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Chronic administration of buprenorphine in combination with samidorphan produces sustained effects in olfactory bulbectomized rats and Wistar-Kyoto rats

Short title: Chronic buprenorphine and samidorphan in rats

Nikita N Burke*1, Yan Li*3, Daniel R Deaver3, David P Finn1, Michelle Roche2, David J. Eyerman3, Connie Sanchez3 and John P Kelly1 (*: contributed equally)

Affiliations 1Pharmacology & Therapeutics and 2Physiology and Centre for Pain Research, School of Medicine, NCBES Galway Neuroscience Centre, National University of Ireland, Galway, Ireland; 3Alkermes, Inc., Waltham, Massachusetts

Corresponding author: Professor John Kelly, Pharmacology and Therapeutics, School of Medicine, NCBES Galway Neuroscience Centre, National University of Ireland, Galway, Ireland.
Telephone: +3538791493268
Fax: +35391525700
Email: john.kelly@nuigalway.ie

Keywords
Opioids, buprenorphine, olfactory bulbectomy, Wistar-Kyoto, forced swim test
Abstract

Background: To harness mood-enhancing effects of opioids without unwanted side-effects such as a risk of addiction, the combination of buprenorphine, a partial mu-opioid receptor agonist and a functional kappa-opioid receptor antagonist, with samidorphan, a functional mu-opioid receptor antagonist, is being developed as an adjunct therapy for major depressive disorder. Acute and subacute administration of the combination of buprenorphine and samidorphan is effective in reducing forced swim immobility in the Wistar-Kyoto rat, but chronic effects have not been examined.

Aims and methods: To assess if chronic (14-day) administration of buprenorphine (0.1 mg/kg, s.c.) alone or in combination with samidorphan (0.3 mg/kg/ s.c.) maintains antidepressant-like activities in the olfactory bullectomised (OB) rat model and the Wistar-Kyoto rat, two models that exhibit ongoing behavioural deficits in tests commonly used to study effects of antidepressants.

Results: OB-induced hyperactivity was attenuated by chronic administration of buprenorphine alone and in combination with samidorphan, to that of sham control activity levels. Neither buprenorphine nor samidorphan altered stress-associated defecation in sham or OB rats in the open field. In Wistar-Kyoto rats, buprenorphine alone significantly reduced forced swim immobility and increased locomotor activity 3 hours post final dosing. Buprenorphine plus samidorphan significantly reduced forced swim immobility without changing locomotor activity at this time point. Buprenorphine alone also significantly reduced forced swim immobility 24 hours post final dosing.

Conclusion: Chronic treatment of buprenorphine alone or buprenorphine plus samidorphan is effective in reversing behavioural deficits in distinct non-clinical paradigms. These non-
clinical results complement the antidepressant effect of this combination observed in clinical studies.

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1. Introduction

The opioid system regulates a host of physiological processes including pain and analgesia, response to stress, respiration, gastrointestinal transit, and endocrine and immune function. Opioids also modulate mood, as recognised by the profound euphoria elicited by exogenous mu-opioid receptor agonists (Keats & Beecher, 1952) and the dysphoric effects produced by kappa-opioid receptor agonists (Pfeiffer, Brantl, Herz, & Emrich, 1986). Mu, kappa and delta, the classic opioid receptors, are highly expressed in corticolimbic brain regions such as the prefrontal cortex and amygdala (Lutz & Kieffer, 2013). There is evidence to suggest that the endogenous opioid system modulation is altered in depressed patients (Pecina et al., 2019). For example, increased mu-opioid receptor density and reduced beta-endorphin levels are detected in the brains of people who died by suicide (Gabilondo, Meana, & Garcia-Sevilla, 1995; Gross-Isseroff, Dillon, Israeli, & Biegon, 1990; Scarone et al., 1990). On the other hand, greater endogenous mu-opioid tone correlates with more positive emotion and better executive function in major depressive disorder (MDD) patients (Light, Bieliauskas, & Zubieta, 2017). In addition, prodynorphin mRNA levels are increased in the periamygdaloid cortex in postmortem tissues of MDD patients (Anderson et al., 2013). The opioid system is therefore being re-examined as a potential therapeutic target for MDD, specifically by attempting to exploit the mood-enhancing qualities of opioids while reducing the risk of unwanted effects such as drug liking and dependence and (Almatroudi, Husbands, Bailey, & Bailey, 2015; Bailey & Husbands, 2018; Ehrich et al., 2015). One such example is the development of a combination of buprenorphine and samidorphan therapy as an adjunct treatment for major depressive disorder (Ehrich et al., 2015).

Buprenorphine, a functional antagonist at the kappa-opioid receptor and partial agonist at the mu-opioid receptor, has an improved safety profile compared to full mu-opioid agonists (Lutfy & Cowan, 2004). Samidorphan is a functional antagonist at the mu-opioid receptor
Evidence from some (but not all) clinical studies suggests the combination of buprenorphine with samidorphan reduces depressive symptoms in patients with MDD and an inadequate response to their current therapy, when administered as an adjunct treatment to monoaminergic antidepressants (Ehrich et al., 2015; Fava et al., 2018; Peckham, De La Cruz, & Dufresne, 2018). In non-clinical studies, acute and subacute administration of buprenorphine with samidorphan induces behavioural changes indicative of antidepressant-like activity (Burke et al., 2019; Smith et al., 2019). It is unknown if the buprenorphine and samidorphan combination induces similar behavioural changes after chronic treatment in well-validated animal models.

Rodent olfactory bulbectomy (OB) has been used as an experimental paradigm to study antidepressants. Following bulbectomy, rats exhibit hyperactivity upon exposure to a novel environment (Stock, Ford, & Wilson, 2000). Other alterations include reduced responsivity to rewarding stimuli, decreased social behaviour, learning and memory deficits, and reduced sexual behaviour (Kelly, Wrynn, & Leonard, 1997; Song & Leonard, 2005). Similar to clinical observations, only chronic antidepressant treatment is effective in reversing these deficits in OB rats (Kelly et al., 1997).

The Wistar-Kyoto rat is an inbred strain with augmented endocrine and behavioural responses to stress. For example, foot shock increases plasma epinephrine and norepinephrine levels significantly more in Wistar-Kyoto rats compared to Sprague-Dawley rats (McCarty & Kopin, 1978). Forced swim increases plasma adrenocorticotropic hormone (ACTH) and corticosterone levels significantly more in Wistar-Kyoto rats compared to Sprague-Dawley rats (Rittenhouse, Lopez-Rubalcava, Stanwood, & Lucki, 2002). Behaviourally, Wistar-Kyoto rats have exaggerated immobility in the forced swim test, reduced sucrose preference and increased marble burying behaviour (Burke et al., 2016; Nam, Clinton, Jackson, & Kerman, 2014; Rittenhouse et al., 2002; Smith et al., 2019; Tejani-Butt, Kluczynski, & Pare,
Wistar-Kyoto rats do not respond to selective serotonin reuptake inhibitors (SSRIs) (Lopez-Rubalcava & Lucki, 2000). However, drugs showing clinical efficacy for treatment-resistant depression are effective in modifying behaviour in Wistar-Kyoto rats, including ketamine (Tizabi, Bhatti, Manaye, Das, & Akinfiresoye, 2012) and buprenorphine (Browne, van Nest, & Lucki, 2015). Thus, Wistar-Kyoto rats may be considered as a genetic model for inadequate response to SSRI antidepressants.

Current antidepressants usually take weeks before a therapeutic benefit is observed (Artigas, Bortolozzi, & Celada, 2018), therefore it is important to assess the effect of chronic treatment regimens in non-clinical research (Cryan, Markou, & Lucki, 2002). We examined the effect of chronic administration of buprenorphine alone or with samidorphan, in OB and Wistar-Kyoto rats.
2. Experimental Procedures

2.1 Animal husbandry

Adult male Sprague-Dawley rats (Harlan, UK) were singly-housed, and adult male Wistar-Kyoto rats (Charles River Labs, Wilmington, MA) were pair-housed. Rats were housed in a temperature-controlled room (22 ± 4°C), relative humidity of 30-70%, with a 12:12h light-dark cycle and ad libitum access to food and water. The OB experiment was carried out after an acclimation period of at least 3 days, in accordance with the guidelines and approval of the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under licence from the Health Products Regulatory Authority, and in compliance with the European Communities Council directive 86/609. The Wistar-Kyoto experiment was approved by the Alkermes Institutional Animal Care and Use Committee, in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2 Experimental design

The doses of buprenorphine and samidorphan were selected based on the dose-response in acute and subacute studies, where the combination of both (0.1 mg/kg buprenorphine and 0.3 mg/kg samidorphan, s.c.) significantly reduced immobility in the forced swim test and blunted buprenorphine-induced increases in extracellular dopamine concentrations in the reward pathway in Wistar-Kyoto rats (Burke et al., 2019; Smith et al., 2019). As the OB model does not respond to acute antidepressant treatment, 14 day treatment is commonly used in this paradigm (Kelly et al., 1997). In addition, 14 day treatment should be sufficient to demonstrate effect of antidepressant in Wistar-Kyoto rats (Tejani-Butt et al., 2003) and other rodent paradigms (Li, Raaby, Sanchez, & Gulinello, 2013). Therefore, a 14-day treatment regimen was used for the following experiments.
Experiment 1

As outlined in Fig 1a, Sprague-Dawley rats (n=7-9/group) underwent sham or OB surgery and were allowed to recover for 2 weeks before the baseline open field test. Rats then received once daily (quaque die – q.d.) subcutaneous (s.c.) injections (1 mL/kg) of vehicle (saline), buprenorphine (0.1 mg/kg, diluted from Bupaq, Chanelle, Ireland), samidorphan (0.3 mg/kg, Alkermes, Inc., Ireland) or a combination of buprenorphine (0.1 mg/kg) with samidorphan (0.3 mg/kg) from Day 14 to Day 28. Doses for this and the following experiment are expressed as the free base form. To avoid the acute effects of drug, the post-treatment open field test was conducted 24 hours after the final dose on Day 29.

Experiment 2

As outlined in Fig 2a, Wistar-Kyoto rats were randomly allocated to the following groups and received q.d. injections (s.c., 1 mL/kg) for 14 days: 1) saline (vehicle); 2) 0.1 mg/kg buprenorphine (diluted from Buprenex, Reckitt Benckiser Pharmaceuticals, Inc., Hull, UK); 3) 0.1 mg/kg buprenorphine and 0.3 mg/kg samidorphan (Alkermes, Inc., Waltham, MA). Rats were tested in the forced swim test (n=14-16/group) or open field test (n=8/group, a separate cohort), 3 hours, 24 hours, and 1 week following the final injection.

2.3 Bilateral olfactory bulbectomy (OB) surgery

Bilateral OB was performed under isoflurane anaesthesia (Abbot Laboratories, UK [5% induction, 2% maintenance in 0.5 l/min O₂]) as described previously (Burke et al., 2010). In brief, two burr holes of 2 mm diameter were drilled into the skull, 5 mm rostral to bregma and 2 mm lateral to the midline and the olfactory bulbs were removed by gentle aspiration with a vacuum pump. The burr holes were plugged with a haemostatic sponge (Septodont, France) to control bleeding. Sham-operated animals were treated in the same manner but the
bulbs were left intact. Animals were handled daily following surgery and lesions were verified by gross inspection after completion of the study. Animals were eliminated from the analysis if the bulbs were not completely removed or if damage extended to the frontal cortex (n=2).

2.4 Forced swim test

Wistar-Kyoto rats display an exaggerated immobility response without prior exposure to the forced swim test (Nam et al., 2014). Therefore, a modified forced swim test protocol was used in this study, consistent with the previous acute study (Smith et al., 2019). This modified protocol omits the traditional conditioning pretest (15 min) before drug administration which permitted us to investigate the duration of action over 3 timepoints following chronic treatment. FST swim sessions were conducted at 3 h, 24 h, and 1 week following the final drug administration. Briefly, rats were placed in Plexiglas tanks (20 cm diameter) filled with 30 cm depth of water (24°C) for 5 minutes, and the test sessions were recorded. The duration of immobility (in seconds) was manually scored offline for the whole duration of 5 minutes. Immobility was defined as no movements other than those necessary to keep the nose above water.

2.5 Open field test

To confirm bulbectomy-induced hyperactivity and evaluate the effect of drug treatments, OB rats were placed into a brightly-lit (200 lux) novel open field arena (diameter 75 cm) with a white floor and reflective walls for 5 minutes (Kelly et al., 1997). Locomotor activity (distance moved, cm) was assessed using a video tracking system (EthoVisionXT®, Noldus, Netherlands) (Burke et al., 2010). The number of faecal pellets was counted at the end of the test as an index of emotionality/anxiety-like behaviour (Hall, 1934).
To assess the locomotor activity, Wistar-Kyoto rats were placed in a dimly lit (4 lux) open field arena (43 cm × 43 cm) for 5 minutes. Distance moved (cm) was analyzed using Activity Monitor software (Med Associates, Fairfax, VT).

2.6 Statistical analyses

The Brown-Forsythe test was used to confirm homogeneity of the data. Data were analysed using GraphPad Prism software. Locomotor activity was analysed by two-way (treatment or OB) ANOVA followed, where appropriate, by Sidak’s post-hoc test. Non-parametric data (faecal pellet count) were analysed with Kruskal-Wallis followed by Mann-Whitney U post-hoc test where appropriate. For the Wistar-Kyoto experiment, immobility in the forced swim test and distance moved in the open field were analysed using one-way ANOVA, followed by post-hoc Dunnett’s test to compare treatment groups to the vehicle group, where appropriate. All data were presented as the mean ± SEM, \( p \leq 0.05 \) was deemed significant.
3. Results

*Chronic buprenorphine, alone and with samidorphan, attenuates hyperactivity in the OB rat*

After recovery from sham or OB surgery, rats were exposed to the open field to confirm bulbectomy-induced hyperactivity and to be allocated to different treatment groups (Fig 1a). Two-way ANOVA revealed that OB animals exhibited greater locomotor activity compared to sham controls (F(1,59) = 37.16, p<0.0001, Fig 1b). Kruskal-Wallis test revealed an overall significant difference in faecal pellet count (χ²(7) = 22.51, p=0.002, Fig 1c). Mann-Whitney U *post-hoc* tests showed that OB rats showed greater faecal pellet output.

Following chronic drug treatment, two-way ANOVA revealed effects of OB (F(1,59) = 19.92, p<0.001) and treatment (F(3,59) = 4.23, p=0.009) on locomotor activity (Fig 1d). Samidorphan alone did not alter OB-induced hyperactivity. Sidak’s *post-hoc* test showed that buprenorphine alone and in combination with samidorphan significantly reduced OB-related hyperactivity.

Kruskal-Wallis test revealed an overall effect for faecal pellet count (χ²(7) = 22.15, p=0.002). Mann-Whitney U *post-hoc* test showed that OB rats had greater faecal pellet output during the open field test, an effect unaltered by drug treatment (Fig 1e).

*Chronic buprenorphine, alone and with samidorphan, reduces immobility of Wistar-Kyoto rats in the forced swim test with different effects on locomotor activity*

When tested at 3 hours after the final drug administration of the 14 day treatment regimen, there was an effect of treatment on the forced swim immobility of Wistar-Kyoto rats (F(2, 43)=16.55, p<0.01, Fig 2b). Dunnett’s *post-hoc* test indicated that buprenorphine alone and in combination with samidorphan significantly reduced immobility. There also was a treatment effect on locomotor activity (F(2, 21)=22.22, p<0.01, Fig 2c). Dunnett’s *post-hoc* test indicated
that buprenorphine significantly increased locomotor activity, but not when buprenorphine was given in combination with samidorphan.

There was also a treatment effect on the forced swim immobility 24 hours post final drug administration ($F(2, 43)=18.02, p<0.01$, Fig 2d). *Post-hoc* Dunnett’s test indicated that only the effect of buprenorphine was significant. Despite an overall treatment effect on locomotor activity ($F(2, 21)=4.68, p<0.05$, Fig 2e), Dunnett’s post-*hoc* test indicated that the effect of buprenorphine alone or in combination with samidorphan was not significantly different from that of vehicle.

One week after the final dose, buprenorphine alone or with samidorphan had no significant effect in either the forced swim test or the open field test (data not shown).
4. Discussion
We examined the effects of chronic treatment of buprenorphine alone or in combination with samidorphan in two rat models commonly used to study antidepressants. Both buprenorphine alone and the buprenorphine-samidorphan combination reduced hyperactivity in OB rats and forced swim immobility in Wistar-Kyoto rats. Buprenorphine also increased locomotor activity in Wistar-Kyoto rats. As acute and subacute buprenorphine and the buprenorphine-samidorphan combination were effective in the forced swim test (Burke et al., 2019; Smith et al., 2019), the results from the current study suggest that these effects are maintained with chronic treatment.

To our knowledge, this is the first study showing efficacy of buprenorphine and the buprenorphine-samidorphan combination in the OB model. Following bulbectomy, rats exhibit hyperactivity upon exposure to a stressful novel environment (Burke, Finn, & Roche, 2015; Burke, Kerr, Moriarty, Finn, & Roche, 2014; Leonard & Tuite, 1981; Roche, Kerr, Hunt, & Kelly, 2012; Stock et al., 2000; Willner & Mitchell, 2002). This stress-induced hyperactivity is the most widely reported behavioural output following bulbectomy, is reproducible and reliable, and is hypothesised to reflect a lack of normal defensive behaviours and disinhibition of the amygdala (Harkin, Kelly, & Leonard, 2003; Leonard & Tuite, 1981). These behavioural changes are not related to anosmia, as peripheral ablation of olfaction does not result in the same behavioural changes (Calcagnotto, Quatrella, & Schechter, 1996; van Rijzingen, Gispen, & Spruijt, 1995). Similar to clinical observations, only chronic, but not acute, administration of antidepressants is effective in reducing these behavioural deficits in OB rats (Cryan et al., 2002; Song & Leonard, 2005).

As buprenorphine and buprenorphine/samidorphan combination have shown evidence of antidepressant efficacy in some (but not all) clinical trials (Ehrich et al., 2015; Fava et al., 2018; Karp et al., 2014; Peckham et al., 2018), our results support the use of the OB model to
study antidepressants with different pharmacological mechanisms. In the OB rat model, an increase of met-enkephalin mRNA expression was detected in limbic brain regions (Primeaux & Holmes, 2000). It will be interesting to examine if the kappa-opioid system is also altered in OB rats. In the current study, OB rats also exhibited increased defecation in the open field, consistent with previous studies (Stock et al., 2000). Studies using administration of antidepressants such as fluoxetine (21 days) to OB animals have demonstrated a reduction in the number of faecal boli (Mar, Spreekmeester, & Rochford, 2000). However, chronic administration of buprenorphine or samidorphan did not alter OB-induced increases in defecation, supporting the observation that defecation and locomotor activity in the open field may not always be correlated (Pisula, 1994), therefore separating these behavioural outputs. Indeed, amitriptyline, desipramine and buspirone have been shown to reduce hyperactivity but not alter defecation, a measure of autonomic nervous system activity (Denenberg, 1969) and visceral reactivity (Mar et al., 2000). Our data suggest that buprenorphine and samidorphan may not exert their effects via the autonomic nervous system.

Growing non-clinical evidence provides support that kappa-opioid receptors are involved in mood regulation (Falcon et al., 2016; Lutz & Kieffer, 2013). In Wistar-Kyoto rats, exaggerated forced-swim immobility is accompanied by increased kappa-opioid receptor and dynorphin A expression (Carr et al., 2010). Our recent work shows that WKY rats have higher mu-opioid receptor and lower nociceptin receptor mRNA expression in the hippocampus; lower mu-opioid receptor mRNA in the striatum; and higher kappa-opioid mRNA expression in the amygdala when compared to Sprague Dawley rats (Burke et al., 2019). A single treatment with buprenorphine (Browne et al., 2015) or the kappa receptor antagonist Nor-BNI (Carr et al., 2010) significantly reduced forced swim immobility in Wistar-Kyoto rats. In Sprague-Dawley rats, 0.1 mg/kg buprenorphine fully occupies kappa- and mu-opioid receptors after a single subcutaneous injection (Alan Pehrson, personal
communication). Therefore, we hypothesize that with increased dynorphin/kappa-opioid tone in Wistar-Kyoto rats, buprenorphine functionally acts as an antagonist at the kappa-opioid receptor and achieves its behavioural effects.

Results from our study support the hypothesis that samidorphan reduces activation of the mu-opioid system by buprenorphine. Buprenorphine alone, but not the buprenorphine-samidorphan combination, increases locomotor activity 3 hours after final dosing in repeatedly-treated Wistar-Kyoto rats. Similar time-dependent locomotor effects were also observed following single dosing in Wistar-Kyoto rats (Smith et al., 2019) and in C57BL mice (Falcon, Maier, Robinson, Hill-Smith, & Lucki, 2015), and after single and repeated treatment in Sprague Dawley rats (Burke et al., 2019). Effects of buprenorphine on locomotor activity confound its apparent anti-immobility effect, and may relate to an activation of the mu-opioid system. Indeed, acute buprenorphine at the same dose used herein increases nucleus accumbens-shell dopamine levels more than the buprenorphine-samidorphan combination (Smith et al., 2019). The buprenorphine-samidorphan combination is less effective than buprenorphine in stimulating mu-opioid receptor-mediated G protein recruitment and downstream signalling (Bidlack et al., 2018). Chronic samidorphan alone does not reduce locomotor activity in rats (sham and OB groups from the OB study), thus the lack of locomotor effects of buprenorphine-samidorphan is less likely a result of any sedative effect of samidorphan. Based on the locomotor activity data in Wistar-Kyoto rats from our study, mu-opioid receptor activation seems to remain lower after repeated buprenorphine-samidorphan combination treatment than buprenorphine treatment alone. Reduced mu-opioid receptor activity may be part of the mechanism for the lowered abuse potential of buprenorphine-samidorphan combination reported in humans (Pathak et al., 2019).

Acute (Smith et al., 2019), subacute (Burke et al., 2019), and chronic (current study) treatment with buprenorphine and buprenorphine-samidorphan is effective in reducing
forced-swim immobility in Wistar-Kyoto rats, indicating that no tolerance developed for this effect. Considering samidorphan alone is not effective after acute and subacute administration, the anti-immobility effect is likely driven by buprenorphine. However, the sustained anti-immobility effect following acute or subacute combination treatment was not observed in the current study after chronic treatment. This may relate to differential adaptive changes at receptor and/or peptide levels following chronic treatment. For example, 7 days of buprenorphine treatment reduced mu-opioid receptor expression in the brains of mice (Falcon et al., 2016). It is unknown if chronic buprenorphine leads to molecular, cellular or circuitry level changes that are further modified by co-administration of samidorphan in Wistar-Kyoto rats. In addition, the difference in signalling pathways may impact the effect of chronic treatment. Buprenorphine partially activates the beta-arrestin pathway, while buprenorphine-samidorphan (at a 1:3 ratio) does not have detectable levels of activation of beta-arrestin in an in vitro system (Bidlack et al., 2018). A limitation of the current study is the lack of cellular mechanistic data to support the behaviour observed here. Testing additional dose combinations and/or dosing periods may also address whether the change in sustained effect results from a different dose response for chronic treatment compared to acute or subacute treatment paradigm (Burke et al., 2019; Smith et al., 2019). It would be of interest for future studies to assess if there is any change in the brain exposure levels of buprenorphine and samidorphan after repeated dosing, compared to those after acute or subacute treatment.

In conclusion, compared to buprenorphine alone, chronic treatment of buprenorphine-samidorphan effectively reversed behavioural deficits in two distinct non-clinical models that have different underlying mechanisms.
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**Declaration of Conflicting Interests.** NNB, DF, JK and MR declare no conflict of interest. At the time of study, YL, CS, DJE, and DD were full time employees of Alkermes, Inc. and own stocks in Alkermes, Inc. DJE owns stock in Alkermes Inc. and Fulcrum Therapeutics. For experiment 1, NNB, DF, JK and MR designed the study. NNB and JK performed the study. NNB analysed the data and performed statistical analysis. For experiment 2, YL, DJE, and CS designed the study. YL performed the study and statistical analysis of the data. NNB and YL wrote the manuscript. All authors contributed to and have approved the final manuscript.

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