Published in final edited form as:

Physiol Behav. 2015 February ; 139: 216–223. doi:10.1016/j.physbeh.2014.11.040.

Cocaine-induced suppression of saccharin intake and morphine modulation of Ca²⁺ channel currents in sensory neurons of OPRM1 A118G mice

Christopher S. Freet^a, Sarah M. Ballard^a, Danielle N. Alexander^a, Taylor A. Cox^a, Caesar G. Imperio^a, Nnaemeka Anosike^a, Alyssa B. Carter^a, Saifeldin Mahmoud^b, Victor Ruiz-Velasco^b, and Patricia S. Grigson^a

^aDepartment of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA

²Department of Anesthesiology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA

Abstract

Several studies have shown that human carriers of the single nucleotide polymorphism of the μ -opioid receptor, OPRM1 A118G, exhibit greater drug and alcohol use, increased sensitivity to pain, and reduced sensitivity to the antinociceptive effects of opiates. In the present study, we employed a ‘humanized’ mouse model containing the wild-type (118AA) or variant (118GG) allele to examine behavior in our model of drug-induced suppression of a natural reward cue and to compare the morphine pharmacological profile in acutely isolated sensory neurons. Compared with 118AA mice, our results demonstrate that homozygous 118GG mice exhibit greater avoidance of the cocaine-paired saccharin cue, a behavior linked to an aversive withdrawal-like state. Electrophysiological recordings confirmed the reduced modulation of Ca²⁺ channels by morphine in trigeminal ganglion (TG) neurons from 118GG mice compared to the 118AA control cells. However, repeated cocaine exposure in 118GG mice led to a leftward shift of the morphine concentration-response relationship when compared with 118GG control mice, while a rightward shift was observed in 118AA mice. These results suggest that cocaine exposure of mice carrying the 118G allele leads to a heightened sensitivity of the reward system and a blunted modulation of Ca²⁺ channels by morphine in sensory neurons.

Keywords

addiction; natural rewards; opiates; pain; reward comparison

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Corresponding Author: Christopher S. Freet, Department of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, 500 University Drive, H181, Hershey, PA 17033 USA, Phone: (717) 531-3758, FAX: (717) 531-6916, csf5@psu.edu.

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1. INTRODUCTION

The National Institute on Drug Abuse estimates that “the total overall costs of substance abuse in the United States, including health- and crime-related costs, as well as losses in productivity, exceed half a trillion dollars annually.” Much of this cost is incurred because addiction is a brain disease characterized by chronic relapse. Thus, while initial use of a drug is voluntary, perhaps even prescribed by a physician for the relief of pain, drugs of abuse alter gene expression and brain circuitry resulting in changes in behavior – i.e., compulsive drug craving, seeking and use [1–4].

Of critical importance, then, is to determine who is most vulnerable to addiction. It is now well understood that the development of addiction is affected by both environmental and hereditary factors. A single nucleotide polymorphism (SNP) of the human mu (μ) opioid receptor gene (OPRM1), A118G, has been linked to addiction to alcohol and heroin [5–8] and an association with an increased risk factor for addiction. Moreover, the presence of the 118G allele may be doubly dangerous, as chronic exposure to opioids can lead to hyperalgesia [9] and carriers of the mutant allele require more opioids in some cases to achieve antinociception [for a review see 10]. As such, this allele may facilitate the progression from the use of a drug to alleviate pain to one of abuse and addiction.

A consequence of the OPRM1 SNP is the substitution of adenine (A) for guanine (G) that results in the replacement of an asparagine for an aspartate residue in the receptor’s N-terminus and decreases the number of glycosylation sites from five to four [11]. The functional consequences of this SNP on the μ -opioid receptor are still under investigation [5, 12, 13]. Behaviorally, as alluded to above, the polymorphism has been linked to addiction in alcoholics and is associated with greater alcohol and drug intake in humans and in non-human primates [5–8]. In addition, ‘humanized’ OPRM1 A118G mice (i.e., mice in which the native exon 1 of the opioid receptor gene was replaced with the human exon 1) have been reported to exhibit increased responsiveness to alcohol [14], blunted morphine pharmacological profile in sensory neurons and significantly lower morphine-mediated antinociception [13].

Although the majority of studies evaluating the A118G SNP in addiction have focused on alcohol and opioids, few have examined the role of this polymorphism in the context of cocaine. This is somewhat surprising given that the endogenous opioid system has been shown to be involved in the rewarding effects of cocaine. For example, cocaine increases β -endorphin levels in the NAc [15, 16], and μ -opioid (not δ - or κ -opioid receptors) receptor-mediated G protein activity is increased with acute and chronic cocaine exposure [17]. In addition, behavioral data support a role for the endogenous opioid system and β -endorphin in cocaine self-administration [16], conditioned place preference and locomotor activity [18], and extinction, [16, 19]. Therefore, the aim of the current set of experiments was to begin to evaluate the effect of chronic cocaine exposure on the behavior and physiology of humanized 118AA and 118GG mice. Experiment 1 examined the effect of this polymorphism on drug-induced avoidance of a saccharin cue following pairing with experimenter delivered cocaine. In this paradigm, greater avoidance of a drug-paired saccharin cue is correlated with greater drug self-administration when rats are employed

[20–22]. We reported previously that morphine exhibits a significantly lower potency and reduced efficacy in sensory neurons from 118GG mice compared with 118AA mice [13], suggesting that the analgesic properties of opiates may be less effective in 118GG mice. Therefore, Experiment 2 measured the modulation of Ca^{2+} currents in acutely isolated trigeminal ganglion (TG) neurons to determine whether and how a history of cocaine exposure in Experiment 1 affects the morphine pharmacological profile of sensory neurons from 118AA and 118GG mice.

2. MATERIALS AND METHODS

2.1 Subjects

The subjects for saccharin-cocaine pairings were 67 adult (60–120 days) naïve male 118AA mice ($n=36$, mean weight: 27.0 g) and 118GG mice ($n=31$, mean weight: 25.9 g). Sixteen mice (118AA: $n=10$, 118GG: $n=6$) and the original breeding pairs were obtained from Dr. Markus Heilig at the National Institutes on Alcoholism and Alcohol Abuse (NIAAA), Bethesda, MD. The remaining mice were bred in the barrier facility at the Pennsylvania State University College of Medicine, Hershey, PA using a low-intensity monogamous stud mating system. A heterozygous mating scheme was used ($\text{AG} \times \text{AG}$); back- and intercross lines were maintained and bred into the population. Pups were weaned at 21 days. Tail cuts were performed, and the DNA isolated (DNeasy Blood & Tissue Kit, Qiagen), amplified (GoTaq DNA Polymerase Kit, Promega), run in gels, extracted (DNA Gel Purification Kit, Qiagen), and sequenced to determine genotype. All subjects were housed individually in standard, clear plastic pan cages in a temperature-controlled (21°C) animal care facility with a 12:12 hour light:dark cycle and mice were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water, except where otherwise noted.

2.2 Experiment 1: Saccharin-cocaine pairings

2.2.1 Apparatus—All behavioral manipulations were conducted in the home cages. Modified serological pipettes (Fisher Scientific; 07-200-574) were used to provide purified water and saccharin access. Pipettes were converted to plastic cylinders by removing the tapered ends. A stainless steel spout (Girton Manufacturing Co., Inc.; ST300C) was then placed in the bottom of the cylinder, while a silicon stopper (Fisher Scientific; 09-704-1D) sealed the top of the cylinder. Intake of water and saccharin was recorded in 1/10 milliliters.

2.2.2 Taste-drug pairing procedure—All subjects were weighed once a day throughout the study. For baseline measurements, all mice were placed on a water restriction schedule which consisted of access to purified water for 1 h in the morning (testing period) and unpurified tap water for 2 h in the afternoon (rehydration period). Baseline intake and body weight were recorded for 1 week and experimental groups were determined by matching terminal baseline morning water intake. All mice remained on the water restriction schedule as described above for the duration of the experiment. For each taste-drug pairing (which occurred every 48 h), all mice received 0.15% saccharin for the morning access period followed immediately by an i.p. injection of either saline (118AA: $n=17$, 118GG: $n=15$) or cocaine (118AA: $n=19$, 118GG: $n=16$). The dose of cocaine increased over trials (trials 1–4: 20 mg/kg, trials 5–7: 40 mg/kg, trials 8–11: 60 mg/kg). Escalating, repeated cocaine is a

common within-subject preparation used in our laboratory; the 20 mg/kg dose is below that required to produce suppression (the minimum required dose in mice similar to the A118G background strain is 30 mg/kg [23]). Although the 60 mg/kg dose can produce seizure-like activity and elevated locomotor activity when administered acutely, these effects are not observed with the repeated escalating doses used in the current preparation, most likely due to the development of tolerance [24, 25]. Thus, taste-drug pairings occurred every other morning (2.5 h into the light phase) for a total of 10 trials and all mice received 1 h access to purified water in the mornings between conditioning trials. An eleventh conditioning trial was conducted with saccharin access only (no injection). All mice received 2 h access to tap water each afternoon to maintain hydration for all conditioning and non-conditioning trials. The saccharin solution was presented at room temperature and cocaine HCl was provided by the National Institute on Drug Abuse.

2.4 Experiment 2: Morphine-mediated modulation of Ca^{2+} channel currents

TG neurons were isolated from mice exposed to saline or cocaine (Experiment 1, above) and were dissociated as previously described (Mahmoud et al., 2011). Briefly, the mice were anesthetized with CO_2 and then quickly decapitated with a laboratory guillotine. Following removal of TG from mice, the ganglia were enzymatically dissociated in Earle's balanced salt solution containing 0.6 mg/ml collagenase (Roche Applied Science, Indianapolis, IN), 0.4 mg/ml trypsin (Worthington Biochemical, Lakewood, NJ) and 0.1 mg/ml DNase (Sigma-Aldrich) for 40 min at 35°C in a shaking water bath. Thereafter, the neurons were dispersed by vigorous shaking (~ 10 sec) and centrifuged twice at $130 \times g$ for 6 min. The neurons were then resuspended in minimal essential medium that was supplemented with 10% fetal calf serum (MidSci, St. Louis, MO), 1% penicillin-streptomycin and 1% glutamine (both from Life Technologies, Carlsbad, CA) and plated onto 35 mm poly-L-lysine coated dishes. The dispersed TG neurons were stored in a humidified atmosphere containing 5% CO_2 /95% air at 37°C .

2.4.1 Electrophysiology and Data Acquisition— Ca^{2+} channel currents were recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) equipped with an 18-bit analog-to-digital converter board (HEKA Instruments, Bellmore, NY), analog filtered at a frequency of 2 kHz (−3dB, 4-pole low-pass Bessel filter) and digitized with custom-designed S5 software written by Stephen R. Ikeda, M.D., Ph.D. (Chief, Laboratory of Molecular Physiology, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA). The patch pipettes were fabricated from borosilicate glass (Garner Glass Co., Claremont, CA) and pulled on a P-97 micropipette puller (Sutter Instrument Co., Novato, CA).

The morphine concentration-response relationship was determined by the sequential application of the agonist at different concentrations. The order of concentration application was random and the initial concentration of drug applied was in some cases higher than the second drug concentration. Due to the short exposure (i.e. less than 40 sec) of agonist to the neurons, this approach did not result in receptor desensitization and baseline currents showed full recovery after drug removal. Also, in order to avoid desensitization, no more than three different concentrations were employed with each neuron. Ca^{2+} current inhibition

(%) was determined as $[1 - (\text{peak current in the presence of agonist} / \text{peak current before agonist})] \times 100\%$. The concentration-response curves were generated by pooling the results for each concentration and fitting the points to the Hill equation. The data points are presented as mean ($\pm$ SE).

2.4.2 Recording Solutions and Drugs—The external Ca^{2+} current external solution contained (in mM): tetraethylammonium hydroxide (TEA-OH) 145, methanesulfonic acid 140, HEPES 10, glucose 15, CaCl_2 2.5, TEACl 15 and TTX 0.0003. The pH of this solution was adjusted to 7.40 with TEA-OH. The solution in the recording pipette consisted of (in mM): N-methyl-D-glucamine 110, TEA-OH 25, HCl 20, EGTA 11, HEPES 10, CsCl_2 20, CaCl_2 1, MgATP 4, Na_2GTP 0.3 and Na-Creatine phosphate 7. The pH of this solution was adjusted to 7.20 with methanesulfonic acid. A stock solution of morphine (from Sigma-Aldrich) was prepared in water and diluted in the external recording solution to the final concentration.

2.4.3 Statistical Analysis—The electrophysiological recordings and statistical analyses were carried out with Igor Pro (WaveMetrics, Inc., Lake Oswego, OR) and Prism (GraphPad Software, Inc., La Jolla, CA) software packages, respectively. The morphine concentration-response fits were compared employing an F test with a p value < 0.05 considered statistically significant.

3. RESULTS

3.1 Experiment 1: Saccharin-cocaine pairings

3.1.1 Saccharin intake (ml/1 h)—Figure 1, panels A and B, shows saccharin intake by 118AA (left panels) and 118GG (right panels) mice treated with saline or increasing concentrations of cocaine. Observation of the intake data shown in the top panels reveals that, while 118AA mice demonstrate suppression of saccharin intake when paired with cocaine, suppression of intake of the taste cue began earlier and was greater in the 118GG mice. In support, saccharin intake was analyzed using a mixed factorial $2 \times 2 \times 11$ analysis of variance (ANOVA) varying genotype (118AA or 118GG), drug (saline or cocaine), and taste-drug trials (1–11). The main effects of genotype, $F(1,63)=7.36$, $p=.009$, drug, $F(1,63)=18.77$, $p<.001$, and trials, $F(10,630)=25.24$, $p<.0001$, were significant. However, the three-way interaction did not reach significance, $F(10,630)=1.31$, $p=.022$. That said, given the robust main effect of genotype, separate 2×11 mixed factorial ANOVAs were conducted varying drug and trials for each genotype. Post hoc Newman-Keuls tests on the significant drug $\times$ trials interaction for each genotype, 118AA: $F(10,340)=12.24$, $p<.001$; 118GG: $F(10,290)=15.63$, $p<.001$, revealed that while cocaine was effective in reducing saccharin intake in 118AA mice on trials 6–11, $p<.05$, 118GG mice suppressed intake of the saccharin cue relative to their saline treated controls following just two saccharin-cocaine pairings, $p<.001$.

3.1.2 Morning water intake (ml/1 h)—Morning intake of water (ml/hr) on the days between conditioning trials (Trials W1–W10) is presented in Figure 1, panels C and D. A $2 \times 2 \times 10$ mixed factorial ANOVA revealed that drug exposure did not significantly alter

morning water intake as a function of genotype, as indicated by a non significant main effect of genotype, $F(1,63) = 1.95$, $p < .17$, genotype $\times$ drug interaction, $F < 1$, and genotype $\times$ drug $\times$ trials interaction, $F < 1$. The main effect of drug also was not significant, $F < 1$, but the main effect of trials was, $F(9,567) = 4.87$, $p < .001$.

3.1.3 Afternoon water intake (ml/2 h)—Intake of water for the 2 h access period in the afternoon for all trials is presented in Figure 2, panels A and B. Although a $2 \times 2 \times 20$ repeated measures ANOVA did not reveal a significant 3-way interaction, $F(19,1197)=1.04$, $p=.41$, or main effect of genotype, $F(1,63)=1.58$, $p=.21$, the main effects of drug, $F(1,63)=4.14$, $p=.05$, and trials, $F(19,1197)=16.45$, $p < .001$, did attain statistical significance.

3.1.4 Body weight—Body weight was analyzed using a $2 \times 2 \times 21$ mixed factorial ANOVA varying genotype (118AA or 118GG), drug (saline or cocaine), and trials (1–11 and W1–W10). Drug exposure did not significantly alter body weight (Figure 2, panels C and D). This conclusion was supported by a non-significant main effect of drug, $F(1,63)=2.45$, $p=.12$, and a non-significant 3-way interaction, $F < 1$. The main effect of trials was significant, $F(20,1260)=20.68$, $p < .0001$, indicating that body weight increased over successive trials for all mice. The main effect of genotype also did not attain statistical significance, $F(1,63)=3.34$, $p=.07$, confirming that 118AA mice were not significantly heavier than 118GG mice overall, (mean 118AA: 27.0 g; mean 118GG: 25.9 g).

3.2 Experiment 2: Morphine-mediated modulation of Ca^{2+} channel currents

Given that 118GG mice treated with cocaine exhibited greater suppression of intake of the cocaine-paired saccharin cue than 118AA mice, the TG neurons from all groups of mice were isolated and μ -opioid receptor-mediated modulation of Ca^{2+} currents was next examined. Previously we reported that in 118GG TG neurons, morphine exhibited a significantly lower potency and reduced efficacy when compared to 118AA neurons [13]. Figure 3A shows the time course of the peak Ca^{2+} current amplitude acquired before and during application of 0.03 and 3 μM morphine on a TG neuron from a control (saline-treated) 118AA mouse. The Ca^{2+} currents were evoked every 10 sec with a 50 msec test pulse to 0 mV from a holding potential of -80 mV (shown in Fig. 3B, top) and the peak Ca^{2+} current amplitude was measured isochronally 10 msec following the initiation of the test pulse. The superimposed Ca^{2+} currents shown in Figure 3B correspond to those plotted in 3A. The initial application of 3 μM morphine caused a 67% block of the currents (trace 2). Following a recovery period, 0.03 μM exposure resulted in a 32% inhibition of Ca^{2+} channels (trace 4). The time course in Figure 3C was recorded from a TG neuron isolated from a 118AA mouse treated with cocaine and the corresponding numbered current traces are shown in Figure 3D. Exposure of the neuron to 0.03 μM (trace 4) and 3 μM (trace 2) morphine inhibited the Ca^{2+} currents 26 and 50%, respectively. The morphine concentration-response curves for control (●) and cocaine-treated (▲) 118AA mice are shown in Figure 5A. The data points in both groups were pooled and fit to the Hill equation. The mean ($\pm$ SE) maximum current inhibition (%) and Hill coefficient values for control and cocaine-treated 118AA mice were $59.8 \pm 1.2\%$, $57.3 \pm 12.6\%$ and 1.26 ± 0.17 , 0.74 ± 0.65 , respectively. The fits for both groups of 118AA mice plots show a slight rightward shift of

the morphine concentration-response curve for the cocaine-treated mice. However, comparison of the fits for both sets of data did not reach significance ($p = 0.17$).

Figures 4A and 4C are the time courses for control and cocaine-treated 118GG mice, respectively, exposed to 0.03 and 3 μM morphine. The numbered current traces are shown to the right. Exposure to morphine produced similar inhibitory effects in both types of neurons. Figure 5B shows the curve fits for both groups of neurons. The statistical comparison of both fits was not significantly different ($p = 0.24$). In this group of data, the mean ($\pm$ SE) maximum current inhibition (%) and Hill coefficient values for control and cocaine-treated 118GG mice were $37.1 \pm 8.7\%$, $39.6 \pm 4.3\%$ and 0.74 ± 0.53 , 1.04 ± 0.56 , respectively. However, opposite to the finding in TG neurons from cocaine-treated 118AA mice, these plots illustrate that TG neurons taken from cocaine-treated 118GG mice exhibited a leftward shift in IC_{50} when compared to 118GG saline-treated controls. Further, the efficacy for morphine was higher in both groups of 118AA mice than 118GG mice (see Figure 5). Finally, a comparison of control TG neurons from both 118AA and 118GG groups showed that the latter had a reduced morphine efficacy and potency. Similar to our previous findings [13], the curve fits obtained between these two groups were significantly different ($p < 0.05$) from each other.

4. DISCUSSION

Exposure to drugs of abuse alters the way in which humans respond to other rewards in the environment. For example, anticipation of access to nicotine (i.e., smoking) [26], as well as a history of cocaine use [27], can decrease sensitivity and responsiveness to monetary gains and losses. The devaluation of alternative rewards observed in addiction can be modeled in rodents when presentation of a saccharin cue elicits anticipation of the availability of a drug of abuse [20, 28, 29]. In this paradigm, saccharin-saline controls continue to consume saccharin over trials, while rats and mice that have had the saccharin cue paired with a drug of abuse (e.g., cocaine or morphine) avoid intake of the same saccharin cue [20, 23]. It is believed that rodents may initially avoid intake of a saccharin cue, at least in part, because they are anticipating the availability of the highly preferred drug of abuse. Using this paradigm, the 118GG mice in the current set of studies demonstrated greater cocaine-induced suppression of saccharin intake compared with 118AA mice (Figure 1). Specifically, the 118GG mice, but not 118AA mice, demonstrated significant suppression of intake of a saccharin cue following just two pairings with 20 mg/kg cocaine. The 118AA mice, on the other hand, did not begin to suppress intake of the cocaine-paired taste cue until the dose of cocaine was increased to 40 mg/kg. The 118GG mice, then, exhibit greater avoidance (perhaps devaluation) of the cocaine-paired saccharin cue than do the 118AA mice.

However, avoidance of the drug-paired cue also can be explained by an alternative account, which is not necessarily mutually exclusive. By this account, with taste-drug experience, rodents may come to avoid intake of a drug-paired cue because of the onset of a conditioned aversive state involving craving and withdrawal [29]. In support, avoidance of a drug-paired taste cue is associated with a conditioned elevation of circulating corticosterone [30], a blunted [31] or reduced [32] accumbens dopamine response to the otherwise palatable

saccharin cue, and frank aversive orofacial responses (i.e., gapes) following the intraoral delivery of the cocaine-paired cue [33]. Recent evidence generated using taste-drug pairings shows that the wait may be a critical component of the onset of the conditioned aversive state, as both rejection of the taste cue (i.e., the occurrence of gapes) and the decrease in accumbens dopamine occur when the cue precedes, but not when it accompanies, cocaine self-administration [32]. Finally, further evidence supports our suggestion that the conditioned aversive state may involve cue-induced craving and withdrawal, as a morphine- or cocaine-paired taste cue elicits withdrawal symptoms (e.g., a precipitous loss in body weight) when intake of the cue is followed by an injection of a low dose of the μ -opioid antagonist, naloxone, rather than drug [34].

Greater avoidance of the cocaine-paired taste cue by the 118GG mice may indicate a greater propensity for drug-seeking and drug-taking. As described, when tested using self-administration in rats, greater avoidance of the drug-paired saccharin cue was correlated with greater cocaine self-administration and greater seeking following an extended period of abstinence [20, 21]. Likewise, greater aversive taste reactivity behavior elicited by intraoral delivery of the cocaine-paired cue is associated with a shorter latency to take drug, a longer load up period, greater drug-taking, and faster acquisition of cocaine self-administration behavior over trials [33]. Importantly, so too in the human study of this kind. Wilson and colleagues found that reduced responsiveness to monetary losses and gains (as indicated by reduced fMRI activation in the ventral striatum of human nicotine addicts) predicts increased preference for a cigarette over money in a subsequent choice test (Wilson, et al., submitted). Future studies must be conducted to test whether the 118GG mice who most greatly avoid intake of the cocaine-paired cue will exhibit the greatest cocaine seeking and taking and whether parallel findings are obtained with opiates and opioids.

A great deal of work suggests that, while drugs can devalue natural rewards, natural rewards can, in turn, devalue drugs of abuse. Indeed, this concept is at the base of all efforts to use contingency management in the treatment of drug and alcohol addiction in humans [35–37]. But all individuals are not equally responsive to natural rewards, and this has bearing on vulnerability to drug. Specifically, we have found that animal models of general reward sensitivity (i.e., increased preference not only for drugs of abuse but for natural rewards as well) exhibit an opposite profile (i.e., lesser suppression of intake of the drug-paired taste cue). This is the case for C57BL/6J mice vs. DBA/2J mice [23] and for NSE-tTA $\times$ TetOp-

FosB mice, a transgenic mouse strain with inducible elevations of the transcription factor FosB [38]. Finally, female Sprague-Dawley rats are known to take more drug and to work harder for drug than males [39–41], but when access to cocaine is preceded by brief access to a sweet, female Sprague-Dawley rats ingest more of the saccharin cue and take less drug than their male counterparts [42]. In contrast to female rats and the mouse strains described above, the 118GG mice appear to have no such protection, at least when 0.15% saccharin serves as the gustatory cue. Thus, avoidance of the saccharin cue is augmented, not reduced, in 118GG mice relative to the 118AA controls. This finding parallels that obtained with addiction-prone Lewis rats who demonstrate greater cocaine-induced suppression of intake of a saccharin cue compared with the relatively reward-insensitive Fischer rats [43, 44, but see 45, 46 regarding opiates] and with male Sprague-Dawley rats with a history of chronic exposure to morphine [47].

Repeated and chronic exposure to drugs of abuse also may result in increased sensitivity to pain, both physical (hyperalgesia) and emotional (hyperkatifeia) [48–50]. In addition, some reports suggest that persistent pain is common following detoxification treatment from drugs of abuse and is associated with poor rates of abstinence [51, 52]. Such an increased sensitivity to pain may contribute to greater avoidance of the saccharin cue, particularly if cue-induced withdrawal contributes to the phenomenon. We have previously shown that morphine exhibits a significantly lower potency and reduced efficacy in TG neurons in drug-naïve 118GG mice compared with drug naïve 118AA mice [13], suggesting that the analgesic properties of opiates may be less effective in 118GG mice. Similar results were obtained in the present study (Figure 5). Interestingly, chronic drug exposure (i.e., a history of cocaine administration) resulted in a rightward shift in Ca^{2+} current inhibition (Figure 3) compared with cocaine naïve 118AA controls. This finding is consistent with reduced sensitivity to the pain-reducing properties of the opiate in drug-experienced 118AA mice. In the 118GG mice, on the other hand, cocaine exposure produced a leftward shift in μ -opioid receptor evoked Ca^{2+} current inhibition relative to their own saline-treated controls (Figure 4). This observation suggests that, while never reaching the sensitivity of drug naïve 118AA subjects, repeated cocaine exposure may attenuate the innate differences in μ -opioid receptor efficacy in TG neurons (at least with morphine as the agonist) in these mice. Further studies are required to understand the generalizability and functional significance of this finding as related to sensitivity to the addictive and pain-reducing properties of opioids.

4.1 Conclusions

In summary, 118GG mice demonstrate greater avoidance of a natural saccharin reward cue compared with 118AA mice when anticipating the availability of cocaine. Thus, relative to 118AA mice, 118GG mice exhibit greater suppression of intake of cocaine-paired saccharin. In addition, μ -opioid receptor potency and efficacy is lower in TG neurons from 118GG mice, although repeated exposure to cocaine decreases the relative difference between the genotypes. The current set of data supports the use of OPRM1 A118G mice as a possible model to elucidate the intersection of reward and pain in the context of drug addiction. Indeed, some data show that the presence of pain can block the rewarding impact of opiates in normal subjects [53–55]. The 118GG mouse, it would seem, may be insensitive to this protective mechanism because they are, at once, more sensitive to pain, less sensitive to the antinociceptive effects of opioids, and more sensitive to the rewarding properties of opioids and other drugs of abuse. Future studies will focus on the exploration of this, and related hypotheses in an effort to better understand neural mechanisms mediating pain, relief from pain, reward, and addiction.

Acknowledgements

This research was supported by the National Institutes of Health National Institute of Drug Addiction [Grant DA009815 to P.S.G. and AR059397 & DA025574 to V.R.-V.].

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Highlights

- We tested the effect of chronic cocaine on behavior and physiology of A118G mice.
- 118GG mice show greater avoidance of a cocaine-paired saccharin cue than 118AA mice.
- μ -opioid receptor potency and efficacy is lower in sensory neurons from 118GG mice.
- Repeated cocaine exposure decreases the relative difference in μ -opioid receptor potency.

Saccharin-Cocaine Pairings in A118G mice

Figure 1.

Saccharin Intake (panels A and B). Mean ($\pm$ SEM) intake (ml/hr) of 0.15% saccharin as a function of pairings with saline (open symbols) or cocaine (closed symbols) in 118AA and 118GG mice. Asterisks indicate significant difference compared with controls, $p < .05$.

Morning water intake (panels C and D). Mean ($\pm$ SEM) intake (ml/hr) of water in the morning in saline (open symbols) and cocaine (closed symbols) treatment groups in 118AA (squares) and 118GG (triangles) mice.

Saccharin-Cocaine Pairings in A118G mice

Figure 2.

Afternoon water intake (panels A and B). Mean ($\pm$ SEM) intake (ml/hr) of water in the afternoon in saline (open symbols) and cocaine (closed symbols) groups in 118AA (left panel) and 118GG (right panel) mice. **Body weight (panels C and D).** Mean ($\pm$ SEM) body weight (g) for saline and cocaine groups over trials in 118AA (left panel) and 118GG (right panel) mice.

A Control 118AA Mice

B

C Cocaine-treated 118AA Mice

D

Figure 3.

The morphine concentration-response relationships of acutely dispersed TG neurons isolated from control (panels A,B) or cocaine-treated (panels C,D) 118AA mice. Time courses of Ca^{2+} current inhibition acquired during morphine exposure in a sensory neuron from control 118AA (A) and cocaine-exposed 118AA (C) mice. The Ca^{2+} currents were evoked every 10 sec by a single 50 msec test pulse to 0 mV from a holding potential of -80 mV (shown top of 5B). The peak Ca^{2+} current amplitudes were measured isochronally 10 ms following depolarization. The filled bars indicate application of 0.03 and 3 μM morphine. B and D show the superimposed Ca^{2+} currents before (black, 1 and 3) and during (grey, 2 and 4) morphine exposure shown in A and C.

A Control 118GG Mice

B

C Cocaine-treated 118GG Mice

D

Figure 4.

The morphine concentration-response relationships of acutely dispersed TG neurons isolated from control (panels A,B) or cocaine-treated (panels C,D) 118GG mice. Time courses of Ca^{2+} current inhibition acquired during morphine exposure in a sensory neuron from control 118GG (A) and cocaine-exposed 118GG (C) mice. The Ca^{2+} currents were evoked every 10 sec by a single 50 msec test pulse to 0 mV from a holding potential of -80 mV (shown top of 5B). The peak Ca^{2+} current amplitudes were measured isochronally 10 ms following depolarization. The filled bars indicate application of 0.03 and 3 μM morphine. B and D show the superimposed Ca^{2+} currents before (black, 1 and 3) and during (grey, 2 and 4) morphine exposure shown in A and C.

A) 118AA Mice

B) 118GG Mice

Figure 5.

Summary of the morphine concentration-response curves of sensory neurons from control and cocaine-treated 118AA mice (panel A) and control and cocaine-treated 118GG mice (panel B). Each point represents the mean ($\pm$ SE) of the morphine-mediated Ca^{2+} current inhibition. The numbers in parenthesis indicate the number of neurons tested. The smooth curves were obtained by fitting the points to the Hill equation and the IC_{50} (nM) values for control (●) and cocaine-treated (▲) mice in each genotype are shown in their respective inset