytic drugs in mice. *Psychopharmacology (Berl)*. 2015; 232:907–915. [PubMed: 25178815]
- Gelernter J, Kranzler H, Cubells J. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry*. 1999; 4:476–483. [PubMed: 10523821]
- Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, Gold MS, Holdcroft A, Lautenbacher S, Mayer EA, Mogil JS, Murphy AZ, Traub RJ, Consensus Working Group of the Sex, G., Pain, S. I. G. o. t. I. Studying sex and gender differences in pain and analgesia: a consensus report. *Pain*. 2007; 132(Suppl 1):S26–45. [PubMed: 17964077]
- Grinnell SG, Ansonoff M, Marrone GF, Lu Z, Narayan A, Xu J, Rossi G, Majumdar S, Pan YX, Bassoni DL, Pintar J, Pasternak GW. Mediation of buprenorphine analgesia by a combination of traditional and truncated mu opioid receptor splice variants. *Synapse*. 2016; 70:395–407. [PubMed: 27223691]

- Hall FS, Goeb M, Li XF, Sora I, Uhl GR. mu-Opioid receptor knockout mice display reduced cocaine conditioned place preference but enhanced sensitization of cocaine-induced locomotion. *Brain Res Mol Brain Res*. 2004; 121:123–130. [PubMed: 14969743]
- Hasvik E, Iordanova Schistad E, Grovle L, Julsrud Haugen A, Roe C, Gjerstad J. Subjective health complaints in patients with lumbar radicular pain and disc herniation are associated with a sex - OPRM1 A118G polymorphism interaction: a prospective 1-year observational study. *BMC Musculoskelet Disord*. 2014; 15:161. [PubMed: 24884878]
- Huang P, Chen C, Mague SD, Blendy JA, Liu-Chen LY. A common single nucleotide polymorphism A118G of the mu opioid receptor alters its N-glycosylation and protein stability. *Biochem J*. 2012; 441:379–386. [PubMed: 21864297]
- Ide S, Minami M, Satoh M, Uhl GR, Sora I, Ikeda K. Buprenorphine antinociception is abolished, but naloxone-sensitive reward is retained, in mu-opioid receptor knockout mice. *Neuropsychopharmacology*. 2004; 29:1656–1663. [PubMed: 15100703]
- Kakko J, von Wachenfeldt J, Svanborg KD, Lidstrom J, Barr CS, Heilig M. Mood and neuroendocrine response to a chemical stressor, metyrapone, in buprenorphine-maintained heroin dependence. *Biol Psychiatry*. 2008; 63:172–177. [PubMed: 17850768]
- Karp JF, Butters MA, Begley AE, Miller MD, Lenze EJ, Blumberger DM, Mulsant BH, Reynolds CF 3rd. Safety, tolerability, and clinical effect of low-dose buprenorphine for treatment-resistant depression in midlife and older adults. *J Clin Psychiatry*. 2014; 75:e785–793. [PubMed: 25191915]
- Kaster MP, Budni J, Santos AR, Rodrigues AL. Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of adenosine in the mouse forced swimming test. *Eur J Pharmacol*. 2007; 576:91–98. [PubMed: 17868670]
- Knapman A, Connor M. Cellular signalling of non-synonymous single-nucleotide polymorphisms of the human mu-opioid receptor (OPRM1). *Br J Pharmacol*. 2015; 172:349–363. [PubMed: 24527749]
- Knapman A, Santiago M, Connor M. Buprenorphine signalling is compromised at the N40D polymorphism of the human mu opioid receptor in vitro. *Br J Pharmacol*. 2014; 171:4273–4288. [PubMed: 24846673]
- Lopez Soto EJ, Catanesi CI. Human population genetic structure detected by pain-related mu opioid receptor gene polymorphisms. *Genet Mol Biol*. 2015; 38:152–155. [PubMed: 26273217]
- Lovallo WR, Enoch MA, Acheson A, Cohoon AJ, Sorocco KH, Hodgkinson CA, Vincent AS, Glahn DC, Goldman D. Cortisol Stress Response in Men and Women Modulated Differentially by the Mu-Opioid Receptor Gene Polymorphism OPRM1 A118G. *Neuropsychopharmacology*. 2015; 40:2546–2554. [PubMed: 25881118]
- Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)*. 2001; 155:315–322. [PubMed: 11432695]
- Lutfy K, Eitan S, Bryant CD, Yang YC, Saliminejad N, Walwyn W, Kieffer BL, Takeshima H, Carroll FI, Maidment NT, Evans CJ. Buprenorphine-induced antinociception is mediated by mu-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors. *J Neurosci*. 2003; 23:10331–10337. [PubMed: 14614092]
- Mague SD, Isiegas C, Huang P, Liu-Chen LY, Lerman C, Blendy JA. Mouse model of OPRM1 (A118G) polymorphism has sex-specific effects on drug-mediated behavior. *Proc Natl Acad Sci U S A*. 2009; 106:10847–10852. [PubMed: 19528658]
- Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC Jr, Jones RM, Portoghese PS, Carlezon WA Jr. Antidepressant-like effects of kappa-opioid receptor antagonists in the forced swim test in rats. *J Pharmacol Exp Ther*. 2003; 305:323–330. [PubMed: 12649385]
- Mahmoud S, Thorsell A, Sommer WH, Heilig M, Holgate JK, Bartlett SE, Ruiz-Velasco V. Pharmacological consequence of the A118G mu opioid receptor polymorphism on morphine- and fentanyl-mediated modulation of Ca(2+)-channels in humanized mouse sensory neurons. *Anesthesiology*. 2011; 115:1054–1062. [PubMed: 21926562]

- Marcus SM, Young EA, Kerber KB, Kornstein S, Farabaugh AH, Mitchell J, Wisniewski SR, Balasubramani GK, Trivedi MH, Rush AJ. Gender differences in depression: findings from the STAR*D study. *J Affect Disord.* 2005; 87:141–150. [PubMed: 15982748]
- McLaughlin JP, Marton-Popovici M, Chavkin C. Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J Neurosci.* 2003; 23:5674–5683. [PubMed: 12843270]
- Melief EJ, Miyatake M, Bruchas MR, Chavkin C. Ligand-directed c-Jun N-terminal kinase activation disrupts opioid receptor signaling. *Proc Natl Acad Sci U S A.* 2010; 107:11608–11613. [PubMed: 20534436]
- Mura E, Govoni S, Racchi M, Carossa V, Ranzani GN, Allegri M, van Schaik RH. Consequences of the 118A>G polymorphism in the OPRM1 gene: translation from bench to bedside? *J Pain Res.* 2013; 6:331–353. [PubMed: 23658496]
- Oertel BG, Doebering A, Roskam B, Kettner M, Hackmann N, Ferreiros N, Schmidt PH, Lotsch J. Genetic-epigenetic interaction modulates mu-opioid receptor regulation. *Hum Mol Genet.* 2012; 21:4751–4760. [PubMed: 22875838]
- Olsen MB, Jacobsen LM, Schistad EI, Pedersen LM, Rygh LJ, Roe C, Gjerstad J. Pain intensity the first year after lumbar disc herniation is associated with the A118G polymorphism in the opioid receptor mu 1 gene: evidence of a sex and genotype interaction. *J Neurosci.* 2012; 32:9831–9834. [PubMed: 22815498]
- Raeal KM, Bohn LM. The role of beta-arrestin2 in the severity of antinociceptive tolerance and physical dependence induced by different opioid pain therapeutics. *Neuropharmacology.* 2011; 60:58–65. [PubMed: 20713067]
- Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M. A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry.* 2011; 16:809–817. [PubMed: 20479755]
- Ray R, Jepson C, Patterson F, Strasser A, Rukstalis M, Perkins K, Lynch KG, O'Malley S, Berrettini WH, Lerman C. Association of OPRM1 A118G variant with the relative reinforcing value of nicotine. *Psychopharmacology (Berl).* 2006; 188:355–363. [PubMed: 16960700]
- Reed B, Butelman ER, Yuferov V, Randesi M, Kreek MJ. Genetics of opiate addiction. *Curr Psychiatry Rep.* 2014; 16:504. [PubMed: 25209027]
- Robinson JE, Vardy E, DiBerto JF, Chefer VI, White KL, Fish EW, Chen M, Gigante E, Krouse MC, Sun H, Thorsell A, Roth BL, Heilig M, Malanga CJ. Receptor Reserve Moderates Mesolimbic Responses to Opioids in a Humanized Mouse Model of the OPRM1 A118G Polymorphism. *Neuropsychopharmacology.* 2015; 40:2614–2622. [PubMed: 25881115]
- Robinson SA, Erickson RL, Browne CA, Lucki I. A role for the mu opioid receptor in the antidepressant effects of buprenorphine. *Behav Brain Res.* 2016; 319:96–103. [PubMed: 27818236]
- Sia AT, Lim Y, Lim EC, Goh RW, Law HY, Landau R, Teo YY, Tan EC. A118G single nucleotide polymorphism of human mu-opioid receptor gene influences pain perception and patient-controlled intravenous morphine consumption after intrathecal morphine for postcesarean analgesia. *Anesthesiology.* 2008; 109:520–526. [PubMed: 18719451]
- Song Z, Du B, Wang K, Shi X. Effects of OPRM1 A118G polymorphism on epidural analgesia with fentanyl during labor: a meta-analysis. *Genet Test Mol Biomarkers.* 2013; 17:743–749. [PubMed: 23909491]
- Wang YJ, Huang P, Blendy JA, Liu-Chen LY. Brain region- and sex-specific alterations in DAMGO-stimulated [(35) S]GTPgammaS binding in mice with Oprm1 A112G. *Addict Biol.* 2014; 19:354–361. [PubMed: 22862850]
- Weller EB, Kloos A, Kang J, Weller RA. Depression in children and adolescents: does gender make a difference? *Curr Psychiatry Rep.* 2006; 8:108–114. [PubMed: 16539885]
- Yovell Y, Bar G, Mashiah M, Baruch Y, Briskman I, Asherov J, Lotan A, Rigbi A, Panksepp J. Ultra-Low-Dose Buprenorphine as a Time-Limited Treatment for Severe Suicidal Ideation: A Randomized Controlled Trial. *Am J Psychiatry.* 2016; 173:491–498. [PubMed: 26684923]

Zhang Y, Picetti R, Butelman ER, Ho A, Blendy JA, Kreek MJ. Mouse model of the OPRM1 (A118G) polymorphism: differential heroin self-administration behavior compared with wild-type mice. *Neuropsychopharmacology*. 2015; 40:1091–1100. [PubMed: 25336208]

Highlights

- The *Oprm1* A112G SNP (G carriers) blunted buprenorphine-mediated antinociception.
- The anxiolytic effects of buprenorphine but not chlorodiazepoxide were blocked in AG and GG mice.
- The motoric effects of BPN were diminished in mice with the GG genotype
- BPN's behavioral effects in the FST were unaffected by *Oprm1* A112G polymorphism.

Figure 1. BPN-induced analgesia is sharp in AG and GG mice

The maximal percentage effect of BPN was significantly impaired by genotype, AA n=12, AG n=20, and GG n=10. GG mice exhibited diminished BPN-induced antinociception compared to AA mice, this difference reached significance at the cumulative doses of 1 and 3 mg/kg BPN, (* = $p < 0.05$). Similarly, BPN-induced antinociception at a cumulative dose of 3 mg/kg was blunted in AG mice compared to their AA littermates (## = $p < 0.01$).

Figure 2. Novelty induced hypophagia

Three doses of BPN were tested in WT female C57BL/6J mice ($n=7-8$ per group) to establish the optimal dose of BPN for investigation in tests of depression and anxiety (Figure 2A). 24 hours following a single injection of BPN, mice were exposed to a novel environment and the latency to consume food was measured. The latency to approach and eat food in WT mice was reduced with 0.25 mg/kg and 0.5 mg/kg of BPN compared to vehicle treated mice, the symbol * indicates $p<0.05$. Figure 2B depicts the effects of the Oprm1 A112G polymorphism on the effects of BPN in the NIH task AA $n=8-10$, AG $n=8$,

and GG n=6. 24 hours following a single injection, BPN (0.25 mg/kg) significantly reduced the latency to approach and consume food in AA but not AG/GG carriers (* represents $p < 0.05$). Conversely, in a separate cohort of mice, CDP (10 mg/kg) tested 30 min post injection readily reduced latency to consume food in all genotypes, AA n=6, AG n=8–9, and GG n=7, Figure 2C. The symbol *** indicates a $p < 0.001$ compared to vehicle treated controls within each genotype.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3. BPN-induced locomotor activity is significantly attenuated in GG mice

Mice were placed in the testing arena 30 minutes post BPN administration and observed for a period of 1 hour, AA n=15–17, AG n=10, and GG n=13. Marked decreases in BPN-induced increases in the number of crosses (Figure 3A) and rearing (Figure 3B) exhibited by GG mice compared to their AA littermates ($^{##} = p < 0.01$ and $^{\#} = p < 0.05$).

Figure 4. BPN's effects on immobility scores in the FST were not altered by genotype 24 hours following a single injection of BPN (0.25 mg/kg), all mice exhibited significant reductions in immobility scores relative to their genotype controls (* indicates $p < 0.05$), AA $n=8$, AG $n=12$, and GG $n=11$. This suggests that a behavioral effect of BPN mediated by KORs was not altered indirectly by the Oprm1 A112G SNP.