Drug and Alcohol Dependence 203 (2019) 19–26

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

# The influence of opioid dependence on salt consumption and related psychological parameters in mice and humans

Craig M. Smith[a,b,c], Joshua B.B. Garfield[d,e], Aparna Attawar[b], Dan I. Lubman[d,e], Andrew J. Lawrence[b,c,*]

[a] Deakin University, School of Medicine, Waurn Ponds, Victoria, 3216, Australia
[b] Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, 3052, Australia
[c] Florey Department of Neuroscience and Mental Health, The University of Melbourne, Victoria, 3010, Australia
[d] Monash Addiction Research Centre and Eastern Health Clinical School, Monash University, Box Hill, Victoria, 3128, Australia
[e] Turning Point, Eastern Health, Richmond, Victoria, 3121, Australia

ARTICLE INFO

*Keywords:*
Opioid dependence
Salt consumption
Human
Animal

ABSTRACT

*Background:* The consumption of dietary salt (NaCl) is controlled by neuronal pathways that are modulated by endogenous opioid signalling. The latter is disrupted by chronic use of exogenous opioid receptor agonists, such as morphine. Therefore, opioid dependence may influence salt consumption, which we investigated in two complimentary studies in humans and mice.

*Methods: Human study:* three groups were recruited: i. Individuals who are currently opioid dependent and receiving opioid substitution treatment (OST); ii. Previously opioid dependent individuals, who are currently abstinent, and; iii. Healthy controls with no history of opioid dependence. Participants tasted solutions containing different salt concentrations and indicated levels of salt 'desire', salt 'liking', and perceptions of 'saltiness'. *Mouse study:* preference for 0.1 M versus 0.2 M NaCl and overall levels of salt consumption were recorded during and after chronic escalating morphine treatment.

*Results: Human study:* Abstinent participants' 'desire' for and 'liking' of salt was shifted towards more highly concentrated salt solutions relative to control and OST individuals. *Mouse study:* Mice increased their total salt consumption during morphine treatment relative to vehicle controls, which persisted for 3 days after cessation of treatment. Preference for 'low' versus 'high' concentrations of salt were unchanged.

*Conclusion:* These findings suggest a possible common mechanistic cross-sensitization to salt that is present in both mice and humans and builds our understanding of how opioid dependence can influence dietary salt consumption. This research may help inform better strategies to improve the diet and overall wellbeing of the growing number of individuals who develop opioid dependence.

## 1. Introduction

Sodium plays a range of crucial cellular and physiological roles and is consequently an essential dietary component that is usually consumed in food as NaCl (salt). Terrestrial animals including humans display salt seeking and consumption behaviours, which are controlled by a variety of highly conserved neuronal pathways within the brain (Denton, 1983). Endogenous opioid signalling is well established as a powerful controller of salt consumption (Smith and Lawrence, 2018). For example, early studies identified that acute morphine injection increases the consumption of highly concentrated (and therefore previously aversive) salt solutions in rats. Conversely, non-selective opioid receptor antagonists significantly reduce salt consumption (Hubbell and McCutcheon, 1993). More recent studies have better characterised this influence and have identified several specific neuronal pathways where opioid signalling acts to modulate salt consumption. These include the mesolimbic dopaminergic system (Lucas et al., 2003, 2007; Zhang and Kelley, 2002) and a host of interconnected regions such as the central amygdala (Smith et al., 2016) and parabrachial nucleus (De Oliveira et al., 2008; Pavan et al., 2015). In addition to influencing salt consumption, endogenous opioid signalling can also powerfully control sweet taste reward value (Eikemo et al., 2016) and the processing of other gustatory information, which compliments its broader roles in controlling feeding and reward more generally (Kelley et al., 2002;

* Corresponding author at: Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne VIC 3010.
*E-mail addresses:* craig.smith@deakin.edu.au (C.M. Smith), joshuag@turningpoint.org.au (J.B.B. Garfield), aparna_attawar@hotmail.com (A. Attawar), dan.lubman@monash.edu (D.I. Lubman), andrew.lawrence@florey.edu.au (A.J. Lawrence).

https://doi.org/10.1016/j.drugalcdep.2019.05.021
Received 14 November 2018; Received in revised form 22 May 2019; Accepted 22 May 2019
Available online 01 August 2019
0376-8716/ 

Nogueiras et al., 2012).

Addiction to exogenous opioid receptor agonists such as morphine, heroin and fentanyl causes long-lasting changes to endogenous opioid signalling, such as down-regulation of endogenous opioid receptors, tolerance, and withdrawal symptoms if drug-taking is ceased (Koob and Volkow, 2016). In addition to the well-established influence of acute opioid receptor agonist / antagonist drugs (Hubbell and McCutcheon, 1993), it is therefore also likely that salt consumption and associated psychological parameters are affected during opioid dependence. However, investigations of the effects of opioid dependence have to-date mainly focussed on metabolic factors and general diet (with an emphasis on sweet foods) rather than salt specifically (Morabia et al., 1989; Nolan and Scagnelli, 2007; Peles et al., 2016; Zador et al., 1996). Therefore, the ability of opioid dependence to influence salt intake specifically remains relatively poorly investigated. This is despite the knowledge that overconsumption of dietary salt is associated with hypertension and increased risk of cerebrovascular accidents, heart and kidney diseases (VicHealth, 2015). Although broader behavioural dysfunction (such as sedentary activity) undoubtedly contributes to high rates of cardiovascular disease in opioid-dependent populations (Hser et al., 2004; Sadeghian et al., 2007), and opioids reduce gastric motility (Nogueiras et al., 2012), neurological and psychological changes that influence dietary choices may also be involved (Morris et al., 2008).

In order to further investigate how opioid dependence alters salt consumption and associated psychological states, we conducted two complimentary studies in humans and mice. We first sought to determine whether patients who are currently opioid dependent and receiving opioid substitution therapy (OST; i.e., methadone or buprenorphine) display altered 'desire' for salt, salt 'liking', or perceptions of 'saltiness'. We hypothesised that patients on OST would display a shift in 'desire' and/or 'liking' towards higher salt concentrations, relative to abstinent and control groups, which may be accompanied with changes in perception of 'saltiness'. Secondly, we examined whether mice alter their salt consumption during and/or after a chronic escalating morphine treatment protocol, which has previously been shown to induce rapid opioid dependence (Goeldner et al., 2011). This study was not designed to mimic the concurrent study in humans but rather served to compliment it by purposely probing different aspects of opioid dependence/ salt intake interactions which are hard to study in humans due to an inability to control factors such as diet. We hypothesised mice would display altered salt consumption during and initially after chronic morphine treatment and, additionally, would display altered preference for 'high' versus 'low' concentration NaCl solutions.

## 2. Methods

### 2.1. Human study

#### 2.1.1. Participants

This study received approval from the Monash University Human Research Ethics Committee (project number CF15/973 – 2015000437) and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Three groups of participants were recruited for this study. The 'OST' group (n = 37) were opioid-dependent individuals, currently prescribed OST (methadone: n = 30; buprenorphine: n = 7), who were recruited through leaflets placed in pharmacies administering OST, substance use rehabilitation services, word-of-mouth referral by other participants, or were participants in previous studies conducted by the authors who had given permission to be contacted regarding other study participation opportunities. The 'abstinent' group (n = 18) were residents of substance use rehabilitation services who had a lifetime history of opioid dependence, according to Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision (DSM-IV-TR; (American Psychiatric Association, 2000)) criteria, but who had been abstinent from all opioids (prescribed or illicit) for at least 8 weeks. The 'control' group (n = 29) reported no history of dependence on any substance other than nicotine and were recruited through word of mouth referral by other participants, advertisements placed in newspapers and online, posters placed in public locations, and through the investigators' institutions. Recruitment of the control group was targeted to match the opioid-dependent groups, at a group level, according to gender and the proportion who were current daily tobacco smokers, to control for effects of these factors on taste preference and perception.

Participants in all groups were required to be aged 18–55 years and to be proficient English speakers. Potential participants were excluded if they reported current major depression; current dependence on any substance other than nicotine (or opioids in the OST group); any history of psychotic or bipolar disorder; any history of head injury that required surgery or rehabilitation, or that resulted in more than 30 min unconsciousness; any current neurological disorder; current acute, severe physical illness; or if they were currently prescribed regular (i.e., more than weekly in the past month) use of any psychotropic medication (other than opioids in the OST group) or any medication known to produce taste perception abnormality as a common side effect. In addition, people were excluded from the control group if they reported any history of dependence on alcohol, illicit drugs, or any other substance other than nicotine or any past month illicit drug use.

#### 2.1.2. Measures

*2.1.2.1. Demographics and substance use history*. An interviewer-administered questionnaire assessed basic demographic information (e.g., date of birth, gender, country of birth), as well as history of use (e.g., age of first use, highest frequency of use, past-year use, and frequency of use in the past 3 months) of tobacco, cannabis, amphetamines, cocaine, hallucinogens, ecstasy, inhalants, illicit/non-prescribed opioids, and illicit/non-prescribed benzodiazepines; history of substance use treatment; and recency of last illicit opioid use and last prescribed opioid use.

*2.1.2.2. Psychiatric and substance use diagnoses*. The Structured Clinical Interview for DSM-IV-TR Disorders (SCID-I/P; (First et al., 2002)) modules assessing current major depressive episode, psychotic symptoms, alcohol abuse and dependence, and non-alcohol substance abuse and dependence were administered to confirm eligibility and determine history of substance dependence diagnoses.

*2.1.2.3. Past-month substance use*. The time-line follow-back (TLFB) interview method (Sobell and Sobell, 1996) was used to record number of days of use and amount of use of tobacco, alcohol, drugs, and medication during the past 30 days.

*2.1.2.4. Severity of opioid dependence*. Opioid-dependent participants completed the Severity of Dependence Scale (SDS) (Gossop et al., 1995) to assess severity of heroin dependence during the past year. Scores on the SDS can range from 0 to 15, with higher scores indicating more severe dependence.

*2.1.2.5. Opioid withdrawal symptom severity*. Opioid-dependent participants completed the Short Opiate Withdrawal Scale (SOWS) (Gossop, 1990) to assess severity of withdrawal symptoms during the past 24 h. SOWS scores can range from 0 to 3, with higher scores indicating more severe withdrawal symptoms.

*2.1.2.6. Taste ratings*. Ratings scales were adapted from a published procedure (Drewnowski et al., 1996). For each sample of broth, participants were presented with 9-point scales assessing "How much did you *like* this broth?", "How strong is your *desire* to consume more of this broth?", and "how *salty* did this broth taste?". Each scale was anchored at 1, 5, and 9 ("Dislike Extremely", "Neither like nor dislike", and "Like Extremely", respectively, for the 'like' scale; "No desire to consume more", "Moderate desire to consume more", and "Extreme

desire to consume more", respectively, for the 'desire' scale; "Not salty at all", "Moderately salty", and "Extremely salty", respectively, for the 'saltiness' scale). Participants were asked to circle one of the 9 numbers on the scale after tasting the sample.

*2.1.2.7. Urine drug test.* To confirm the presence of OST in the OST group, and the absence of any recent illicit drug use in the abstinent and control groups, participants were required to provide a urine sample, which was tested by a contract pathology service for opiates, amphetamine-type substances, benzodiazepines, THC metabolites, cocaine metabolites, methadone metabolites, and buprenorphine.

*2.1.2.8. Procedure.* Participants were presented with verbal and written information describing study procedures before providing written, informed consent to participate. A research psychologist then conducted the interviewer-administered measures (demographics, substance use history, SCID-I/P, and TLFB). Participants were then provided the SDS and SOWS to self-complete while the researcher prepared the tasting samples. Eight 50 ml samples were made using Pacific Organic Low Sodium Vegetable Broth, which has a sodium concentration of 0.027 mol/l (confirmed using a Radiometer ABL800 BASIC electrolyte analyser), with 0.038, 0.155, 0.389, 0.622, 0.856, 1.09, 1.32, and 1.79 g of table salt added to achieve sodium concentrations of 0.04, 0.08, 0.16, 0.24, 0.32, 0.40, 0.48, and 0.64 mol/l respectively. Samples were heated to 40–55 °C before being presented to participants.

Samples were presented in a randomised order, using the online random.org sequence generator (www.random.org/sequences/). Participants were blind to order (i.e., single-blind design). Participants were asked to taste each sample, complete the taste ratings, and then to rinse their mouths with room temperature tap water prior to tasting the next sample to avoid the flavour of the previous sample interfering with their perception of the subsequent sample. Overall, the questionnaires and taste tests typically took approximately 2 h to complete. Participants were paid AUD60 for completing these procedures.

*2.1.2.9. Analysis.* Analyses were conducted using IBM® SPSS® Version 25. Comparisons between groups on demographic and clinical variables were conducted using univariate analyses of variance (ANOVA) for continuous variables and Pearson's $X^2$ for categorical variables, with Bonferroni pairwise post-hoc comparisons used where relevant. Four participants were missing data for less than half of the taste ratings: 1 participant prescribed methadone was missing the 'liking' rating for the 0.40 mol/l sample; 2 controls were missing the 'liking' rating for the 0.48 mol/l sample, and 1 participant prescribed methadone was missing 'liking', 'desire', and 'saltiness' ratings for the 0.16, 0.24, and 0.40 mol/l samples. Thus, the overall rate of missing data was 0.4% for 'desire' and 'saltiness' ratings and 0.9% for 'liking' ratings. To allow inclusion of their non-missing data in repeated-measures analyses, single linear regression imputation of missing values, based on the non-missing values, was used to impute these participants' missing ratings.

Repeated measures ANOVA (RM-ANOVA) were used to examine whether group moderated the effect of salt concentration on 'liking' and 'desire' ratings. Since *a priori* hypotheses predicted that the effect of concentration on these ratings would follow a quadratic function, which would be shifted to the right in participants with current opioid dependence, and since a rightward shift in a quadratic function reflects a change in the linear component of that function, hypothesis-driven analyses focused on interactions involving the linear term in tests of within-subjects contrasts. Since salt perception and preferences may vary with age (Barragan et al., 2018), and the control group was significantly younger than the opioid-dependent groups, age was controlled for by including it as a covariate in all analyses. RM-ANOVA effect sizes were interpreted using $\eta_p^2$, following Cohen (1988) suggestion that values of .01, .06, and .14 indicate small, medium, and large effects, respectively.

### 2.2. Mouse study

#### 2.2.1. Animals

All animal experimentation received approval from the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health and was performed in accordance with the Prevention of Cruelty to Animals Act, 1986. Forty male 7-week old C57BL/6 J mice were acquired from the Australian Research Centre (Canning Vale, WA, Australia). Mice were housed in a temperature-controlled room (˜21 °C) on a 12 h light-dark cycle (lights on at 0700–1900) and were individually housed in open top cages with standard mouse diet (0.2% w/w sodium content; Barastoc Rat and Mouse, Ridley AgriProducts, Melbourne, Australia) and water available *ad libitum*.

#### 2.2.2. Measurement of NaCl intake

After 1 week acclimatization, standard mouse diet was permanently replaced with low sodium diet (0.02% w/w sodium content, SF02-020, Specialty Feeds Pty Ltd). In addition to the water sipper, mice were provided with *ad libitum* access to two additional sippers that contained 0.1 M and 0.2 M NaCl solutions for the duration of the experiment. Intake from all three sippers was recorded at ˜0900 daily, and the locations of these NaCl sippers relative to the cage lid were alternated twice a week to control for position preferences.

#### 2.2.3. Morphine dependence and withdrawal

After 2 weeks acclimatisation to the low sodium diet and 0.1 and 0.2 M NaCl sippers, chronic morphine or vehicle treatment began. Mice were randomly assigned into one of four groups: 1. chronic vehicle + final acute vehicle (n = 10); 2. chronic morphine + final acute vehicle (n = 10); 3. chronic vehicle + final acute naltrexone (n = 10), and; 4. chronic morphine + final acute naltrexone (n = 9; data from a 10$^{th}$ mouse was excluded due to a leaking sodium sipper). Mice in the chronic vehicle groups received twice-daily (˜0900 and ˜1500) intraperitoneal (IP) sterile $dH_20$ injections (0.1 ml / 10 g body weight, alternating between 4 different abdominal quadrants to avoid local inflammation) for 7 days. Mice in the chronic morphine groups received equivalent twice-daily injections, which contained escalating doses of morphine hydrochloride (Glaxo Australia Pty Ltd; 20,40,60,80, 100 mg/kg, dissolved in $dH_20$) for 5 days, followed by two further doses of 100 mg/kg on days 6 and 7. Doses for these experiments were similar to those used in a previously published study (Goeldner et al., 2011) and are sufficient to induce morphine dependence and subsequent withdrawal (which was also confirmed here – data not shown). Two hours after the final scheduled morphine or vehicle injection on day 7, mice in the final acute naltrexone groups were given an injection of naltrexone hydrochloride (Tocris Bioscience; IP, 1 mg/kg, 0.1 ml /10 g body weight) to precipitate morphine withdrawal, while mice in the final acute vehicle groups were given a corresponding injection of $dH_2O$ vehicle.

#### 2.2.4. Statistics

The amount of 0.1 M and 0.2 M NaCl that mice consumed was recorded daily and averaged to form 3-day time bins to minimise daily variations in data. The 'basal' time bin corresponds to the 3 days immediately before chronic injections commenced. The 'chronic treatment period days 1-3′ corresponds to the period immediately following the second injection on day 1 to the second injection on day 4. The 'chronic treatment period days 4-6′ corresponds to the period immediately following the second injection of day 4 until after the final acute injection on day 7. The subsequent two 3-day time bins are designated 'post-treatment period days 1-3' and 'post-treatment period days 4-6'. All statistical analyses were conducted using GraphPad Prism 7.03 software. Data within each time bin were assessed via 2-way ANOVA and expressed as the mean ± the standard error of the mean (SEM).

**Table 1**
Demographic and clinical characteristics of human participant groups.

| Variable | Controls (n = 29) | OST (n = 37) | Abstinent (n = 18) | $X^2$ | F | p |
|---|---|---|---|---|---|---|
| Age mean (SD) | 28.5 (9.7)[a,p] | 39.6 (8.2)[c] | 37.2 (6.6)[c] | | 14.584 | < .001 |
| Male gender [% (n)] | 65.5 (19) | 67.6 (25) | 77.8 (14) | 0.849 | | .654 |
| Current daily tobacco smoker [% (n)] | 86.2 (25) | 91.9 (34) | 83.3 (15) | 0.996 | | .608 |
| Cigarettes per day [mean (SD)] | 9.5 (6.3) | 11.6 (8.7) | 7.2 (6.1) | | 2.171 | .121 |
| Any alcohol use (past 30 days) [% (n)] | 72.4 (21) | 56.8 (21) | n.a.[***] | 1.722 | | .189 |
| Days alcohol use (past 30 days) [mean (SD)] | 3.8 (4.9) | 4.0 (7.4) | n.a.[***] | | 0.013 | .911 |
| Number of standard drinks[*] of alcohol (past 30 days) [mean (SD)] | 17.8 (25.8) | 12.9 (21.0) | n.a.[***] | | 0.735 | .395 |
| SDS score [mean (SD)] | n.a. | 9.9 (3.2) | 10.7 (4.8) | | 0.439 | .511 |
| SOWS score[**] [mean (SD)] | n.a. | 0.5 (0.4)[a] | 0.3 (0.3)[p] | | 4.601 | .037 |
| Past alcohol dependence [% (n)] | n.a. | 29.7 (11) | 55.6 (10) | 3.422 | | .064 |
| Past cocaine dependence [% (n)] | n.a. | 8.1 (3) | 5.6 (1) | 0.117 | | .732 |
| Past cannabis dependence [% (n)] | n.a. | 18.9 (7)[a] | 44.4 (8)[p] | 3.978 | | .046 |
| Past sedative dependence [% (n)] | n.a. | 16.2 (6) | 33.3 (6) | 2.080 | | .149 |
| Past amphetamine dependence [% (n)] | n.a. | 24.3 (9)[a] | 66.7 (12)[p] | 9.198 | | .002 |

For categorical variables, statistics shown are: % (n). OST: opioid substitution treatment; SD: standard deviation; SDS: Severity of dependence scale; SOWS: Short Opiate Withdrawal Scale.

* A standard drink in Australia is defined as 10 g of pure ethanol.

** SOWS data was missing for two participants in the OST group.

*** Abstinent participants were residents of abstinence-based rehabilitation or supported accommodation services and alcohol use data for this group is therefore not shown because participants in this group generally had no opportunity to consume alcohol. However one participant in this group reported past-month alcohol consumption (1 day only, 5 standard drinks).

[a] Significant pairwise difference from abstinent group.

[c] Significant pairwise difference from controls.

[p] Significant pairwise difference from OST group.

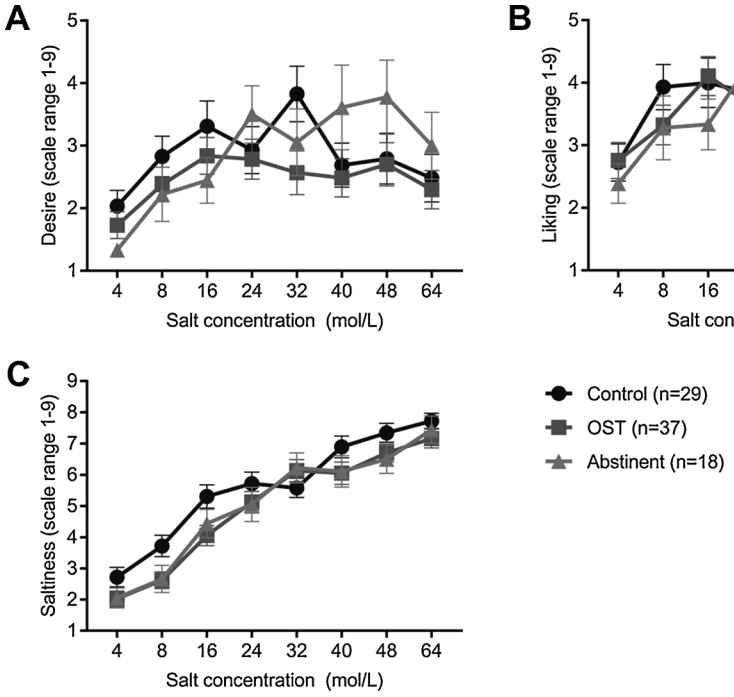


**Fig. 1.** Measurements of salt 'desire', salt 'liking', and perceptions of 'saltiness' in control, OST and abstinent individuals. Human volunteers tasted eight liquid broths that contained different salt concentrations (randomized order, single-blind design). After tasting each one, participants answered the following questions using a 9-point scale (high numbers indicate positive responses): **(A)** "How strong is your *desire* to consume more of this broth?; **(B)** "How much did you *like* this broth?", and; **(C)** "how *salty* did this broth taste?". Participants on OST (opioid substitution therapy, with methadone or buprenorphine) had received their last previous dose of opioid a mean of 10.4 h prior to testing. Abstinent volunteers were previously opioid dependent and had abstained from opioids for a mean of 9 months (minimum 68 days). Control participants had no prior history of opioid dependence. Data are presented as mean ± SEM.

## 3. Results

### 3.1. Human study

#### 3.1.1. Demographic and clinical characteristics of participant groups

Table 1 shows demographic and selected substance use data. As is typical for Australian opioid-dependent samples, approximately 70% were male, and the majority were tobacco smokers. We were successful at closely matching the control group to the opioid-dependent groups on these variables, though the control group was significantly younger. Twenty-seven (73.0%) of the OST group had used illicit opioids in the past month (range 1–30 days), which was almost exclusively heroin use (used by all 27 of these participants), though three of these participants also used illicit oxycodone and/or illicitly used more than their prescribed dose of OST. Participants in the OST group also reported illicit/non-prescribed past-month use of other non-opioid substances, including benzodiazepines (n = 7, range 1–4 days use), amphetamines (n = 7, range 1–5 days use), cannabis (n = 12, range 1–15 days), illicitly-obtained antipsychotics (n = 2, range 1–9 days use), cocaine (n = 1, 1 day use), and MDMA (n = 1, 1 day use). Regarding OST, the most recent dose taken by those prescribed methadone ranged from 5 to 155 mg (mean = 57.7 mg; median = 50 mg), while for those prescribed buprenorphine, most recent doses ranged from 0.75 to 20 mg (mean = 10.4 mg; median = 10 mg). Self-reported time since last OST dose at the start of the questionnaire session ranged from 0.5 to 72 hours (mean = 11.9 h; median = 6 h); however, since three

participants had used heroin more recently than their most recent OST dose, self-reported time since most recent opioid (whether prescribed or illicit) was 0.5–25.5 hours (mean = 10.4 h; median = 6 h).

Abstinent participants had abstained from opioids (prescribed or illicit) for 68-1,002 days (mean = 280 days; median = 183 days). Given that participants in the OST group were prescribed long-acting opioid agonists, and the abstinent participants had not used opioids for at least 2 months, SOWS scores were generally low in both groups, indicating absent or mild withdrawal symptoms. Despite this, SOWS scores were significantly higher in the OST group relative to abstinent individuals. This may reflect the fact that, at the time of participation, some OST participants had not had opioids since the previous day.

#### 3.1.2. Analysis of salt concentration versus 'Desire' ratings

Salt concentration had a moderate, significant linear effect on desire ratings ($F(1,80) = 4.424$, $p = .039$, $\eta_p^2 = .052$; see Fig. 1A), but, contrary to expectations, the quadratic main effect was non-significant ($F(1,80) = 1.449$, $p = .232$, $\eta_p^2 = .018$). Group significantly moderated this linear effect ($F(2,80) = 3.877$, $p = .025$, $\eta_p^2 = .088$). Examination of Fig. 1A suggests that this interaction was due to a more pronounced linear effect of salt concentration on 'desire' in the abstinent group than in other groups, whereby abstinent participants showed a clear tendency for 'desire' ratings to increase with increasing salt concentration. A consequence of this interaction was that at the highest salt concentrations (0.4, 0.48 and 0.64 mol/l), abstinent patients recorded noticeably higher 'desire' ratings compared to the other two groups (see Fig. 1A).

Post-hoc analyses confirmed this: in the control and OST groups, the linear effect of concentration was non-significant (controls: $F(1,27) = 1.672$, $p = .207$, $\eta_p^2 = .058$; OST: $F(1,35) = .026$, $p = .872$, $\eta_p^2 = .001$). However, in the abstinent group, there was a large linear effect ($F(1,16) = 6.895$, $p = .018$, $\eta_p^2 = .301$). To further confirm whether it was the abstinent group that differed from the other two groups, and to test whether the OST group showed any difference from controls, three further pairwise post-hoc RM-ANOVAs were conducted comparing pairs of groups. RM-ANOVAs in which the abstinent group was compared only to the control group ($F(1,44) = 8.725$, $p = .005$, $\eta_p^2 = .165$) or only to the OST group ($F(1,52) = 4.088$, $p = .048$, $\eta_p^2 = .073$) both showed significant interactions with moderate-large effect size between group and the linear effect of salt concentration on 'desire', but no significant interaction was present when including only the OST and control groups ($F(1,63) = 0.435$, $p = .512$, $\eta_p^2 = .007$).

#### 3.1.3. Analysis of salt concentration versus 'liking' ratings

Analysis of 'liking' ratings (Fig. 1B) found no significant linear ($F(1,80) = 2.779$, $p = .099$, $\eta_p^2 = .034$) or quadratic ($F(1,80) = 0.506$, $p = .479$, $\eta_p^2 = .006$) main effects of salt concentration. However, there was a significant interaction between group and the linear effect with moderate effect size ($F(2,80) = 3.373$, $p = .039$, $\eta_p^2 = .078$). The inherent psychological and biological relationship between 'liking' and 'desire' is evident when visually comparing both sets of data, where again abstinent participants tended to give higher ratings of the highest salt concentrations (0.4, 0.48 and 0.64 mol/l) compared to the other two groups.

Post-hoc tests confirmed that the linear effect of salt concentration on liking was non-significant in control ($F(1,27) = 1.337$, $p = .258$, $\eta_p^2 = .047$) and OST ($F(1,35) = 0.500$, $p = .484$, $\eta_p^2 = .014$) groups but that there was a very large, significant effect in the abstinent group ($F(1,16) = 5.833$, $p = .028$, $\eta_p^2 = .267$). Pairwise comparisons between groups suggested that the interaction between group and concentration was specifically due to a difference between abstinent and control groups. The interaction involving the linear effect was large and significant when abstinent participants were compared to controls ($F(1,44) = 8.507$, $p = .006$, $\eta_p^2 = .162$) but was non-significant when the abstinent and OST groups were compared ($F(1,52) = 2.458$, $p = .123$, $\eta_p^2 = .045$) or when the OST and control groups were compared ($F(1,63) = 1.031$, $p = .314$, $\eta_p^2 = .016$).

#### 3.1.4. Analysis of salt concentration versus perceived 'saltiness' ratings

Analysis of perceived 'saltiness' ratings, shown in Fig. 1C, suggested no reason to believe that the effect of group on 'desire' ratings was due to changes in perceptual sensitivity. As expected, the linear main effect of concentration on 'saltiness' ratings was very large ($F(1,80) = 31.324$, $p < .0001$, $\eta_p^2 = .281$), and there was also a large quadratic main effect ($F(1,80) = 30.365$, $p < .001$, $\eta_p^2 = .165$). However, these effects did not significantly interact with group (linear: $F(2,80) = 0.437$, $p = .647$, $\eta_p^2 = .011$; quadratic: $F(2,80) = 2.423$, $p = .095$, $\eta_p^2 = .057$), nor was there a significant main effect of group on 'saltiness' ratings ($F(2,80) = 1.348$, $p = .266$, $\eta_p^2 = .033$). Thus, all groups displayed similar overall mean ratings of 'saltiness' and showed similar ability to distinguish between low and high salt concentrations.

### 3.2. Mouse study

#### 3.2.1. Increased NaCl consumption in mice during and following chronic morphine treatment

Both spontaneous and naltrexone-precipitated morphine withdrawal elicited strong withdrawal behaviours (data not shown), indicating that morphine dependence was successfully achieved in agreement with other studies that have used a similar chronic dose schedule (Goeldner et al., 2011). Across all time-points, chronic morphine treatment or acute final naltrexone injection did not alter the preference that mice displayed for 0.1 M versus 0.2 M NaCl solutions compared to controls (Fig. 2A; main effects of chronic treatment and final acute injection, within each time point, all $p > 0.05$). Importantly, however, chronic morphine treatment significantly increased the total amount of NaCl consumed (expressed as mmol) relative to chronic vehicle controls during the last 3 days of morphine treatment, which persisted into the first 3 days of morphine withdrawal (Fig. 2B; main effect of chronic treatment, within 2nd morphine and 1st

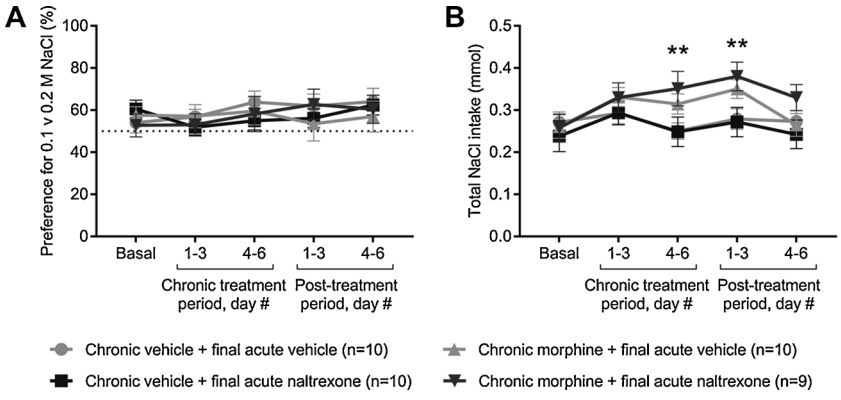


**Fig. 2.** Salt preference and intake in mice during and after chronic morphine treatment. Mice were maintained on low sodium food and provided free access to 0.1 M and 0.2 M NaCl sippers (in addition to normal drinking water) throughout the study. The percentage preference for 0.1 M versus 0.2 M NaCl **(A)**, and the total amount of NaCl consumed **(B)** was recorded daily and represented in 3-day time bins to minimize day-to-day variability. During the 'chronic treatment period', mice were injected twice daily for six days with either an ascending dose of morphine, or vehicle. At the conclusion of this chronic treatment period, mice were given a final acute injection of either naltrexone or vehicle. Data expressed as mean ± SEM. Two-way ANOVA, post-tests between chronic treatments, within time-bin, $^{**}p < 0.01$.

withdrawal time points, both $p < 0.01$). The magnitude of this increase was approximately similar in chronic morphine treated mice, irrespective of whether they received a final injection of naltrexone or vehicle (main effects of final injection, and chronic treatment x final injection interactions, within all time points, all $p > 0.05$).

## 4. Discussion

Due to the well-established involvement of endogenous opioid signalling in the neuronal control of salt consumption, this study sought to determine whether psychological salt 'desire', 'liking' or perceptions of 'saltiness' were altered in currently opioid dependent or abstinent human patients. Concurrently, preference for 0.1 M versus 0.2 M NaCl, and overall amounts of salt consumption, were assessed in mice during and after chronic opioid treatment. The purpose of this animal study was to impose consistent environmental conditions across all subjects in order to investigate the influence of chronic morphine treatment on salt preference and consumption, something difficult to achieve in a human cohort. Despite the nuances between study designs and measurements taken in humans versus mice, similarities in findings were nonetheless observed, whereby opioid dependence increased direct or psychological aspects of salt consumption – although this was only expressed in formerly-dependent, abstinent participants in the human study.

In currently abstinent but previously opioid dependent humans, individuals displayed a significant linear effect of salt concentration on 'desire' and 'liking' ratings, with ratings tending to increase with increasing salt concentration, while these effects were non-significant in the control and OST groups. In other words, although we did not observe the expected significant quadratic main effects (i.e., inverted U-shaped patterns of 'desire' and 'liking' ratings over increasing salt concentrations), the abstinent group's preferences were shifted towards the higher salt concentrations relative to control and OST groups. A somewhat analogous finding was that mice consumed significantly more salt after morphine treatment had ended, although they also consumed more salt *during* the latter period of chronic morphine treatment, while humans on chronic opioid treatment (the OST group) did not show an analogous shift in 'desire' or 'liking' ratings.

### 4.1. Does opioid dependence cause cross-sensitization to salt in mice and humans?

The similarities in the findings in both mice and humans after opioid withdrawal suggest that a common mechanistic process might underlie these observations. In the human study, the mean duration of abstinence in the abstinent group was 9 months, and the minimum duration was 68 days, by which time opioid receptor re-sensitization and many key neuronal functions could be expected to have recovered somewhat (Ieong and Yuan, 2017). However, several long-term neurological changes have been suggested and/or identified, which may be responsible for persistent changes such as an increased risk of relapse that might last for decades (Hyman and Malenka, 2001; Nestler, 2001). It is therefore possible that in abstinent individuals, hypersensitivity to drug cues and/or drug prime (which normally drives 'desire' for drug and subsequent drug seeking/ consumption behaviour) has cross-sensitized to salt, whereby tasting of high salt solutions (in the 0.4-0.64 mol/l range) has driven increased incentive salience, influencing 'desire' and 'liking' ratings. At a neurobiological level, this may be due to consumption of high salt solutions causing a release of endogenous opioids in key limbic structures such as the central amygdala (Smith et al., 2016) that could mimic an exogenous opioid prime, which abstinent individuals are abnormally sensitive to (Cocores and Gold, 2009). Cross-sensitization has been well studied in non-human animals, and rodents that are (or were) previously dependent on one drug can display increased locomotor activity and other heightened responses when administered a different drug (Avena et al., 2008; Kalivas et al., 1992). Cross-sensitization involving natural rewards such as sweet tastants has also been observed. For example, behavioural cross-sensitization can occur between sucrose and psychostimulants (Avena et al., 2008; Gosnell, 2005) and between opioids and food intake (Bakshi and Kelley, 1994), while a prior history of salt depletion can cross-sensitize to sugar intake (Santos et al., 2016). Further highlighting the possibility of food-drug cross-sensitization in salt consumption neuronal circuits, previous bouts of salt depletion in rats increases the locomotor effects of several drugs including morphine (Acerbo and Johnson, 2011; Clark and Bernstein, 2004; Na et al., 2009). Although increased salt consumption renormalised to control levels from 4 days of abstinence onwards in mice, this does not necessarily indicate different neuronal mechanisms in mice versus humans. Instead, this may merely reflect the vastly more extensive prior drug exposure experienced by the human participants, which may have given rise to more persistent salt cross-sensitization.

Cross-sensitization from opioids to salt may also help explain two other observations, which initially seem disparate. Firstly, data from OST patients was similar to controls. Although this contradicted our initial hypothesis, this finding is consistent with a pilot study in which OST patients and controls displayed equivalent 'desire' and 'eagerness' to consume highly salted potato chips (Nolan and Scagnelli, 2007). Secondly, increased salt consumption in mice emerged during the last 3 days of chronic morphine treatment. Although this time period in mice superficially seems similar to OST patients (as both species were currently receiving opioid drug), a shift from control levels in mice, but not humans, may be due to mice experiencing opioid withdrawal during the continuous 24 h recording period, while most human OST patients were not experiencing significant levels of withdrawal at the time they were engaged in the study. Rather than a continual infusion, morphine was administered in mice twice daily at 0900 and 1500, as this strategy is more ethologically relevant and is sufficient to induce opioid dependence (Goeldner et al., 2011), which we also confirmed. However, the half-life of morphine in male mice following IP administration is less than 30 min. (Diaz et al., 2007). As a consequence, mice in the present study would have been oscillating between phases of acute morphine intoxication during the hours following administration and opioid withdrawal during the hours before administration. This withdrawal was qualitatively noticed from day 4 of morphine administration onwards. Furthermore, a significant increase in withdrawal behaviours (including piloerection, wet dog shakes and tremors) was quantified the day after the last morphine injection in mice that received a final injection of vehicle (or naltrexone; data not shown). During an oscillating pattern of drug intoxication and withdrawal, the incentive salience for drug in response to a cue or prime is greatest during the withdrawal phase (Koob and Le Moal, 2005). It is therefore possible that cross-sensitization-induced increases in salt intake occurred during the periods that mice were experiencing withdrawal, which was sufficient to result in an overall statistically significant difference relative to controls.

In contrast, human participants were stabilised on OST pharmacotherapies (i.e., methadone and buprenorphine), and most participants (92%) received their most recent OST dose within the past 24 h. Indeed, withdrawal (SOWS) scores were low across the sample (see Table 1) with only 3 participants reporting the need split their dose to manage withdrawal symptoms. The relative 'saturation' of opioid receptors with OST may have therefore attenuated any priming effects that may have otherwise been elicited by salt. To investigate this theory further, it would be interesting to explore whether salt 'desire' or 'liking' fluctuates according to withdrawal state in opioid-dependent humans. Additionally, 24 -h measurements of salt consumption in mice (using automated circadian metabolic cages, for example) would also be worthwhile, to clarify whether increased salt consumption during chronic opioid treatment occurs during periods of intoxication or withdrawal.

### 4.2. Other potential mechanisms

It is also possible that the observed differences in abstinent human patients do not directly involve opioid signalling at all and instead relate to broader behavioural, dietary, and/or other factors that may have confounded analyses. The wide range of other substances used and histories of dependence exhibited by the OST group, which was itself a combination of methadone and buprenorphine treatments at various doses, and the higher rate of past dependence on non-opioid substances in the abstinent group (see Table 1), may have influenced taste preferences. However, insufficient numbers of patients precluded stratification by sub-groups.

In relation to the mouse study in isolation, an additional possible explanation is that during withdrawal mice increased consumption of the rewarding salt stimulus in an effort to combat the negative affective state associated with withdrawal (Parylak et al., 2011). In humans this is often described as 'comfort eating' and relies on the observation that food and drugs share overlapping reward systems. Therefore, the consumption of a food that elicits opioid signalling might be able to 'substitute' or 'compensate' for absence of an exogenous opioid (Avena et al., 2008; Gosnell et al., 1995). Cocores and Gold (2009) make a direct link with salt in their "salted food addiction hypothesis" and found that human patients consume more highly salted foods during opioid withdrawal.

Given the established ability of acute pharmacological and optogenetic manipulations (Matsuda et al., 2016) to impact salt intake, it is important to consider the role that acute bouts of morphine intoxication might have played in explaining the mouse data. Acute opiate treatment increases the consumption of previously aversive high salt concentrations in salt depleted mice (Na et al., 2012). However, this phenomenon is unlikely to explain the present findings. Firstly, mice in the present study were not salt depleted and had constant access to salt. Although it would be informative to examine the effects of current and prior opioid dependence and withdrawal under both salt replete and salt depleted states, additional manipulation of homeostatic salt status was outside the scope of the present study. Secondly, 0.1 M and 0.2 M NaCl are not considered aversive, and approximately equal preference for these two salt sources was observed throughout the study. Thirdly, an increase in salt consumption was not present during the first three days of escalating morphine treatment.

Finally, it should be acknowledged that our ability to detect effects in the human study may have been limited by low palatability of the broth used in the taste test. While we selected a broth that we hoped would be palatable to participants at moderate salt concentrations, 'liking' ratings suggested otherwise, with all groups' mean ratings of all concentrations falling below the scale mid-point (i.e., below 5 on a scale of 1–9; see Fig. 1B). Taha (2010) notes that modulation of opioid neurotransmission appears to selectively modify consumption of highly-palatable, or relatively more-preferred, foods. Thus, we may have found stronger effects, particularly in the OST group, if we had used a more palatable broth (or other food), or if there was a larger contrast between the hedonic value of the least- and most-preferred samples.

### 4.3. Conclusion

These studies revealed that opioid dependence increased salt consumption or related psychological parameters in mice and humans. Firstly, previously opioid dependent human participants who had been abstinent for a mean of 9 months displayed a shift in preference towards more concentrated salt solutions. Secondly, mice exhibited increased salt consumption during the last three days of chronic morphine treatment, which persisted for three days of abstinence. These observations may represent a common neuronal mechanism; however, this remains to be verified. Taken together, these studies highlight the need for future research to better characterise the influence of opioid dependence on salt consumption behaviour. Given that rates of opioid dependence are drastically rising across the USA and the globe (Peacock et al., 2018), this research may help inform better strategies to improve the diet and overall wellbeing of this population.

## Contributors

Smith CM: Design and execution of mouse study, data analysis, and compilation of article. Garfield JBB: Design and execution of human study, data analysis, and compilation of article. Attawar A: Execution of mouse study, data analysis. Lubman DI: Design and overview of human study, data analysis, and compilation of article. Lawrence AL: Design and overview of mouse and human studies, data analysis, and compilation of article. All authors have contributed to and approved the final article.

## Role of funding source

This research was supported by the National Health and Medical Research Council (NHMRC Project grant 1079891). AJL is an NHMRC Principal Research Fellow (1116930). We also acknowledge the Victorian Government's state infrastructure program.

## Declaration of Competing Interest

No conflict declared

## Acknowledgments

We would like to thank the pharmacies that distributed our recruitment leaflets to their patients on OST, as well as the rehabilitation and supported accommodation services (Bridge Program, Odyssey House, Oxford Houses, Quin House, Raymond Hader Clinic, Reconstructing Life After Dependency (RLAD), Self Help Addiction Resource Centre (SHARC), and Windana Drug and Alcohol Recovery), who generously allowed us access to their residents and facilities. We would also like to thank Cassandra Jovic for her assistance developing online advertising used to help recruit control participants.

## References

Acerbo, M.J., Johnson, A.K., 2011. Behavioral cross-sensitization between DOCA-induced sodium appetite and cocaine-induced locomotor behavior. Pharmacol. Biochem. Behav. 98, 440–448.
American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Association, Washington, DC Text Revision.
Avena, N.M., Rada, P., Hoebel, B.G., 2008. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci. Biobehav. Rev. 32, 20–39.
Bakshi, V.P., Kelley, A.E., 1994. Sensitization and conditioning of feeding following multiple morphine microinjections into the nucleus accumbens. Brain Res. 648, 342–346.
Barragan, R., Coltell, O., Portoles, O., Asensio, E.M., Sorli, J.V., Ortega-Azorin, C., Gonzalez, J.I., Saiz, C., Fernandez-Carrion, R., Ordovas, J.M., Corella, D., 2018. Bitter, sweet, salty, sour and umami taste perception decreases with age: sex-specific analysis, modulation by genetic variants and taste-preference associations in 18 to 80 year-old subjects. Nutrients 10, e1539.
Clark, J.J., Bernstein, I.L., 2004. Reciprocal cross-sensitization between amphetamine and salt appetite. Pharmacol. Biochem. Behav. 78, 691–698.
Cocores, J.A., Gold, M.S., 2009. The salted food addiction hypothesis may explain overeating and the obesity epidemic. Med. Hypotheses 73, 892–899.
Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2nd edition. Lawrence Erlbaum Associates, Hillsdale, NJ.
De Oliveira, L.B., De Luca Jr., L.A., Menani, J.V., 2008. Opioid activation in the lateral parabrachial nucleus induces hypertonic sodium intake. Neuroscience 155, 350–358.
Denton, D.A., 1983. The Hunger for Salt. An Anthropological, Physiological and Medical Analysis. Springer, Berlin.
Diaz, S.L., Hermida, M.P., Joannas, L.D., Olivera, M., Ridolfi, A., Villaamil, E.C., Balerio, G.N., 2007. Pharmacokinetic aspects of naloxone-precipitated morphine withdrawal in male and female prepubertal mice. Biopharm. Drug Dispos. 28, 283–289.
Drewnowski, A., Henderson, S.A., Driscoll, A., Rolls, B.J., 1996. Salt taste perceptions and preferences are unrelated to sodium consumption in healthy older adults. J. Am. Diet. Assoc. 96, 471–474.

Eikemo, M., Loseth, G.E., Johnstone, T., Gjerstad, J., Willoch, F., Leknes, S., 2016. Sweet taste pleasantness is modulated by morphine and naltrexone. Psychopharmacology 233, 3711–3723.
First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P). Biometrics Research, New York State Psychiatric Institute, New York.
Goeldner, C., Lutz, P.E., Darcq, E., Halter, T., Clesse, D., Ouagazzal, A.M., Kieffer, B.L., 2011. Impaired emotional-like behavior and serotonergic function during protracted abstinence from chronic morphine. Biol. Psychiatry 69, 236–244.
Gosnell, B.A., 2005. Sucrose intake enhances behavioral sensitization produced by cocaine. Brain Res. 1031, 194–201.
Gosnell, B.A., Lane, K.E., Bell, S.M., Krahn, D.D., 1995. Intravenous morphine self-administration by rats with low versus high saccharin preferences. Psychopharmacology 117, 248–252.
Gossop, M., 1990. The development of a short opiate withdrawal scale (SOWS). Addict. Behav. 15, 487–490.
Gossop, M., Darke, S., Griffiths, P., Hando, J., Powis, B., Hall, W., Strang, J., 1995. The Severity of Dependence Scale (SDS): psychometric properties of the SDS in English and Australian samples of heroin, cocaine and amphetamine users. Addiction 90, 607–614.
Hser, Y.I., Gelberg, L., Hoffman, V., Grella, C.E., McCarthy, W., Anglin, M.D., 2004. Health conditions among aging narcotics addicts: medical examination results. J. Behav. Med. 27, 607–622.
Hubbell, C.L., McCutcheon, N.B., 1993. Opioidergic manipulations affect intake of 3% NaCl in sodium-deficient rats. Pharmacol. Biochem. Behav. 46, 473–476.
Hyman, S.E., Malenka, R.C., 2001. Addiction and the brain: the neurobiology of compulsion and its persistence. Nat. Rev. Neurosci. 2, 695–703.
Ieong, H.F., Yuan, Z., 2017. Resting-state neuroimaging and neuropsychological findings in opioid use disorder during abstinence: a review. Front. Hum. Neurosci. 11, 169.
Kalivas, P.W., Striplin, C.D., Steketee, J.D., Klitenick, M.A., Duffy, P., 1992. Cellular mechanisms of behavioral sensitization to drugs of abuse. Ann. N. Y. Acad. Sci. 654, 128–135.
Kelley, A.E., Bakshi, V.P., Haber, S.N., Steininger, T.L., Will, M.J., Zhang, M., 2002. Opioid modulation of taste hedonics within the ventral striatum. Physiol. Behav. 76, 365–377.
Koob, G.F., Le Moal, M., 2005. Plasticity of reward neurocircuitry and the' dark side' of drug addiction. Nat. Neurosci. 8, 1442–1444.
Koob, G.F., Volkow, N.D., 2016. Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 3, 760–773.
Lucas, L.R., Grillo, C.A., McEwen, B.S., 2003. Involvement of mesolimbic structures in short-term sodium depletion: in situ hybridization and ligand-binding analyses. Neuroendocrinology 77, 406–415.
Lucas, L.R., Grillo, C.A., McEwen, B.S., 2007. Salt appetite in sodium-depleted or sodium-replete conditions: possible role of opioid receptors. Neuroendocrinology 85, 139–147.
Matsuda, T., Hiyama, T.Y., Niimura, F., Matsusaka, T., Fukamizu, A., Kobayashi, K., Noda, M., 2016. Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. Nat. Neurosci. 20, 230–241.
Morabia, A., Fabre, J., Chee, E., Zeger, S., Orsat, E., Robert, A., 1989. Diet and opiate addiction: a quantitative assessment of the diet of non-institutionalized opiate addicts. Br. J. Addict. 84, 173–180.
Morris, M.J., Na, E.S., Johnson, A.K., 2008. Salt craving: the psychobiology of pathogenic sodium intake. Physiol. Behav. 94, 709–721.
Na, E.S., Morris, M.J., Johnson, A.K., 2009. Behavioral cross-sensitization between morphine-induced locomotion and sodium depletion-induced salt appetite. Pharmacol. Biochem. Behav. 93, 368–374.
Na, E.S., Morris, M.J., Johnson, A.K., 2012. Opioid mechanisms that mediate the palatability of and appetite for salt in sodium replete and deficient states. Physiol. Behav. 106, 164–170.
Nestler, E.J., 2001. Molecular basis of long-term plasticity underlying addiction. Nat. Rev. Neurosci. 2, 119–128.
Nogueiras, R., Romero-Pico, A., Vazquez, M.J., Novelle, M.G., Lopez, M., Dieguez, C., 2012. The opioid system and food intake: homeostatic and hedonic mechanisms. Obes. Facts 5, 196–207.
Nolan, L.J., Scagnelli, L.M., 2007. Preference for sweet foods and higher body mass index in patients being treated in long-term methadone maintenance. Subst. Use Misuse 42, 1555–1566.
Parylak, S.L., Koob, G.F., Zorrilla, E.P., 2011. The dark side of food addiction. Physiol. Behav. 104, 149–156.
Pavan, C.G., Roncari, C.F., Barbosa, S.P., De Paula, P.M., Colombari, D.S., De Luca Jr., L.A., Colombari, E., Menani, J.V., 2015. Activation of mu opioid receptors in the LPBN facilitates sodium intake in rats. Behav. Brain Res. 288, 20–25.
Peacock, A., Leung, J., Larney, S., Colledge, S., Hickman, M., Rehm, J., Giovino, G.A., West, R., Hall, W., Griffiths, P., Ali, R., Gowing, L., Marsden, J., Ferrari, A.J., Grebely, J., Farrell, M., Degenhardt, L., 2018. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. Addiction 113, 1905–1926.
Peles, E., Schreiber, S., Sason, A., Adelson, M., 2016. Risk factors for weight gain during methadone maintenance treatment. Subst. Abuse 37, 613–618.
Sadeghian, S., Darvish, S., Davoodi, G., Salarifar, M., Mahmoodian, M., Fallah, N., Karimi, A.A., 2007. The association of opium with coronary artery disease. Eur. J. Cardiovasc. Prev. Rehabil. 14, 715–717.
Santos, B.M., de Andrade, C.A.F., Menani, J.V., De Luca Jr., L.A., 2016. Short-term cross-sensitizion of need-free sugar intake by combining sodium depletion and hypertonic NaCl intake. Appetite 107, 79–85.
Smith, C.M., Lawrence, A.J., 2018. Salt appetite, and the influence of opioids. Neurochem. Res. 43, 3–9.
Smith, C.M., Walker, L.L., Leeboonngam, T., McKinley, M.J., Denton, D.A., Lawrence, A.J., 2016. Endogenous central amygdala mu-opioid receptor signaling promotes sodium appetite in mice. Proc. Natl. Acad. Sci. U. S. A. 113, 13893–13898.
Sobell, L., Sobell, M., 1996. Timeline Followback User's Guide: a Calendar Method for Assessing Alcohol and Drug Use. Addiction Research Foundation, Toronto.
Taha, S.A., 2010. Preference or fat? Revisiting opioid effects on food intake. Physiol. Behav. 100, 429–437.
VicHealth, 2015. State of Salt: the Case for Salt Reduction in Victoria. vichealth, Melbourne.
Zador, D., Lyons Wall, P.M., Webster, I., 1996. High sugar intake in a group of women on methadone maintenance in south western Sydney. Australia. Addiction 91, 1053–1061.
Zhang, M., Kelley, A.E., 2002. Intake of saccharin, salt, and ethanol solutions is increased by infusion of a mu opioid agonist into the nucleus accumbens. Psychopharmacology 159, 415–423.