μ-Opioid Modulation of Reported Wanting of Palatable Food Images

A pharmacological fMRI study in healthy humans

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Abstract

The endogenous μ-opioid receptor (MOR) system in the brain is central to reward behaviors across species, and brain areas implicated in reward are dense with μ-opioid receptors. The MOR system has received the most interest through its involvement in pleasure mediation ('liking'), but there is much evidence to suggest a role for the MOR system in motivated 'wanting' as well. Nevertheless, we still know very little about the mechanisms of MOR modulation in reward motivation in healthy humans. Further, it is unclear to what extent the animal research on MOR modulation of reward-processing in the brain can be extended to humans, as very few studies have explored this relationship directly in the human brain. We examined the effects of a low dose (10mg) of per oral morphine (a μ-opioid agonist) on reported food wanting, and of applying a cognitive regulation task to downregulate this wanting, in healthy human participants. We also measured neural activity as approximated by functional magnetic resonance imaging. The study was designed to minimize the risk of potential confound effects of the drug manipulation. In a within-subject, counterbalanced, placebo-controlled, double-blind design, 63 participants (31 male, mean age 27 ±5) were tested in a morphine and placebo session on two separate days. In line with our expectations, morphine did not significantly affect subjective mood or state, respiration- or heart rate, or motor coordination. Morphine also did not appear to alter global BOLD, measured by a simple visual control task. The food wanting task elicited significant activation in reward related regions compared to baseline, and cognitive regulation produced the expected decrease in food wanting, together with increased activity in ventral prefrontal regions. Activation in extrastriate occipital regions was observed across tasks. Preliminary analyses confirmed our hypothesis that MOR agonism would increase food wanting, but did not confirm our hypothesis of associated activity increase in the striatum and medial prefrontal areas. Instead, increased activity in regulation-related regions may be required for successful downregulation of wanting after morphine treatment. In summary, we have now validated the paradigm and task design of this study. Thus, a complete analysis of the drug effects of interest can be conducted and the results interpreted to draw meaningful conclusions regarding the effects of MOR stimulation with morphine on BOLD signals relating to 'wanting' for palatable food images.

Table of Contents

1 Introduction

The concept of reward is fundamental for understanding how adaptive behavior develops and is maintained (O'Doherty et al., 2004). The mechanisms by which reward can shape behavior were described in detail by B.F. Skinner (e.g. 1938) in the framework of operant conditioning. Although Skinner himself was more preoccupied with the behavioral outcomes of reward (or positive reinforcement), later authors have attempted to establish the neural underpinnings of reward as well (e.g. Carver & White, 1994; Schultz, Dayan, & Montague, 1997).

Reward is a fundamental motivation for behavior in day to day life. The word can refer both to a rewarding object, like chocolate, and the feelings associated with seeking and consuming said chocolate. The latter will be main focus of this thesis. A distinction is usually made between primary and secondary rewards. Primary rewards, like food and sex, are desired in and of themselves, and have inherent value for the organism. Secondary rewards are only reward insofar as they are (ultimately) associated with the obtainment of a primary reward, and are not themselves essential for survival. Money is a good example of a secondary reward (Sescousse, Caldú, Segura, & Dreher, 2013).

1.1 Parsing reward

An influential theory of reward was originally proposed by Kent Berridge and colleagues over 20 years ago (T. E. Robinson & Berridge, 1993) and has been reiterated and refined many times since (Berridge & Kringelbach, 2008; Berridge & Robinson, 2003; Berridge, Robinson, & Aldridge, 2009; Kringelbach & Berridge, 2009). The theory defines reward in terms of three core psychological components: (1) ‘wanting’, related to the motivational aspect of acquiring a reward, (2) ‘liking’, denoting the pleasurable feeling related to consuming a reward, and (3) learning, the predictive associations and cognitions formed between cue and reward (Berridge & Robinson, 1998). The theory will be referred to as the LWL framework in this thesis.

Wanting and liking can refer to consciously experienced affective states, but denoted in single quotes, these terms refer to more implicit psychological processes that do not rely on conscious awareness (Berridge et al., 2009). ‘Wanting’ occurs before a reward is attained, and is related to anticipation and reactions to cues that signal reward, etc. Of course, if one wants

food and gets it, one must also have the motivation to eat until satiety, so ‘wanting’ also occurs during reward consumption. ‘Liking’ represents a precursor to the pleasurable feeling that often arise during and sometimes after the attainment and/or consumption of a reward. Learning, usually assumed to be a process of updating hedonic expectations of a stimulus based on experienced vs expected reward, occurs throughout this process (Kringelbach, Stein, & van Hartevelt, 2012).

In a typical reward process, wanting and liking will cooccur. However, these processes can be dissociated. Studies of the rat brain have identified distinct areas within the that when stimulated with particular pharmacological agents can produce behaviors indicative of either ‘wanting’ (defined as approach behavior, lever pressing, and consumption) or ‘liking’ (defined as orofacial responses like licking, assumed to signal pleasure in the animal), depending on the specific areas being stimulated (Mahler & Berridge, 2012; Peciña, 2008; Peciña & Berridge, 2005, 2013; K. S. Smith & Berridge, 2007; Zhang, Balmadrid, & Kelley, 2003, see section "Microinjections in in rats with MOR agonists.").

Another line of evidence for dissociation of wanting and liking comes from studies of reward processing in mental disorders such as depression, schizophrenia and drug addiction (Strauss, Waltz, & Gold, 2014; Volkow, Wang, & Baler, 2011). The symptom of *anhedonia* refers to the impaired capacity to experience pleasure, while *amotivation* describes the lack of motivation to obtain reward. Anhedonia and amotivation sometimes appear together, but not always (Gorwood, 2008; Rømer Thomsen, Whybrow, & Kringelbach, 2015). Further, in drug addiction and other motivational disorders, maladaptively high levels of ‘wanting’, usually referred to as craving, can develop, even as ‘liking’ for the drug appears to decrease (M. J. F. Robinson, Fischer, Ahuja, Lesser, & Maniates, 2015; T. E. Robinson & Berridge, 1993).

1.2 Reward neurocircuitry

Early influential work into the neural correlates of reward processing was the experiments of Olds and Milner (1954). In these experiments, electrodes were implanted in various regions of the brain in rats, and the rats could press a lever to electrically stimulate the area in question. The results showed that in regions spanning the brain from the brainstem to frontal portions, including the tegmentum, striatal regions, and cingulate cortex, rats would either increase or decrease lever-pressing in response to electrical stimulation. These results were interpreted as a reflection of pleasure or displeasure from the stimulation. Since these

early discoveries, an enormous body of research has amassed on the structural layout and connectivity of the systems involved in reward. This section will give a general outline of the most consistently implicated anatomical systems in reward-related processing.

1.2.1 Brain areas implicated in reward processing

The majority of neuroscientific research on reward has been conducted using animal models. The amount of human neuroimaging studies on the subject has grown substantially within the last two decades however (e.g. Delgado, 2007; Haber & Knutson, 2010). There are important strengths and limitations related to the use of both of these approaches that are worth mentioning. In general, animal subjects allow for much more invasive paradigms like lesioning, genetic modification, and single cell recording, which provide precise and direct ways of manipulating and recording signaling in the brain. However, the translation of results from say, rats, to humans is not always straightforward (Wallis, 2012; Xiong, Mahmood, & Chopp, 2013). Studying the human brain avoids the issues of translating results across species, but usually requires much less invasive and indirect methods to be applied due to ethical concerns. For example, functional magnetic resonance imaging (fMRI) has been widely adopted as a way of studying the brain, but must infer neuronal activity from oxygen changes in the brains blood supply, which severely reduce spatial and temporal resolution.

Brain areas involved in reward processing across species includes regions of the striatum, (ventral striatum [VS] and nucleus accumbens [NAc] in particular), ventral tegmental area (VTA), substantia nigra, ventral pallidum (VP), amygdala, hippocampus, thalamus, hypothalamus, orbitofrontal (OFC) and medial prefrontal cortex (vmPFC), and anterior cingulate cortex, all of which are anatomically interconnected in complex ways (Haber & Knutson, 2010; Namburi, Al-Hasani, Calhoon, Bruchas, & Tye, 2015). Of these areas, some appear to play a more non-specific role in reward. For instance the amygdala has been proposed to track arousal in general (e.g. Anderson et al., 2003; Fastenrath et al., 2014), although it has also been linked to ‘liking’ and associative reward learning (Mahler & Berridge, 2012; Wassum, Cely, Balleine, & Maidment, 2011). Other areas seem to be involved more specifically in reward processing, like the medial OFC/vmPFC, thought to be important for value encoding of positive stimuli (Bartra, McGuire, & Kable, 2013; Sescousse et al., 2013; Sescousse, Redouté, & Dreher, 2010), and the VTA and the NAc, implicated in, amongst other things, reward prediction (Haber & Knutson, 2010; Hikosaka, Bromberg-Martin, Hong, & Matsumoto, 2008; Nestler, 2005; Schultz et al., 1997). The NAc, amygdala

and VP have also been extensively studied the context of the LWL framework, and research in animals suggests that they can be separated into subregions that, are preferentially associated with ‘wanting’, ‘liking’, or learning (Mahler & Berridge, 2012; Peciña, 2008; Peciña & Berridge, 2005, 2013; K. S. Smith & Berridge, 2007; Wassum et al., 2011; Zhang et al., 2003).

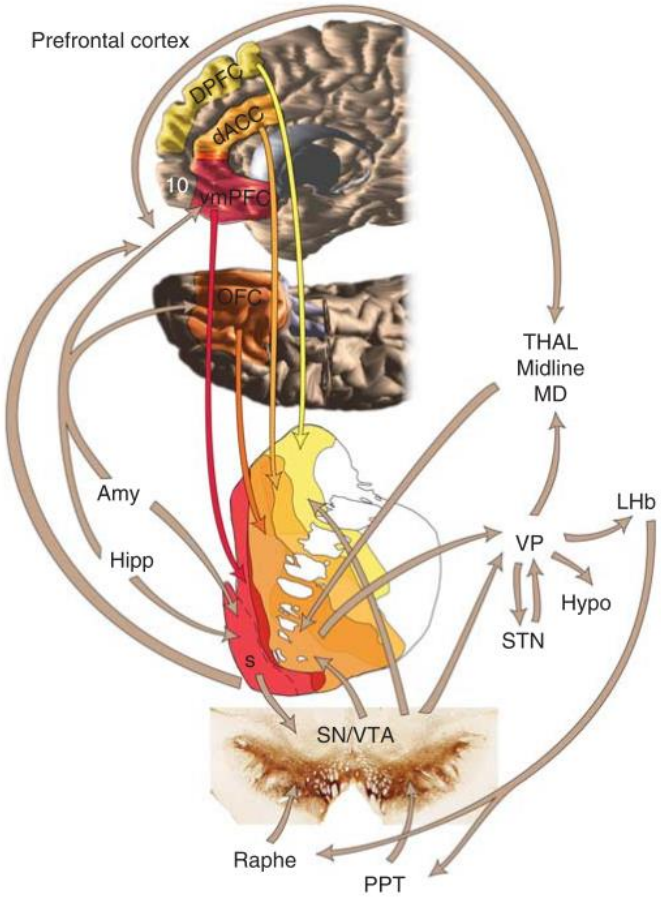


Figure 1: Simplified overview of the anatomical connections of parts of the reward system. Center structure represents the ventral striatum. Reprinted from Haber and Knutson (2010).

1.2.2 Neurochemistry of reward processing

In addition to a complex anatomy, several neurotransmitter systems interact during reward processing. Early theories of neurotransmitter involvement in reward centered on monoamines (Wise, 2008). Throughout the 1970s, this research focus shifted towards a theory of dopamine as the key neurotransmitter for mediating reward behavior (Fouriezos, Hansson, & Wise, 1978; Fouriezos & Wise, 1976). The notion that dopamine is “the reward transmitter” has since been very influential.

However, the dopamine theory has been hotly debated during the last decades (Berridge & Robinson, 1998; Nutt, Lingford-Hughes, Erritzoe, & Stokes, 2015; Salamone & Correa, 2012; Spanagel & Weiss, 1999; Wise, 2008), Numerous more recent studies demonstrating involvement of several other neurotransmitters in reward processing, such as opioids, GABA, glutamate, endocannabinoids, oxytocin, and serotonin (e.g. Chartoff, Connery, Marini, Perez, & Bidlack, 2014; Fallon, Shearman, Sershen, & Lajtha, 2007; Fields & Margolis, 2015; Johnson & North, 1992; Le Merrer, Becker, Befort, & Kieffer, 2009; Nestler, 2005; Peciña & Berridge, 2013; van Zessen, Phillips, Budygin, & Stuber, 2012; Young, Liu, Gobrogge, Wang, & Wang, 2014).

Dopamine and reward

The mesolimbic dopamine pathway consists of dopamine neurons in the VTA that innervates receptors in the NAc (Nestler & Carlezon, 2006). This pathway has been extensively tied to reward in non-human animals (for reviews, see: Berridge & Robinson, 1998; Kelley & Berridge, 2002; Namburi et al., 2015; Wise, 2008). Based on these animal models, neuroimaging studies using positron emission tomography (PET) have demonstrated the involvement of dopaminergic transmission in both the VTA (D'ardenne, McClure, Nystrom, & Cohen, 2008) and the ventral striatum (Cox et al., 2009; Martinez et al., 2003) during human reward processing as well.

Extensive animal research shows a key role of mesolimbic dopamine in behavioral responses to rewarding stimuli. For instance, Kiyatkin and Gratton (1994) demonstrated increased dopamine levels in the rat NAc during lever pressing for a food reward as well as during food consumption. Conversely, dopamine antagonist drugs like haloperidol that block dopaminergic receptors have been shown to decrease the reinforcement value of food in rats (Ettenberg & Camp, 1986).

Dopamine also plays a key role in anticipation of reward. For example, Schultz and colleagues measured dopaminergic cell firing patterns in the monkey VTA using single cell recordings during anticipated and unanticipated reward (Schultz et al., 1997). They found that dopamine neurons would increase firing in response to an unexpected reward, but that the same neurons would fire in response to the reward-predicting cue after a cue-reward association had been established. Moreover, dopamine cell firing would decrease when a cue was not followed by an expected reward, and this decrease would occur when the reward would normally have been presented. These experiments indicate that midbrain dopamine activity does not signal reward outcome per se. Rather, dopamine seems to be involved in

signaling events that are more and less rewarding than expected, as well as signaling cues that will lead to reward (but see: Haber & Knutson, 2010).

Within Robinson and Berridge's framework, dopamine is considered necessary for motivational processes, but not for 'liking' or reward learning. In particular, dopamine is believed to specifically mediate the motivational 'wanting' that drives effortful behavior towards reward and reward cues (Berridge, 2007).

Dopamine criticism. The strongest evidence against dopamine involvement in 'liking' and learning comes from studies in rats with chemical lesioning of >99% of striatal/accumbens dopamine neurons (Berridge & Robinson, 1998). The rats displayed intact orofacial 'liking' responses to pleasant sweet taste. A study in humans also reported that the dopamine antagonist pimozide did not reduce self-reported amphetamine liking (Brauer & De Wit, 1997). Dopamine-deficient rats were also capable of learning paired associations between a previously liked taste conditioned stimulus and an aversive unconditioned stimulus. In addition, later studies were not able to show increases in instrumental learning in rats with a genetically enhanced dopamine response (Yin, Zhuang, & Balleine, 2006). What previous studies do show, is that dopamine levels appears to code reward-motivated behavior (e.g. Kiyatkin & Gratton, 1994). This is pointed out by Berridge and Robinson (1998), who argues that the LWL framework is able to account for previous findings and explain the role of dopamine as a necessary component of 'wanting' driven behavior (Berridge et al., 2009).

Despite decades of debate, there has also been a longstanding recognition that dopamine is one part of a larger neurocircuitry of reward: "It is clear that reward circuitry is multisynaptic, and since dopamine cells do not send axons to each other or receive axons from each other, dopamine can at best serve as but a single link in this circuitry." – Wise and Rompre (1989, p. 220). In particular, the μ-opioid receptor (MOR) system appears to play an essential role in 'liking' and associative reward learning (Barbano & Cador, 2007; Berridge & Kringelbach, 2008, 2015; Corbett, Henderson, McKnight, & Paterson, 2006; Fields & Margolis, 2015). There is much evidence to also believe that MOR influence the 'wanting' aspect of reward.

Direct μ-opioid modulation of reward motivation?

There are several different endogenous opioid ligands in the brain, the most common being dynorphins, endorphins, and enkephalins (Akil et al., 1984). These ligands bind to one (or more) of a handful of opioid receptors in the central nervous system. The most widely known receptors to date is the μ (mu) opioid receptor (MOR), κ (kappa) opioid receptor, and the δ (delta) opioid receptor, although other receptors and ligands have been identified as well (e.g. Whiteside & Kyle, 2013). The different ligands and receptors are associated with different behavioral functions, and with different modulation of the same function (Feng et al., 2012). Across domains, the MOR system is the most widely studied of these receptors.

Kent Berridge's research has shown that the MOR system plays a special role in rodent 'liking' responses. MOR modulation of 'liking' is often contrasted with the dopamine modulation of 'wanting' (Barbano & Cador, 2007). Of course, since we typically want what we like, opioid-induced increases in 'liking' would be expected to indirectly increase 'wanting' too. However, evidence from Kent Berridge's lab also points to important direct effects of opioids on measures on motivation that mirror the well-known dopamine effects on 'wanting' (Berridge, 2007).

In the following sections I will review three lines of evidence supporting a role for the MOR system in 'reward' processing and behavior, with a particular focus on studies of motivation/'wanting'; (1) Drug microinjection studies in rodents; (2) systemic MOR manipulations across species, and (3) evidence from drug addiction research.

Microinjections in in rats with MOR agonists. Some of the most compelling evidence for MOR system involvement in reward behavior comes from studies in rodents where drug microinjections have been used to study drug effects in very localized areas of the brain. These studies show clear modulation of both 'wanting' and 'liking' behavior following μ-opioid drug injections into subcortical parts of the forebrain. Here I describe a selection of the studies that have shown modulation of reward behavior after μ-opioid microinjections into various parts of the rodent brain.

The NAc can be functionally and anatomically divided into a "core" and a "shell" section (Corbit, Muir, & Balleine, 2001; Zahm & Brog, 1992). Studies of the rat NAc have found that the shell seems to be critical for pleasure generation (Peciña, 2008). 'Liking' reactions, measured by orofacial reactions to sucrose, are considerably increased by opioid drug microinjections in a specific $1mm^3$ of the NAc shell. This area is often referred to as a "hedonic hotspot". However, microinjections into widely-distributed areas of the NAc shell

and core appear to enhance ‘wanting’ reactions, measured as for example increased food seeking, intake or lever pressing for food. (Peciña & Berridge, 2005, 2013; K. S. Smith & Berridge, 2007; Zhang et al., 2003).

For example, Peciña and Berridge (2013) showed that microinjections of both amphetamine (dopamine agonist drug that stimulates dopaminergic receptors) and DAMGO (a μ-opioid selective synthetic agonist peptide that stimulates MOR receptors) throughout the NAc shell produced enhanced ‘wanting’ responses to sucrose reward cues, even without affecting ‘liking’. DAMGO injections into the posterior and central VP will also increase eating behavior and sucrose intake (K. S. Smith & Berridge, 2005). These regions likely interact in ‘wanting’ mediation, as it has been shown that naloxone microinjections into the NAc can attenuate the eating that is normally followed by DAMGO injections in the VP. However, naloxone microinjections in the VP will not affect eating following NAc DAMGO injections(K. S. Smith & Berridge, 2007).

DAMGO also increases ‘wanting’ behavior when injected into the central nucleus of the amygdala (CeA). Mahler and Berridge (Mahler & Berridge, 2012) showed that DAMGO injections into the CeA can increase sucrose intake even while decreasing orofacial ‘liking’ responses to the same stimulus, and that not only eating, but also sexual ‘wanting’ of an estrous female was increased by MOR agonism in male rats.

Systemic μ-opioid manipulations across species. While studies using drug microinjections have provided valuable knowledge regarding drug effects in very localized areas in the brain, many studies using systemic manipulations also demonstrate a general role for the MOR system in reward. In rodents, it is well established that systemic MOR agonism enhances, and MOR antagonism reduces typical ‘wanting’ behavior such as food intake, and specifically for for high calorie food options (e.g. Cleary, Weldon, O'Hare, Billington, & Levine, 1996; Doyle, Berridge, & Gosnell, 1993; Taha, 2010; Taha et al., 2006). In rats for example, systemic injection of naloxone decreases consumption of a highly palatable sucrose diet in a dose-dependent manner (Glass, Grace, Cleary, Billington, & Levine, 2001). Naltrexone, another MOR antagonist with high μ affinity, produces similar effects (Gosnell et al., 2010). Conversely, systemic morphine (a MOR agonist with high μ affinity) injections increase food intake (Doyle et al., 1993).

In addition to altering food intake, systemic MOR agonism enhances and antagonism decreases effort exerted to obtain palatable food, another behavioral signature of ‘wanting’. For example, naltrexone dose-dependently decreases the amount of times a rat is willing to

lever-press to obtain a sucrose reward (Cleary et al., 1996; Gosnell et al., 2010). The opposite effect, increased effort exerted to obtain food, has been shown following systemic injection of an agonist (Solinas & Goldberg, 2005).

Systemic manipulations, unlike microinjections, have also been used to study reward behavior in humans. Human data largely corroborates the animal literature, and show decreased appetite and food intake following MOR antagonism (e.g. Bertino, Beauchamp, & Engelman, 1991; Eikemo et al., 2016; Yeomans & Gray, 2002; Ziauddeen et al., 2013).

In contrast to the animal literature however, few studies have been published on effects of MOR agonism on food reward in humans. The few that exist (Drewnowski, Krahn, Demitrack, Nairn, & Gosnell, 1992; Eikemo et al., 2016; Morley, Parker, & Levine, 1985) do not provide consistent results regarding agonistic effects. For instance, Morley et al. (1985) found increased food consumption, while Drewnowski et al. (1992) and Eikemo et al. (2016) did not. Notably, the two former studies had quite limited sample sizes and used the mixed MOR agonist and antagonist butorphanol, which complicates inferences from these results. However, one recent study found that morphine increased 'wanting' behavior, measured by how much effort healthy men exerted to view images of female faces of varying attractiveness. Morphine only increased motivation to view the most attractive female faces. MOR agonism has also been shown to increase motivation to obtain monetary rewards in healthy humans(Eikemo, Biele, Willoch, Thomsen, & Leknes, 2017). Notably, Chelnokova et al. (2014), and Eikemo et al. (2017), also found reduced motivation for reward following opioid antagonist treatment.

MOR system and pathological wanting (craving). In addition to studies demonstrating MOR system involvement in wanting and liking in *healthy* humans and rodents, many studies show that the MOR system is involved in responses to drugs of abuse. Positron emission tomography (PET) studies measuring the binding potential of [^{11}C]carfentanil, a MOR ligand have demonstrated an increase in endogenous μ-opioid levels (inferred by reduced ligand binding potential) following administration of amphetamine (Colasanti et al., 2012; Mick et al., 2014) and nicotine (Domino, Hirasawa-Fujita, Ni, Guthrie, & Zubieta, 2015; Ray et al., 2011).

Further, long-term substance abuse has also been associated with changes in MOR system function. PET has been used to study various patient groups with substance use disorder, and have demonstrated altered endogenous μ-opioid levels in patients addicted to cocaine (Ghitza et al., 2010; Gorelick et al., 2008; Zubieta et al., 1996) and alcohol (Bencherif

et al., 2004; Weerts et al., 2011; Williams et al., 2009). Further, decreased MOR binding potential correlated with increases in craving, indicating that increased endogenous ligand binding is associated with increased 'wanting'. PET has also recently been used to show decreased endogenous opioid release to an oral amphetamine challenge in pathological gamblers (Mick et al., 2016), suggesting that the MOR system (and MOR-DA interplay) may also be altered in behavioral addictions, though no difference in baseline μ-opioid levels between pathological gamblers and controls were found. Together, these PET studies indicate that the endogenous μ-opioid system changes following drug exposure and addiction.

Another line of evidence for MOR involvement in wanting behavior comes from clinical use of MOR antagonist drugs in addiction treatment. Opioid antagonist drugs such as naltrexone are used as pharmacotherapy to reduce symptoms of craving (excessive 'wanting') across both drug- and behavioral addictions (Lobmaier, Kunøe, Gossop, & Waal, 2011). A recent meta-analysis of randomized clinical trials on naltrexone treatment of alcohol dependence showed overall reduced craving for, and self-administration of, alcohol during naltrexone treatment (Hendershot, Wardell, Samokhvalov, & Rehm, 2016). Reduced craving following MOR antagonism with naltrexone has also been shown for heroin- (Sullivan, Vosburg, & Comer, 2006), nicotine- (King & Meyer, 2000) and amphetamine dependent patients (Nitya Jayaram-Lindstrom et al., 2007). Further, naltrexone also reduced self-reported wanting of amphetamine in healthy participants following an initial amphetamine administration (N. Jayaram-Lindstrom, Wennberg, Hurd, & Franck, 2004). Naltrexone has also been used to attenuate urges to gamble in patients with pathological gambling (Jon E Grant, Kim, & Hartman, 2008) and to steal in patients with kleptomania (Jon E. Grant, Kim, & Odlaug, 2009). In summary, there is substantial evidence to suggest that blockade of MORs reduces excessive wanting/craving in impulse control and substance use disorders.

In conclusion, based on multiple lines of evidence there is good reason to suspect that the MOR system is intricately involved human wanting. We hypothesize that since opioid mechanisms in general appears to be highly conserved across species (Berridge, 2003; Chelnokova et al., 2014; Eikemo et al., 2016; Fields & Margolis, 2015; Mahler & Berridge, 2012) opioids should modulate wanting in healthy humans in a way comparable to rodents. Behavioral studies from our own lab already provide support for this hypothesis (Chelnokova et al., 2014; Eikemo et al., 2017; Eikemo et al., 2016), but brain mechanisms underlying opioid modulation of non-pathologic wanting in healthy humans remains poorly understood. In addition, the majority of studies on opioid modulation of reward have focused only on

subjective feelings such as pain relief and reduced food 'liking' (Leknes & Tracey, 2008). We therefore decided to study consciously experienced wanting in healthy humans and implement neuroimaging to record simultaneous neural activity.

PET is an ideal candidate for studying MOR modulations in the human brain because the MOR system can be targeted specifically by a tracer ligand. As mentioned above, it has previously been used to study excessive wanting in several studies (Bencherif et al., 2004; Ghitza et al., 2010; Gorelick et al., 2008; Mick et al., 2016; Weerts et al., 2011; Williams et al., 2009; Zubieta et al., 1996). However, PET is a very expensive technique to implement and requires a radioactive tracer to be synthesized on site and injected into participants. In addition, PET requires a stable mood state to be maintained for over 20 minutes to get a reliable measure of signal change. Due to these challenges, we opted instead to use fMRI with a systemic MOR manipulation to study wanting-related activity in the human brain. However, this technique has its own set of limitations that must be addressed.

Pharmacological functional magnetic resonance imaging

Pharmacological MRI (phMRI) describes fMRI designs with one or more drug conditions vs a placebo control, allowing one to infer that changes in blood oxygenation level dependent signal (BOLD) between conditions is ultimately caused by the drug manipulation. However, in addition to the general caveats of fMRI (indirect measure of neural activity, not a quantitative measurement, movement restrictions, etc.) special considerations must be made when interpreting differences in BOLD signal measured with and without a drug. For instance, caffeine has strong vasoconstrictive properties that can alter the BOLD signal due to changes in cerebral blood flow (Bourke & Wall, 2015; Jenkins, 2012; Murphy & Mackay, 2011).

One important potential caveat of pharmacological fMRI using opioid agonist drugs is that they may alter respiration rate and consequently the end-tidal CO_2 (Pattinson, 2008) which can produce BOLD signal changes between drug conditions in ways unrelated to neural activity (Cohen, Ugurbil, & Kim, 2002). There are ways to overcome this caveat. For example, some studies have provided additional oxygen to ensure comparable O_2 and CO_2 levels across conditions (e.g. Wanigasekera, Lee, Rogers, Hu, & Tracey, 2011). Other recommendations include thorough controlling for extraneous effects (Bourke & Wall, 2015).

Furthermore, pharmacological fMRI studies must take into account potential caveats related to psychopharmacological methods more generally. Importantly, expectations towards drugs can lead to effects that are as large as and even contrary to the actual effects of the drug

itself, e.g. (Bingel et al., 2011). Drugs can also affect behavioral and brain measures in non-specific ways such as through altered subjective state and/or motoric function. Large doses of opiates are known to cause sedation, drug high and motor slowing (e.g. Zacny & Lichtor, 2008). Having mentioned some of the caveats of phMRI, the next section reviews phMRI research that has been done on human reward processing.

Pharmacological neuroimaging studies in humans. Systemic manipulations of the MOR system have in the last decade been used to study modulation of human brain responses to rewards in addition to behavior. phMRI has been used to explore the relationship between systemic MOR manipulation and BOLD response to rewards in several studies. A literature search in PubMed of papers mentioning various μ-opioid agonists and antagonists and fMRI revealed 13 papers that were specifically using fMRI to study reward, while administrating a MOR agonist or antagonist.

Nine of these studies assessed effects of MOR antagonism on reward related brain-activity. Across reward paradigm and antagonist applied, the general finding appears to be that MOR antagonists attenuates reward-associated activity and increases prefrontal activation, which correlates with reductions in measures of pleasure, 'wanting', and craving (Lukas et al., 2013; Myrick et al., 2008; Petrovic et al., 2008; Quelch et al., 2017). However, one study fails to find behavioral effects at all, despite reporting alterations in BOLD (Murray et al., 2014), and one fails to find a main effect of the antagonist whatsoever (Schacht et al., 2013). The spatial localization of MOR antagonism on neural activation also varies extensively across these studies. Some, but not all, studies report increased BOLD responses in the insula and superior frontal areas (Lukas et al., 2013; Murray et al., 2014; Myrick et al., 2008; Petrovic et al., 2008). Decreased VS responses is found in some studies (Myrick et al., 2008; Quelch et al., 2017), but not others (Schacht et al., 2013; G.-J. Wang et al., 2014). A reason for this variation may be variations in design across studies, including drug used, anticipation- (e.g. Myrick et al., 2008) vs outcome-focused tasks (e.g. Murray et al., 2014), and studying both healthy (e.g. Petrovic et al., 2008) and clinical populations (e.g. Lukas et al., 2013). Control measures of physiology or potential CO_2 changes in the brain was rarely mentioned in these studies.

Further, four studies investigated effects of a μ-opioid agonist on reward related activity (Becerra, Harter, Gonzalez, & Borsook, 2006; Langleben et al., 2008; Mei, Zhang, & Xiao, 2010; Wardle et al., 2014). Again, the results vary extensively across studies. For example, Langleben et al. (2008) and Wardle et al. (2014) find decreased OFC activations, while Mei et

al. (2010) do not. And while Becerra et al. (2006) report BOLD signal increase in NAc to a euphoria-inducing morphine administration, no study finds agonist modulation of NAc during a reward task (Langleben et al., 2008; Mei et al., 2010; Wardle et al., 2014). The use of different reward tasks (heroin cues, emotional image task, morphine administration), drugs (methadone, buprenorphine, oxycodone, morphine), and administration routes (oral, intravenous) across studies may be one reason for the variation in results. In addition, the four studies were all quite low-powered (mean number of participants was 13). Two of the studies report having controlled for changes in physiology (Becerra et al., 2006; Wardle et al., 2014). No changes is reported in either case.

In summary, there is good reason to hypothesize that the MOR system is intricately involved in the processing of wanting in the human brain. However, the majority of studies on MOR modulation of motivation for reward in humans have used antagonist manipulations, likely due to the abuse liability associated with opioid agonist drugs. Little is known about the effects of MOR agonism on wanting in healthy humans. In addition, while animal research has demonstrated the involvement of a number of specific regions in MOR modulation of reward processing, few studies have tested whether these findings extend to the human brain. Notably, the available neuroimaging studies show inconsistent results. For example, while it is well-established that the NAc is central for MOR mediated reward in rodents, fMRI neuroimaging studies have yet to extend this finding to humans (although PET studies implicate the accumbens in relief and to some extent wanting, see e.g. Hsu et al., 2013). Further, activation patterns are in general inconsistent across human imaging studies, likely due to low sample sizes.

Increased knowledge about MOR system modulation of wanting in the human brain may increase our understanding of MOR mechanisms in pathological ‘wanting’ observed in conditions like substance use disorders, pathological gambling and binge eating disorders.

2 The current study

To study the mechanisms of MOR agonist modulation of wanting in the healthy human brain, we designed and conducted a pharmacological fMRI study. We administered a low but clinically significant dose (10mg per-oral) of the μ-opioid receptor agonist morphine in healthy volunteers. Two reward tasks were included to gauge opioid modulation of distinct aspects of reward function: (1) a 'Food Wanting and Regulation task' where participants either passively viewed or consciously regulated their desire (wanting) for images of palatable foods; and (2) a commonly used fMRI task to assess responses to monetary reward and losses, the Monetary Incentive Delay (MID) task. After presenting results from a series of control tasks and measures conducted to ensure the interpretability of fMRI data after morphine administration, I will report preliminary analyses of data from the Food Wanting and Regulation task in this thesis.

2.1 Study rationale

Controlling for changes in mood and subjective state. We controlled for participants' subjective state and mood during each session. It has been shown that large doses of morphine can induce various changes in subjective state including sedation, nausea, and drug high (Zacny & Lichtor, 2008), which could conceivably confound the task measures reported here. Importantly, we chose a morphine dose that is not expected to cause changes in subjective state, mood, or drug effects such as 'high' or euphoria based on previous research (Chelnokova et al., 2016; Eikemo et al., 2016; Zacny & Lichtor, 2008). To control for potential drug effects on mood or subjective state, we also developed a subjective state questionnaire that was administered before drug administration, during estimated peak effect, and at end of each session (figure 2).

Controlling for changes in motor coordination. In addition to subjective state, larger doses of morphine can also reduce motor function (Zacny & Lichtor, 2008). In order to control for this potential confound in our results, we administered a short eye-hand coordination task (Giovannoni, Van Schalkwyk, Fritz, & Lees, 1999). Based on previous studies from our lab, we do not expect any reduction in motor coordination with a 10mg per oral dose of morphine (Chelnokova et al., 2016; Eikemo et al., 2016).

Controlling for global changes in BOLD signal. We measured changes in heart rate and respiration during fMRI scanning in both sessions. We also implemented a simple visual

task to control for potential global BOLD differences between drug sessions, by looking at differences between morphine and placebo in the primary visual cortex. This type of task has been recommended as a control measure in pharmacological fMRI designs (Murphy & Mackay, 2011), and has previously been implemented in fMRI studies using opioid agonists in healthy participants (Tracey et al., 2000; Wardle et al., 2014), although the sample size in both cases were very small.

Studies in the macaque brain have shown that the primary visual cortex contains very few μ-opioid receptors (Lewis et al., 1981). However, if the morphine dose influences physiological measures like respiration, there could be a difference in BOLD signal contrast merely due to increased CO_2 levels in the blood throughout the brain. Increased CO_2 levels in the blood will increase the baseline ratio of deoxygenated/oxygenated blood. This which will in turn decrease the average BOLD response, which measures changes in this relationship from baseline (Cohen et al., 2002). This would present a serious confound to the interpretation of task-related BOLD activity. If the BOLD response is systematically weakened in the entire brain because of the drug manipulation, we would not know whether a task-related difference in BOLD between the drug and placebo session is caused by a task-related neural activity difference, or a task-unrelated difference in signal baseline between the drug conditions.

Food wanting rationale. Food images were selected as an appropriate reward stimulus for two primary reasons. First, food is a robust natural reward stimulus that have been widely used as an outcome measure in reward research across species (e.g. Berridge, 1996; Kelley & Berridge, 2002; Mahler & Berridge, 2012; Peciña & Berridge, 2013; Taha et al., 2006; Volkow et al., 2011; Wassum et al., 2011), and in fMRI research on human reward processes specifically (Sescousse et al., 2013). In animals it has even been shown that food is often preferred to drug in mutually exclusive reward choice paradigms (e.g. Lenoir, Serre, Cantin, & Ahmed, 2007). Although many studies have used actual food consummation to study reward processes, images of food still consistently activate reward regions in healthy human participants (van der Laan, De Ridder, Viergever, & Smeets, 2011).

Second, while a prevalent view of the role of MOR in reward is that it is involved in the consummatory 'liking' aspects of reward, it is clear that MOR has independent influence on anticipatory 'wanting' behaviors as well (Chelnokova et al., 2014; Hendershot et al., 2016; Peciña & Berridge, 2013). Based on rodent findings (Mahler & Berridge, 2009; Peciña & Berridge, 2005, 2013; K. S. Smith & Berridge, 2005) , we expected that MOR modulation of

desire to eat palatable foods in humans would be reflected in increased activity in similar circuitry, including ventral striatum.

Primary aims of the thesis

1) To establish whether the opioid administration was associated with physiological or other non-specific side effects that could confound the behavioral and fMRI measures and…
2) To assess whether the Food Wanting and Regulation task elicited the expected effects on behavior and BOLD signal, i.e. typical BOLD activity patterns associated with (a) palatable food images and (b) regulatory behavior

As such, the principal goal of this thesis was to ensure that any potential drug effects observed in the fMRI reward tasks were not due to confounds such as CO_2-related BOLD changes, slowed motor coordination, or drug effects on mood, nausea, hunger, sedation, and general drug experience. In addition to these primary aims, preliminary results of drug effects in the Food Wanting and Regulation task will also be presented and discussed briefly.

2.2 Hypotheses

2.2.1 Control measures

Based on control task measurements from a previous study performed in our research group, we expected minimal drug side effects in healthy, pain-free participants (Hanks, O'Neill, Simpson, & Wesnes, 1995; O'Neill et al., 2000; Zacny & Lichtor, 2008). We predicted that:

1. Participants would be unable to distinguish between the morphine and placebo sessions (i.e. successful drug blinding). Accordingly,
    a. Morphine (10 mg per-oral) would not influence ratings of mood, somatic state (e.g. typical medication side effects) or motor-coordination to an extent believed to interfere with task performance.
2. Morphine (10 mg per-oral) would not significantly reduce respiration or heart rate.
3. Morphine administration would not interfere with global BOLD signal compared to placebo, as assessed using a visual checkerboard control task.

2.2.2 Food Wanting and Regulation task

Behavioral task effects

1) After observing images of palatable food, participants would report *decreased* food wanting when asked to actively regulate 'food wanting' compared to blocks in which they were asked to passively observe images.
2) Participants would report *increased* food wanting in the morphine session compared to the placebo session. We did not expect 10 mg per oral morphine to interfere with the ability to cognitively downregulate 'food wanting' in this task.

fMRI BOLD activity

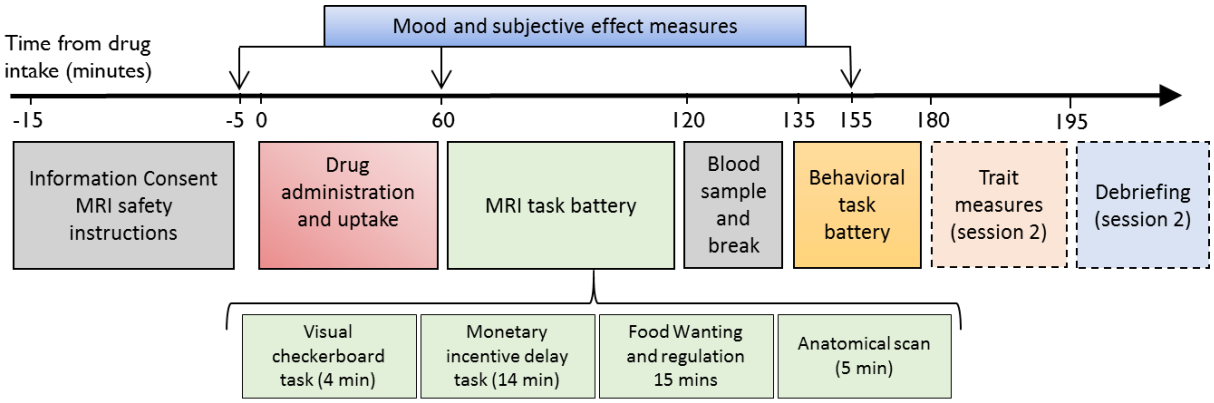
3) Passive viewing of palatable food images (*observe condition*) would be associated with significantly increased BOLD activity in brain areas previously associated with hedonic value and reward anticipation (Bartra et al., 2013; Sescousse et al., 2013; van der Laan et al., 2011), compared to a rest. This includes medial orbitofrontal/prefrontal cortex, ventral striatum, thalamus, amygdala, and insula.
4) Cognitive regulation of food wanting, compared to passive viewing, would be associated with significantly increased activity in areas previously associated with regulation of nicotine craving and emotion regulation (Buhle et al., 2014; Johnstone, van Reekum, Urry, Kalin, & Davidson, 2007; Kober et al., 2010). This includes ventromedial PFC, and inferior and medial frontal gyrus.
5) In the *observe condition*, morphine would be associated with significantly increased BOLD activity compared to placebo in MOR-rich brain regions previously associated with hedonic value and reward anticipation, especially value-responsive areas like the ventral striatum and orbitofrontal cortex.

3 Methods

3.1 Design

A double-blind, placebo-controlled crossover design with per oral administration of morphine (10 mg) or placebo over two sessions was employed. The order of drug administration was randomized and counterbalanced. Participants completed a subjective state and drug effect questionnaire before and twice after drug administration. Scanning occurred at 60-120 minutes after drug intake.

The experiment reported here was conducted as part of a larger study investigating the effects of morphine on various behavioral reward measures, as well as its effect on BOLD signal activity measured with fMRI. Measurements and tasks unrelated to the topic of this study will be reported elsewhere.



B

3.1.1 Participants

Sixty-eight healthy adult participants were recruited for this study through flyers around the University of Oslo and the Oslo and Akershus University College campus, and through online advertisement. Four participants did not return for the second session. One participant was mistakenly given placebo in both sessions and was excluded from further analysis. In total, 63 (31 males, age 19-45, *Mean*=27, *SD*=5) participants completed testing with morphine and placebo and were included in the statistical analyses (see table 1 for sample characteristics). Participants were screened to exclude those with a history of depression or other major psychiatric illness, current ongoing psychiatric or medical illness, multiple complex allergies, prior drug dependence or addiction, current use of medication (except for

antihistamines). Exclusion criteria also included history of chronic use of opioids, use of any strong opioids in the two last years, and use of codeine drugs in the last four months. All participants reported to have normal or corrected-to-normal vision.

Self-report data revealed that 23 of the 32 female participants were taking hormonal contraceptive medication. For another six women, both test sessions were determined from self-report to fall within the same phase of the hormonal cycle. The final three women who were not on contraceptives were unable to state the day since their last ovulation.

Participants were requested not to consume alcohol on the evening before each test day and were asked to refrain from using tobacco in the hour before each test session. They were advised not to drive a vehicle for six hours after drug administration. All participants were asked to eat a few hours or less before testing, and were offered food if they reported being hungry upon arrival.

3.1.2 Procedure

Experimental procedures were approved by the Regional Ethics Committee ((2011/1337/REK sør-øst D). All participants gave written consent and were informed about their right to withdraw consent at any time. The minimum inter-session interval was two days to ensure adequate wash-out of potential drugs from the first session (M = 3.9, SD = 6.3).

In psychopharmacological studies, there is a general issue of recruiting biases and, more importantly, the ethics of exposing healthy participants to a potentially addictive substance. To minimize potential problems related to these issues participants went through careful medical screening prior to participation. The study was implemented in a double-blind fashion to prevent participants' and experimenters' drug effect expectations to systematically bias results.

Each session lasted on average 3 hours. After giving written consent, participants completed a MRI safety questionnaire and completed baseline behavioral measurements including a subjective state questionnaire (See table 1). Participants then received either 10 mg morphine or placebo (double-blind) and were told to swallow the pills immediately with water, and to not look at the pills while ingesting. As in a previous pharmacological study conducted in our lab (Chelnokova et al., 2014; Chelnokova et al., 2016; Eikemo et al., 2016) participants were told that the pills could be either morphine, placebo or naltrexone. Although naltrexone was not included in the present design, the information was kept to avoid

recruitment of participants attracted specifically by the prospect of morphine treatment. An experimenter was always present during drug administration.

After drug administration, participants viewed a nature documentary for ~30 minutes before completing a practice version of the fMRI-specific tests outside of the scanner. The documentary was included so that onset of the test-period inside the MRI scanner could be timed to start within the period of peak drug plasma concentration (30-90 minutes, see figure 3).

In the scanner, the participants were connected to equipment measuring pulse, respiration and end-tidal CO_2. They then completed a subjective state questionnaire followed by a flickering checkerboard control fMRI task, a customized version of the monetary incentive delayed (MID) task (Knutson, Westdorp, Kaiser, & Hommer, 2000), the wanting regulation task for food images, blip images in the up/down directions for magnetic field correction, and finally a high resolution structural scan. All tasks were presented using an MRI-compatible computer screen placed behind the head of the participant, and a mirror system. Between each scan, participants were asked about their general comfort level, and were given the opportunity to take a short rest before moving on.

After scanning the participants were offered a short break before a blood sample was collected. After the fMRI session, participants completed a behavioral task battery including a hand-eye coordination test, an emotional perception/sensitivity task, a social dominance questionnaire, subjective ratings of the stimuli from the MID and food wanting tasks, and a third round of the subjective state questionnaire.

At the end of the second session the participants were debriefed and asked to guess the identity of the drug given each session. Participants were reimbursed on average 340 NOK (about 40 USD) for their participation, ±20 NOK based on their performance on the MID task.

3.1.3 Drug administration

Morphine is a mu-opioid selective drug widely used in treatment of severe pain (Vindenes, Handal, Ripel, Boix, & Morland, 2006). To minimize subjective effects such as sedation and euphoria that could confound with outcome measures, we used a small analgesic dose of per oral morphine (10 mg, Morfin®, Nycomed Pharma, Asker, Norway). It has been shown that morphine per-oral morphine doses between 10 and 30 mg are associated with very few changes in subjective effects or mood in healthy pain-free humans (Hanks et al., 1995;

O'Neill et al., 2000; Zacny & Lichtor, 2008). For oral absorption, peak plasma concentrations occur between 30-90 minutes after administration with a half-life of 2-4 hours (see figure 3). The bioavailability or per oral morphine is quite variable, but is on average around 30-40 % (Lugo & Kern, 2002). Cherry-flavored breath mints were used as placebo, and a small amount of the flavored placebo pills was added to the drug dosages. This was done to mask any difference in taste between the drug sessions. The test interval between 60 and 180 min after drug administration was chosen to ensure relatively high and stable levels of morphine throughout the session.

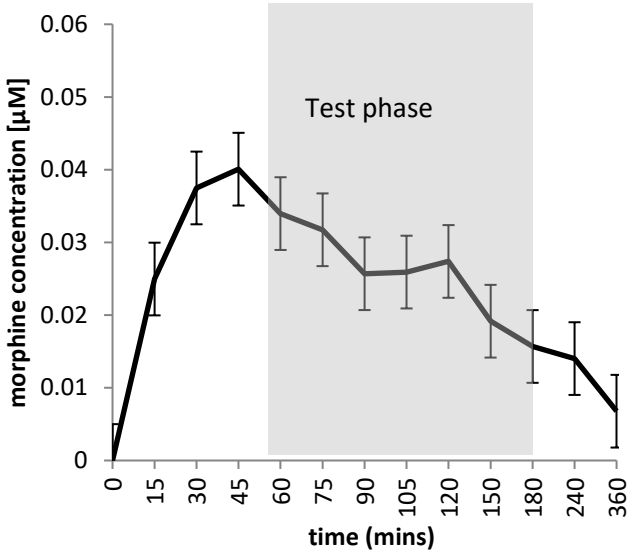


Figure 3. The timeline of morphine concentration in blood after oral intake based on unpublished data courtesy of the Norwegian Institute of Public Health. The shaded area marks the time of fMRI and behavioral tasks in the current study. Adapted and reprinted with permission from ©Marie Eikemo (2017), PhD thesis.

3.1.4 Control measures

Subjective state questionnaires

Measurement of mood and subjective state (21 items, see table 1) were collected three times during each session. We used a locally developed questionnaire based on previous studies of opioid side effects (e.g. Zacny & Lichtor, 2008). Questions were presented in Norwegian, and the version has been used in several previous studies (Chelnokova et al., 2016; Eikemo et al., 2016). Items were rated on an 11-point electronic visual analogue scales (VAS) of 100mm, anchored at ‘Not at all’ and ‘Very much’.

The questionnaire was administered five minutes prior to drug administration, immediately before the first sequence inside the scanner (t = 60), and after completion of all tasks (t 155). The questionnaires administered outside the scanner were presented in MATLAB (version 7.10.0. Natick, Massachusetts: The MathWorks Inc., 2010). The

questionnaire administered inside the scanner was presented in E-prime 2.0 (Psychology Software Tools, Pittsburgh, PA). The two versions differed slightly in visual appearance, but the questions and VAS descriptions were identical. One participant completed the subjective state questionnaire inside the scanner with enlarged font size and MR-compatible glasses due to reduced eyesight.

‘

Table 1. Locally developed subjective state and drug effects questionnaire. Questions are listed in the same order as they were presented to subjects.

Right now I feel…
1) good
2) dry in my mouth
3) irritable
4) happy
5) dizzy
6) blunted
7) discomfort in muscles and joints
8) numb
9) nauseous
10) not quite myself
11) high
12) hungry
13) tired
14) confident
15) spaced out
16) anxious
17) red/warm in my face
Drug effects questionnaire
1) Do you feel an effect of the tablets?
2) Do you like the effect of the tablets?
3) Do you dislike the effect of the tablets?
4) How much would you agree with the statement: 'I would like to take these tablets again on a later occasion'?"

Physiological measures

Several authors have pointed out specific methodological challenges related to using pharmacological agents in combination with fMRI (Bourke & Wall, 2015; Jenkins, 2012; Murphy & Mackay, 2011). A particularly important problem here is that μ-opioid agonists are known to decrease respiration and end-tidal CO_2 (Wanigasekera et al., 2012). Increases in

baseline blood CO_2 can substantially decrease task-related BOLD signal changes unrelated to neural activity (Cohen et al., 2002), which would erroneously bias any drug-related contrasts towards showing decreased activity in the morphine- compared to the placebo session.

To control for this potential confound we measured heart rate (HR) and respiration rate (RR) continuously during all functional scans. Heart rate was measured using a pulse-oximeter on the left middle-finger, and respiration was measured using a pneumatic respiratory belt. Both had a sample rate of 500hz.

Visual checkerboard task

We also implemented a short task in the beginning of each scan session to control for potential changes in global BOLD signal unrelated to assess opioid-receptor activity (Phan et al., 2008). The visual checkerboard task consisted of passive viewing task where participants observed 12 trials of a flickering checkerboard pattern for one second followed by a fixation cross for 20 seconds (see figure 4). The task was designed to produce strong and reliable activation in primary visual cortex.

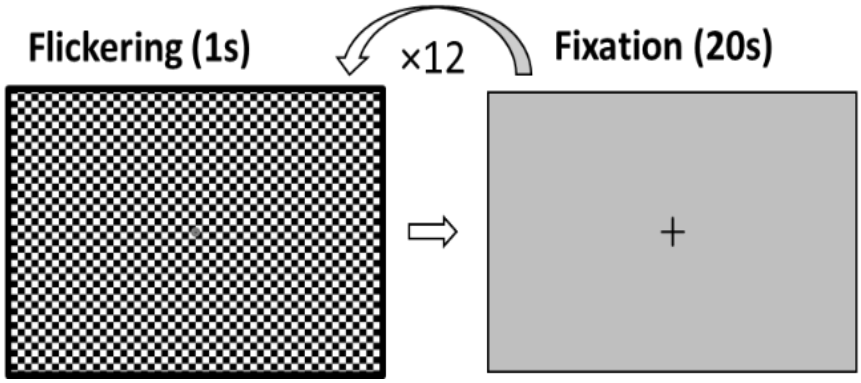


Figure 4: The visual checkerboard task. Participants passively viewed all trials on a screen inside the scanner. Duration of each event displayed in brackets at the top of the figure.

Motor coordination task

To assess the potential effects of morphine on motor control, an eye-hand coordination test was administered approximately 140 minutes after drug administration (Bradykinesia Akinesia Incoordination test [BRAIN], Giovannoni et al., 1999). The test is a computerized finger-tap task in which participants are instructed to, as fast and as accurately as possible, alternatingly press two buttons on a keyboard for 30 seconds using their dominant index finger. The task is designed to measure upper limb motor function and generates several scores. The dysmetria score was most relevant for our study, as it is a weighted index of incorrectly hit keys corrected for time, and represents an overall measure of task performance.

The test has previously been used to study the effect of dopamine drugs on motor function (Pizzagalli et al., 2008).

Blood sampling

A blood sample was collected after the fMRI tasks were completed (about 120 minutes after start of session). The sample was collected in vacutainer tubes via venipuncture of the arm. The samples were collected for two purposes. We wanted to measure individual uptake of morphine to use as a covariate in analyses, using recently developed methods (Johnsen, Leknes, Wilson, & Lundanes, 2015). We also wanted to identify individuals' alleles of the μ-opioid receptor coding gene OPRM1. Although the sample size in the current study is too low for meaningful analyses of the contribution of genetic variance, we hope to combine this with genetic data collected in previous experiments. These analyses were not completed in time to be reported here.

3.1.5 Food Wanting and Regulation task

The food wanting regulation task was designed to measure changes in BOLD associated with viewing highly appetitive food images. In addition, a cognitive regulation exercise was added as part of the task, allowing an exploration of a potential interaction between MOR system agonism and cognitive regulation of food desirability. Cognitive regulation of emotional responses has primarily been studied in the context of negative emotions (e.g. Johnstone et al., 2007). More recently, studies of cognitive regulation of drug and food craving (Giuliani, Calcott, & Berkman, 2013; Kober et al., 2010), have begun to emerge, likely due to potential implications for understanding pathologies like addiction.

In both sessions, participants completed the food wanting regulation task. In each session, in the drug uptake period before entering the scanner, participants completed a short practice run with detailed instructions about the task and the strategies for regulating.

The task is adapted from a similar paradigm for measuring cigarette cravings in smokers (Kober et al., 2010). Participants viewed 20 21-second blocks of full color photographs of highly palatable high-caloric food items (e.g. hamburger, chocolate cake, ice-cream, see figure 5). Each trial began with a fixation cross displayed for 10 seconds, followed by an instruction (1.5 s) to either "observe" or "regulate" while viewing an upcoming block of images. *Observe* and *regulate* blocks were presented in a fixed, alternating order.

In the *observe* condition, participants were instructed to observe upcoming images without controlling their reaction to or wanting of the food items in any way. In the *regulate*

condition, participants were told to use either of two possible regulation strategies while observing the upcoming images. One was to focus on the negative consequences of giving in to their desire for the food items, and think of reasons not to consume fatty/sugary food.

Alternatively, they could pretend that the food displayed was "not real", e.g. that it was a toy made of plastic. Importantly, participants were told to keep their attention on the image and not simply distract themselves by thinking about something else. After the instruction cue there was a blank screen for 500ms, followed by a block of four food images displayed for 5 seconds each. There were four sets of images. All image sets were counterbalanced across instruction conditions. Participants never saw the same image twice, neither within nor across sessions. After each image block, participants were given seven seconds to rate how much they craved food after viewing the images in that block. The task lasted for a total of 13 minutes.

In each session, in the 'drug uptake' period before entering the scanner, participants completed a short practice run with detailed instructions about the task and the strategies for regulating.

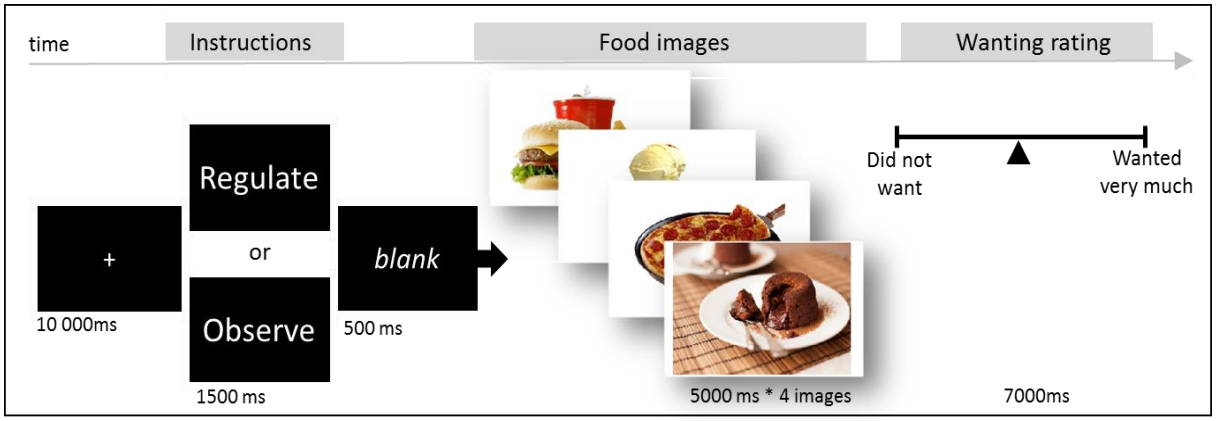


Figure 5: Trial structure for the Food Wanting and Regulation task. Duration of each trial event reported in milliseconds (ms) below each event. Each trial lasted 21 seconds in total.

3.1.6 Imaging parameters

MRI scanning was performed on a 3 T Philips Ingenia whole body MRI scanner with a 32-channel SENSE head-coil (Philips Medical Systems, Best, Netherlands). High-resolution T1 weighted images were acquired for anatomical reference and co-registration to standard space (voxel size 1 × 1 × 1mm, TR/TE 4.7/2.3, 184 slices, field of view (FOV) 256x184, overcontiguous sampling). Functional images were acquired using gradient, echo planar imaging (GE-EPI, 42 transverse 3 × 3 × 3mm thick slices, 0.3 mm interslice gap, descending

slice order, TR = 2208ms, TE = 30ms, flip angle 80°, FOV 240 x 138mm, 80 × 79 matrix). Phase-encoding direction was from anterior to posterior. SENSE factor was set to three. Detailed justification for the slice acquisition and SENSE factor parameters has been reported elsewhere (Lie, 2015). For the food wanting regulation task, 365 volumes were collected. For the checkerboard task, 115 volumes were collected.

3.1.7 Statistical analyses

Mood and subjective state

Subjective state questions were analyzed using paired sample t-tests in SPSS (Version 22.0. Armonk, NY: IBM Corp.). Prior to analysis, ratings from the two measures of subjective state (mood and somatic effects) following drug administration were averaged and baseline corrected by subtracting the average from the pre-drug ratings. Ratings from the four drug effect questions were not baseline corrected, but averaged across the pre-fMRI and post-fMRI measures.

Physiological measures

Heart rate (HR) and Respiration rate (RR) data collected during scanning were extracted in MATLAB (R2015a). Signal loss was removed from each time series, and wave-peaks were extracted. Mean HR and RR for each participant was calculated for each session. The means of HR and RR were analyzed using paired sample t-tests in SPSS.

Motor coordination

The dysmetria scores from the BRAIN task for each session were analyzed using a paired sample t-test in SPSS.

Food wanting subjective ratings

Ratings of the image blocks inside the scanner were analyzed using a linear mixed effects model in R (version 3.3.3) using the "nlme" package (version 3.1-131, Pinheiro, Bates, DebRoy, & Sarkar, 2007). The mixed effects approach was deemed appropriate after visual inspection of the data revealed large individual variation in ratings. Further, mixed effects regression provides a flexible approach for including time-varying covariates while adjusting for correlated measures within-subject (Baayen, Davidson, & Bates, 2008; Gueorguieva & Krystal, 2004; Quené & van den Bergh, 2008). The mixed effects approach offers several advantages of the more commonly used repeated measures (rm)ANOVA. For one, trials can be modeled directly without first averaging across trial type, which makes it less likely that

one underestimates variance in the dataset. Another big advantage of the approach is the robust handling of randomly missing data. In rmANOVAs, randomly missing data points at one level (e.g. subject with missing data from one condition) requires the whole subject to be removed, but in a mixed effects approach the remaining data can still be used for estimation (Quené & van den Bergh, 2008). The biggest disadvantage of the linear mixed effects approach is the complexity of the design and interpretations (Gueorguieva & Krystal, 2004). For these preliminary analyses, a relatively simple model with only one randomly varying intercept was chosen. Model fit for added effects was assessed by chi-square tests on the log-likelihood values to compare models with and without the effects included. This could be done as all models contained the exact same set of dependent measure data points.

fMRI analysis

fMRI image processing was conducted in FSL (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl) version 5.0.9. Before submitting functional images to analysis, we corrected for magnetic field inhomogeneity induced distortions in the raw images. Fieldmap correction in FSL is typically performed by collecting B0 field map images that are uploaded in the default processing pipeline. However, standard B0 fieldmap sequences collected from Philips MRI scanners are not compatible with this pipeline (originally developed for use with Siemens scanners at the FMRIB center). As a work-around for this issue, we collected dual phase-encode blip images, and calculated phase maps for each session for each participant using the *topup* program in FSL (for a detailed explanation of this method, see Andersson, Skare, & Ashburner, 2003). This procedure for fieldmap correction is more commonly used for distortion correction of diffusion tensor imaging (DTI) sequences, but can also be used with fMRI data. Using the *applytopup* function, all functional scans were corrected for distortions caused by susceptibility-induced off-resonance fields, and the corrected images was submitted as input to first level analyses.

fMRI analysis was performed using the FEAT (fMRI Expert Analysis Tool) version 6.00, part of FSL. The following preprocessing steps were applied: Motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002); non-brain removal using BET (S. M. Smith, 2002); spatial smoothing using a Gaussian kernel (FWHM = 5mm); high-pass temporal filtering with a 90s cutoff. Functional data was registered to the high-resolution image from each participant, and then to MNI152 standard space (Montreal Neurological Institute), using FLIRT (Jenkinson & Smith, 2001). Time-series statistical analysis was performed using FILM with correction for local autocorrelation (Woolrich, Ripley, Brady, &

Smith, 2001). A temporal derivative was added to all main explanatory variables (EVs). In addition, the fsl_motion_outliers function was used with default parameters to create EVs for large motion artifacts, which were then added as confound EVs. Z statistic images were thresholded at Z=2.3 in the checkerboard analyses, and at Z=3.1 in the food wanting analyses. The latter value has been recommended by FSL developers after the recent discussions of family-wise error underestimation in fMRI software, and is shown to perform reasonably well in the simulations that sparked this discussion (Eklund, Nichols, & Knutsson, 2016). In contrast, we consciously kept the low Z threshold in the checkerboard task in order to allow a liberal cluster discovery, since this was a control task. Cluster significance threshold of analyses were set to $p<.05$ in both tasks.

Across tasks, three separate analyses were run to produce main effects for the *placebo* condition, for the *morphine* condition, and for the drug contrasts (*morphine* > *placebo* and *placebo* > *morphine*)

All fMRI data analyses were processed on the Abel computer cluster (HPC, University of Oslo).

Checkerboard task

The checkerboard time series were analyzed using a general linear model (GLM) with a single EV modeling the flickering checkerboard pattern. The analysis was designed to model the average activation of each drug session, as well as the comparing them using the contrasts *morphine* > *placebo*, and *placebo* > *morphine*.

In addition to the FEAT analyses, the featquery tool in FSL was used to extract summary statistics and peristimulus raw data from selected regions of interest. Combined ROIs of ventral and dorsal area V1 (see figure 9B) were selected based on the probabilistic atlas by Wang et al. (2014). The ROI masks were thresholded at 50% probability and binarized, as FSL can have difficulty handling probabilistic maps outside of those included in the suite. Peristimulus data for the checkerboard stimulus parameter estimate were submitted to R (v. 3.3.3) to model and compare the activity change from baseline for each drug session.

Food wanting regulation task

Time series from the food wanting regulation task were analyzed in a GLM with the following six EVs included in the design matrix: The trial instruction, images displayed in the *regulate* condition, images displayed in the *observe* condition, the VAS rating, as well as two covariate EVs. One covariate predicted increased activity with increasing self-reported food

wanting after viewing a block in the *observe* condition. This was included to identify regions with activity correlated with self-reported wanting. The other covariate predicted increased activity with decreasing self-reported food wanting in the *regulate* condition. This was included to investigate activity correlated with successful regulation. Contrasts entered at first level included the main effects of each task condition, the contrasts *regulate* > *observe* and *observe* > *regulate*, and the main effects of each covariate EV. At group level, the mean activation in all contrasts were analyzed in each drug session, and the group level contrasts for the drug session were entered.

In addition to the FEAT analysis, we again used featquery to extract summary statistics from ROIs. We created a priori defined masks derived from Neurosynth, an online meta-analytic tool for synthesizing BOLD-activation maps (for details, see Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011) by downloading the probabilistic map generated for the term "value". This map was then thresholded at 10% probability, and divided into a left and right striatal, as well as a frontal ROI (see figure 11A). The masks were then binarized and analyzed in the same way as the checkerboard ROIs.

4 Results

4.1.1 Drug blinding

Participants were not able to guess in which session they had received morphine at levels above chance. In the placebo session, 52 % of participants believed that they had received placebo. In the morphine session however, only 19 % of participants believed that they had received morphine. The same proportion believed they had received naltrexone, yielding a total of 38% correct guesses of an active drug condition. Overall, participants were only 45% correct in estimating whether they had received placebo or an active drug.

4.1.2 Subjective state

Relevant measures are illustrated in figure 6. Uncorrected p-values are reported. Since we are trying to demonstrate the null-hypothesis that drug does not alter subjective state, the strictest correction for multiple comparisons will in this case be no correction for multiple comparisons. Since the point of the questionnaire was to measure *any* possible effect of morphine on subjective state, we did not want to overlook any systematic differences. For the subjective state questionnaires, participants had higher ratings of feeling dry in the mouth in the morphine- (M=3.18, SD_{pooled}=2.45) vs the placebo (M=2.35, SD_{pooled} =2.08) condition; M_{diff}=.73, t(62)=3.71, p=0.0004, two-tailed, uncorrected. Participants also had higher ratings of feeling numb in the morphine- (M=1.97, SD_{pooled}=2.24) vs the placebo (M=1.63, SD_{pooled}=2.05) condition; M_{diff}=.34, t(61)=2.47, p=0.016, two-tailed, uncorrected. There were no other significant differences in subjective state between conditions (p in all other tests >.05).

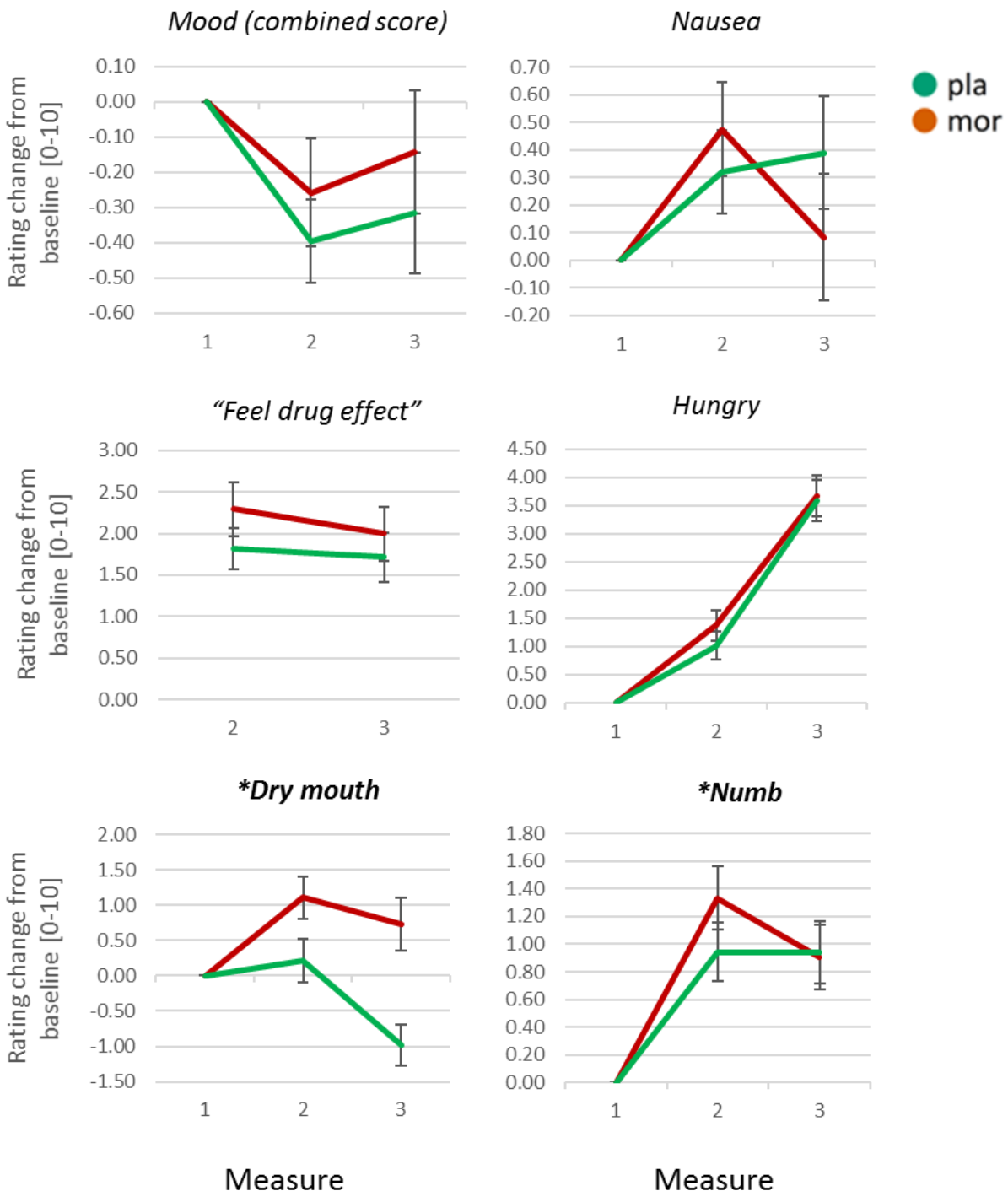


Figure 6. Lines represent mean change from baseline (first rating in session prior to drug administration, scale = 0-10) for selected ratings from the subjective state questionnaire, The "*Feel drug effect"* is only plotted for measure 2 and 3, as their baseline was always zero (no drug had been given yet). For *Dry mouth* and *Numb* there was a significant main effect of drug across measures. Error bars represent the standard error of the change scores.

4.1.3 Motor coordination

A two-tailed paired t-test of dysmetria scores indicated no significant difference in motor function between the morphine (*M*=1.161, *SD*=.28) and placebo (*M*=1.156, *SD*=.26) condition; *t*(50)=.202, *p*=.841.

4.1.4 Respiration and heart rate

As illustrated in figure 7, the difference in respiration rate between the morphine (*M*=16.20, *SD*=3.26) and the placebo (*M*=16.31, *SD*=3.01) condition was not significant; M_{diff} = -.12, *t*(54)=.41, *p*=.683, two-tailed, uncorrected (figure 7A). Nor was there a difference in heart rate between the morphine-(*M*=66.81, *SD*=11.18) and the placebo (*M*=66.94, *SD*=9.50) condition; M_{diff}=.12, *t*(53)=.106, *p*=.916, two-tailed, uncorrected (figure 7B).

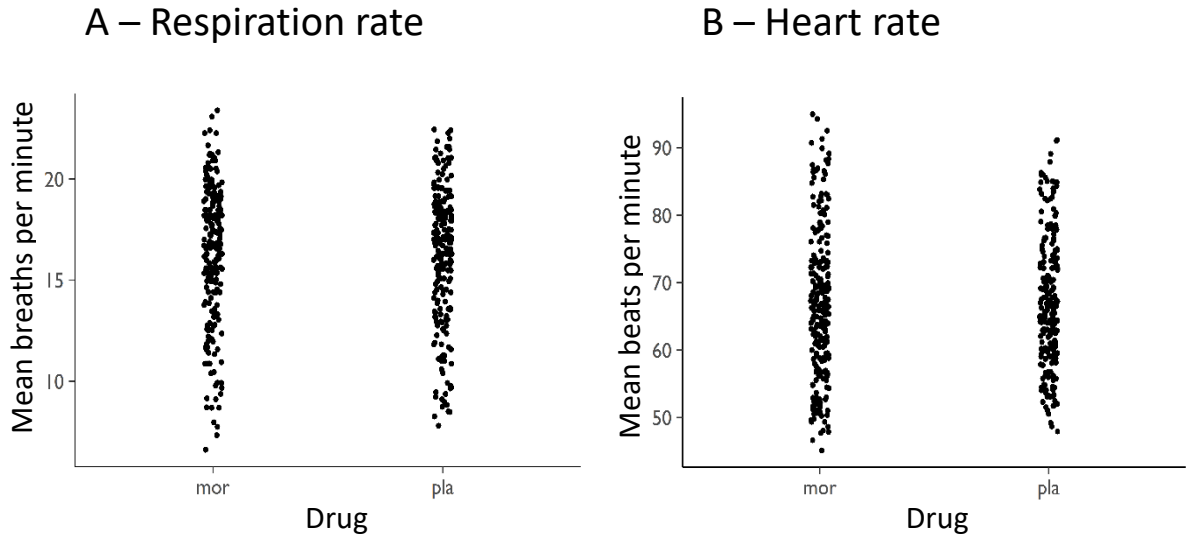


Figure 7. A) Respiration rate subject means for each session. **B)** Heart rate subject means for each session. Dots represent the mean respiration rate/heart rate while inside the scanner, for one subject for one session.

4.1.5 Food wanting behavioral

There was a substantial improvement in model fit after including participant intercepts as a random effect (χ^2=521.14, *p*<.001). After establishing this, a base model containing hypothesis-driven effects of drug, condition, session, and gender, (main effects, 2-way and 3-way interactions), was created. The three-way interactions and drug*session interaction were removed as they were highly correlated with other predictors, and did not contribute meaningfully to model fit. Participants' ratings of hunger and their weight were considered a priori to be important covariates and were added separately. The contribution to model fit of

hunger VAS scores measured at 60 minutes post drug administration was compared with self-reports of "time since last meal". Self-reported hunger provided the best overall model fit and was included as a main effect term. Interactions with the two main predictors, drug and condition, did not improve the overall fit of the model (p of all $\chi^2 > .05$). Weight was then added as a main effect term and interaction with the main explanatory variables. Adding all terms provided the best model fit ($\chi^2 = 16.1$, $p = .003$). The final model for the VAS ratings in the scanner included the main effects of drug, condition, session, gender, hunger, weight, and the interactions drug*condition, condition*session, drug*gender, condition*gender, weight*condition, weight*drug, weight*drug*condition.

Food wanting block ratings from fMRI task

There was a significant main effect of drug, $F(1, 2446) = 4.48$, $p = .03$, such that food wanting was rated higher in the morphine- than in the placebo session (figure 8A). There was also a significant effect of condition, $F(1, 2446) = 963.4$, $p < .001$, such that vas ratings in the *regulate* condition were rated lower than in the *observe* condition in both drug sessions, but there was no significant interaction between drug and condition, $F(1, 2446) = 0.34$, $p = .56$ (see figure 8B). There was a significant main effect of hunger, $F(1, 2446) = 36.54$, $p < .001$, such that food images elicited higher wanting ratings in hungry participants (figure 8C). There was significant interaction between gender and condition, $F(1, 2446) = 14.53$, $p < .001$, such that females in general had lower vas scores than males in the *observe* condition (figure 8D). Finally, a three-way wight*condition*drug interaction was significant, $F(1, 2446) = 6.73$, $p = .01$, showing a small increase in hunger ratings with increasing weight in all sessions and conditions, except during passive observation in the morphine session. No other effects were significant.

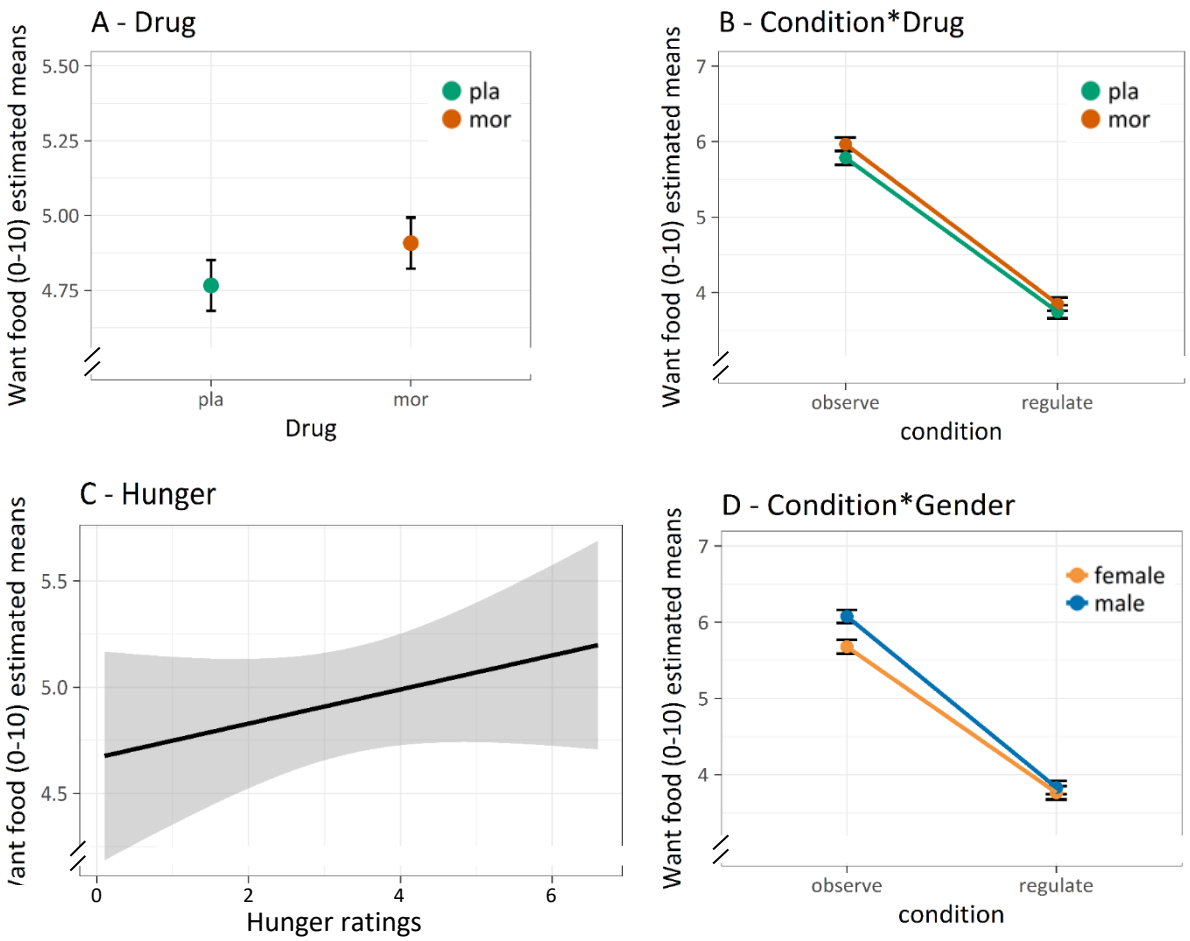


Figure 8: plots of significant effects in the analysis of visual analogue scale (VAS, 11-point scale) ratings of self-reported food wanting in the Food Wanting and Regulation task. **A)** main effect of drug, **B)** condition*drug interaction, **C)** main effect of subjectively rated hunger, **D)** interaction between condition and Gender, E) Interaction between weight, drug and condition. Error bars represent within-subject confidence interval=95%.

4.2 fMRI results

4.2.1 Checkerboard task

Coordinates and statistics for peaks activations pertaining to the results reported below are listed in table 2 of the appendix.

Whole brain fixed effects analyses revealed significant activations in occipital cortex, including primary visual cortex, in both the morphine and placebo sessions (see table 2 and figure 9A). There was also a significantly higher activation in several regions of the occipital lobe in the morphine- compared with the placebo session. However, no voxels with greater activity during placebo compared to morphine survived even the lenient ($Z=2.3$, $p<0.05$) cluster thresholding used for this control analysis. Thus, we found no indication of a global change in BOLD signal caused by putative opioid drug effects on respiration and/or end-tidal CO_2. Mean time series were also extracted from the a priori ROIs, left and right V1, and plotted in Figure 9C to further examine the BOLD signal in this visual control task. Overall, we did not find evidence of morphine modulation of activity-irrelevant BOLD signal changes using this task.

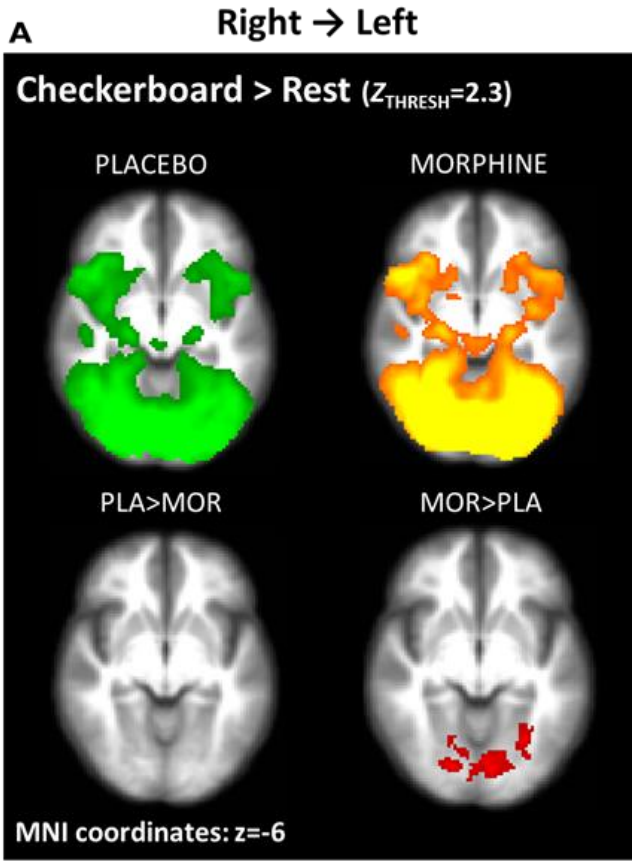
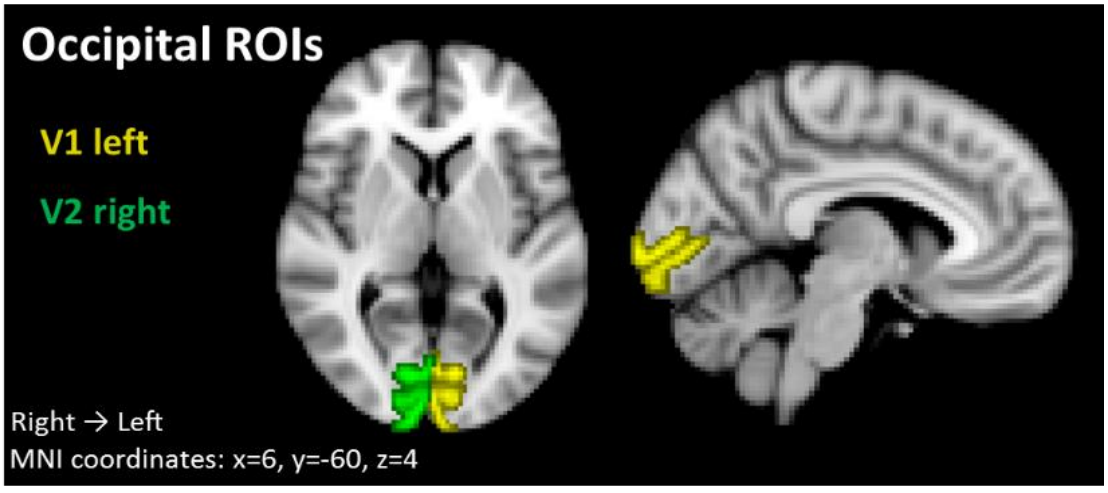
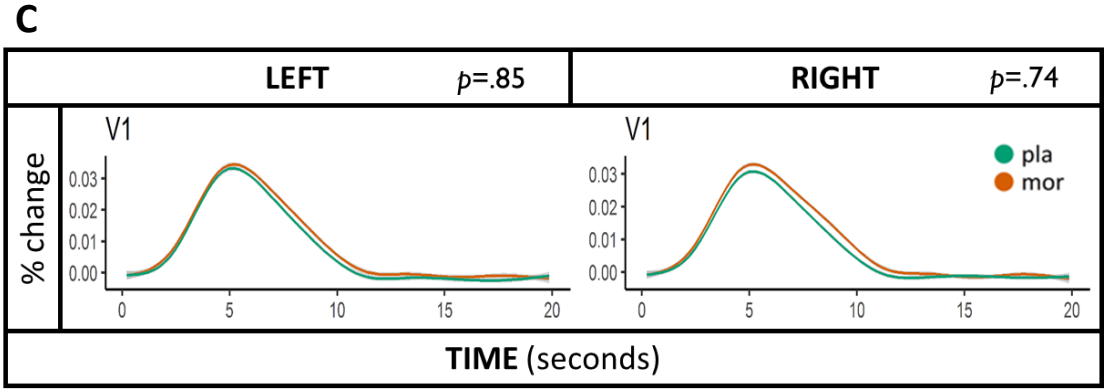


Figure 9. A) Significant activations in the checkerboard paradigm, modeling visual stimuli against baseline (rest/fixation cross). Activation in the placebo condition is colored green, morphine condition Is colored in orange. and the morphine>placebo contrast in red. **B)** Masks created for the left (yellow) and right (green) V1 ROIs, shown in MNI space. Lightness represents the probability of the voxel belonging to the designated area. The binarization process took place within the featquery tool. **C)** Peristimulus plotted data from each of the a priori selected visual ROIs. Lines were fitted to baseline corrected peristimulus data from featquery using a "gam" method in the ggplot2 package in R.





4.2.2 Food wanting and regulation

Coordinates and statistics for peak activations pertaining to the results reported below are listed in tables 3, 4, and 5 of the appendix.

Task activation during observation: Whole brain analysis revealed significant activations in several of the expected regions related to visual food reward during passive viewing of palatable food images in both drug conditions (see figure 10A left and middle, and table 3). This included visual cortices, left and right insula, lateral inferior frontal regions, medial orbitofrontal cortex, and thalamus. Activity in striatal regions survived thresholding for the placebo condition only.

Higher activation during regulation compared to observation: Comparing the task conditions revealed expected differences, in *regulate > observe* direction, in ventrolateral PFC regions, including left inferior frontal gyrus and medial frontal gyrus (See Suppl. table 4). In addition, there was significantly higher activation in right posterior cingulate in the *observe > regulate* direction. These findings were similar for both the placebo and the morphine sessions.

Drug contrasts: When simply observing the food images, there was significantly higher activation in the morphine session *morphine > placebo* in right ventral occipital cortex, corresponding to visual area V2 and V3. In the placebo session, there was significantly higher activation (*placebo > morphine)* in lateral orbitofrontal cortex.

During blocks of cognitive regulation of food wanting, there was again significantly higher activation in the morphine session in right ventral occipital cortex. No areas were significantly more activated in the opposite contrast, placebo>morphine.

The most extensive differences between drug conditions were found for the contrast comparing activation during regulation to observing (*regulate > observe*). Participants showed significantly higher activation in the morphine session (*morphine > placebo*) in various regions, including inferior frontal and superior frontal gyrus, cerebellum, caudate, and anterior occipital cortex (See figure 10B and table 5). No regions survived thresholding for the opposite drug contrast, i.e. we found no regions significantly more active during regulation > observation for the *placebo > morphine* contrast.

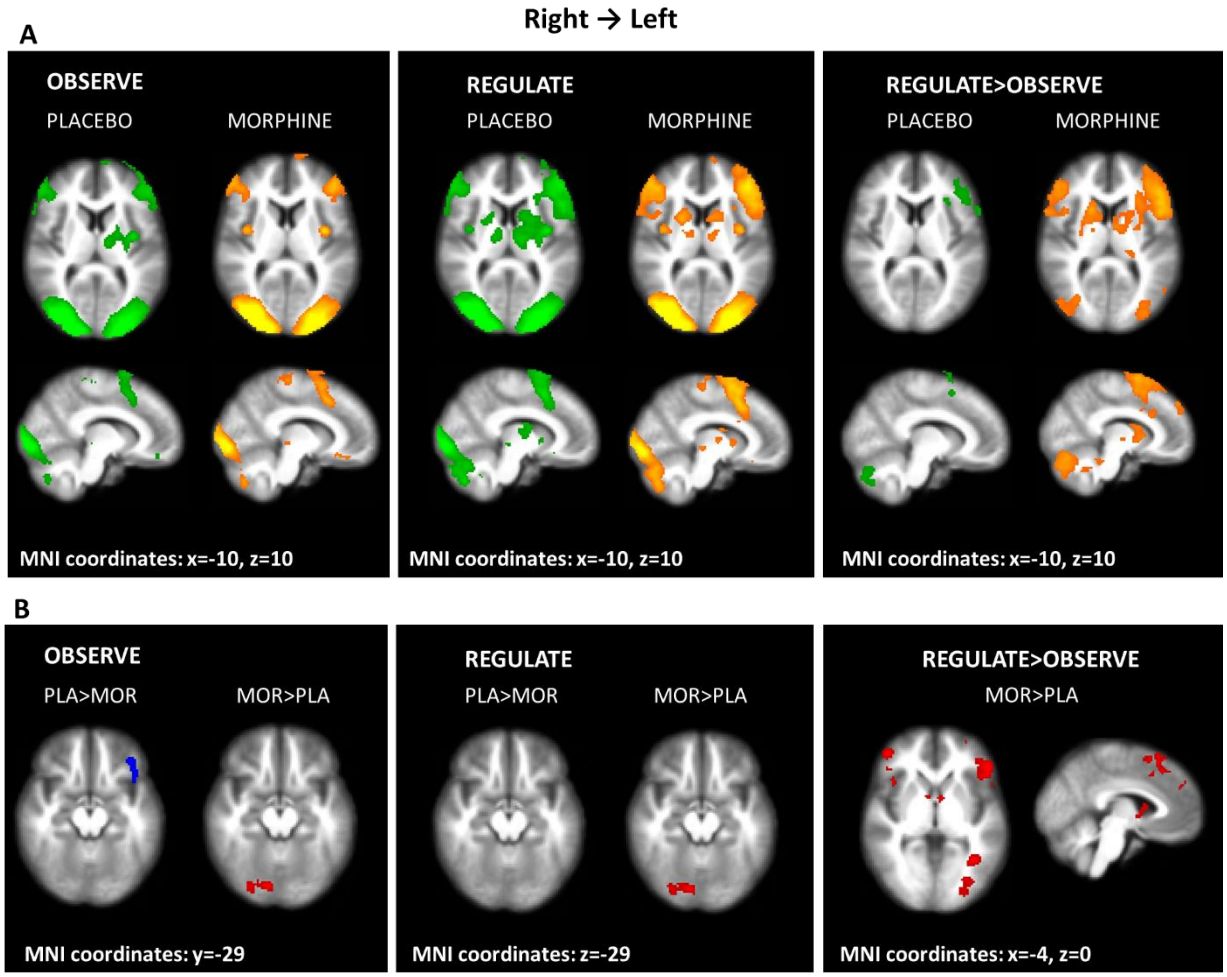


Figure 10: A) Brain activation elicited during image presentation in each drug condition for the *observe>baseline*, *regulate>baseline*, and *regulate>observe* contrasts). Activation in the placebo condition is colored in green, the morphine condition in orange. **B)** Drug contrasts for the same condition contrasts. Activation in the placebo>morphine contrast is colored in blue, and the morphine>placebo contrast in red. Slice coordinates is given at the bottom of each figure. Image left side is right side of brain. Activation threshold in all images is set to Z=3.1. PLA=placebo, MOR=morphine.

ROI analyses: Analysis of peak percentage signal change within the a priori defined ROIs did not reveal a significant difference between drug sessions (figure 11B/C/D), in either the left (M_{diff}=0.03, t(60)=0.78, p=.44) or right (M_{diff}=0.03, t(60)=-0.97, p=.34) striatal, or the frontal ROI (M_{diff}=0.05, t(60)=-0.72, p=.47).

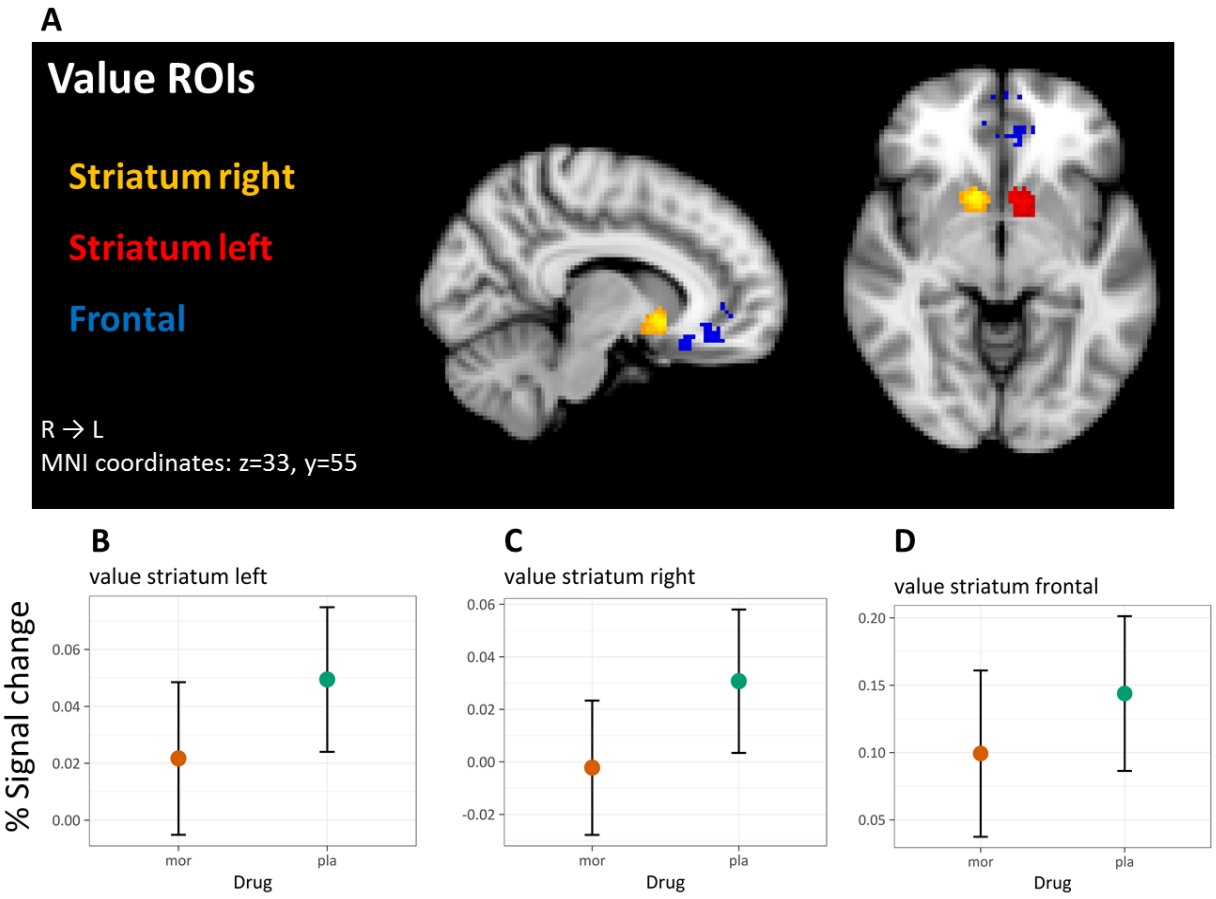


Figure 11: A) Masks created for the left (red) and right (orange) striatum, frontal regions (blue), shown in MNI space. Lightness represents the probability of the voxel belonging to the designated area in any given brain. The binarization process took place within the featquery tool. Mean percentage signal change are shown for each drug condition in **B)** left ventral striatal **C)** right ventral striatal **D)** frontal ROI from Neurosynth. Error bars represent between-subject standard errors.

5 Discussion

This study was designed to measure the effect of pharmacological stimulation of μ-opioid receptors in the healthy human brain on ratings of food wanting due to viewing palatable food images, and associated fMRI activity. Due to the potential complexity of interpreting results from pharmacological studies, especially in the context of BOLD signal analysis, the aim of this thesis was to assess the internal validity of the tasks and drug manipulation employed. There were two primary goals. The first was to analyze and interpret any drug-related differences in subjective state measures, motor coordination, physiology, and BOLD signal in a visual control task, to assess whether drug effects observed in the experimental tasks could be confounded by extraneous variables such as mood differences or changes in respiration. The second goal was to verify that the task itself produced the expected results in terms of behavior and neural response, irrespective of the drug manipulation.

Results from the control analyses revealed no significant effects on breathing and heart rate, nor any significant effects of morphine on a measure of motor coordination. Participants reported somewhat higher ratings of dry-mouth and numbness after morphine compared to placebo treatment. No other measures of subjective state differed significantly between morphine and placebo even with a lenient statistical threshold, i.e. no correction for multiple comparisons. Indeed, participants performed around chance level when asked to guess which session they had received an active drug, indicating that any effects of morphine on task behaviors would be unlikely to stem from expectations rather than specific drug effects. Importantly, we also found no areas displaying significantly higher BOLD signal during placebo than morphine in the analysis of the visual control task. Together with the comparable breathing rates, this result renders it unlikely that the morphine dose should have caused altered global BOLD signal due to respiratory depression and/or increased CO_2 in the blood.

The behavioral results showed a large decrease in subjective ratings of food wanting in regulation blocks. In addition, analysis of drug effects in the Food Wanting and Regulation task revealed a small, but significant increase in ratings of wanting in the morphine session compared to the placebo session.

Whole brain analysis of the Food Wanting and Regulation task showed that viewing of palatable food images was associated with significant activity in a set of regions typically

observed during viewing of palatable food images. Further, as expected, cognitive regulation of food wanting regulation (*regulate* > *observe*) was associated with neural activation of areas previously reported during cognitive regulation such as the dorsolateral PFC, inferior frontal gyrus (IFG) and medial frontal gyrus (MFG) (e.g. Giuliani et al., 2013; Johnstone et al., 2007; Kober et al., 2010). The behavioral results showed a large decrease in subjective ratings of food wanting in regulation blocks. In addition, preliminary analyses of drug effects in the Food Wanting and Regulation task revealed somewhat higher ratings of wanting in the morphine session compared to the placebo session.

Whole-brain analyses of the drug contrasts showed significant occipital activity in both the *regulate* and the *observe* conditions. There was a difference in left lateral orbitofrontal cortex (OFC) in the *regulate* > *observe* contrast in the placebo session. BOLD responses were not significantly different between drug sessions in either frontal or the striatal a priori "value"-related ROIs. In addition to the effects observed during passive viewing of the images, there was an increased *regulate* > *observe* contrast difference in the morphine session than in the placebo session. This difference encompassed several regions, including bilateral IFG and the caudate. The Neurosynth-extracted ROIs did not differ significantly between drug conditions.

5.1 Control measures

Subjective state measures. It was important to verify that participants did not feel high or sedated in the morphine session, since such effects of opioid drugs are commonly observed at larger dosages and if present, could be expected to influence task performance. In addition, it was important to check for task-relevant mood or other subjective state effects such as nausea caused by the drug, as this could potentially influence food wanting and other task measures. Orally administered morphine to healthy, pain-free individuals has previously been shown to influence a variety of subjective states, both positive and negative (Zacny & Lichtor, 2008). However, the doses used by Zacny and Lichtor were three to six times the amount administered in this study. A previous study from our lab reported no significant changes in subjective state in healthy participants using a 10mg per oral dose of morphine (Chelnokova et al., 2016; Eikemo et al., 2016; Hanks et al., 1995).

In line with previous findings, the current results suggest that 10 mg per-oral morphine is associated with very few, mild changes in mood and subjective state. The small significant

increases in numbness and dry-mouth did not seem to be recognized by participants as drug-related, since drug blinding was successful. However, feeling dry-mouthed may nonetheless influence ratings of food wanting. The dry-mouth measure was not included in the preliminary analyses of behavioral or brain measures, but should be considered as a relevant covariate in future analyses of drug modulation of food related effects.

Motor task performance. Previous studies have also shown that μ-opioid agonists can alter motor performance (Zacny & Lichtor, 2008), and decrease respiration rate (Khalili-Mahani et al., 2012; Wanigasekera et al., 2011; Wanigasekera et al., 2012; Zacny & Lichtor, 2008). A previous study from our lab suggests that a 10mg per oral dose of morphine does not affect eye-hand coordination as measured by the dysmetria score of the BRAIN task (Eikemo et al., 2016). The results in the current study replicate the previous null result of a 10 mg morphine pill.

Checkerboard task. We demonstrate that a 10mg per oral dose of morphine does not appear to cause changes in either heart rate or respiration in healthy human participants. As expected after finding no changes in respiration between participants, there was no signal increase in the placebo- compared to morphine session in either of the a priori selected V1 ROIs in the checkerboard task. A decreased BOLD contrast between the checkerboard stimuli and rest would be expected in the morphine condition if respiration rate was reduced (Cohen et al., 2002).

Whole brain analysis did however reveal an unexpected increase in activity in more anterior occipital regions in the *morphine > placebo* contrast. If there was a global BOLD signal difference present in the data and assuming it was largely homogenous across the brain, it would also be reasonable to assume that this difference would be most pronounced in whatever areas most strongly activated at any given moment. Visual inspection of BOLD responses revealed the highest signal change in both drug conditions to occur in ventral medial occipital regions closely overlapping with the V1 ROIs. The ROIs, which revealed comparable responses to visual stimuli across drug conditions, had only a minimal overlap with the regions observed in the *morphine > placebo* contrast. Another argument against global BOLD change as an explanation for these results is that exogenous opioid administration does not appear to affect cerebral blood flow directly (e.g. as a vasodilator, which could also affect global BOLD signal, Benyo & Wahl, 1995).

A different explanation for this result might be activation increase due to increased amount of MOR receptors in visual area V3. Lewis et al. (1981) have shown binding of

naloxone in more anterior and lateral portions of the occipital cortex in rhesus monkeys. For example, they mention increased μ-opioid binding in the "fusiform". Visual inspection revealed a significant overlap between the occipital fusiform gyrus (identified using the Harvard-Oxford Structural Atlas in FSL) and regions with a significant *morphine > placebo* contrast in the checkerboard task. A further possibility is an effect of morphine on visual attentional mechanisms. While larger doses of morphine makes people sedated, drowsy, and dizzy, which may impede attention, small doses of morphine, similar to the one administered in the current study, have in some studies been shown to slightly improve attention (Hanks et al., 1995; O'Neill et al., 2000). An argument against an attentional explanation however, is that the task required very little actual attention from the participants. Notably, none of these explanations are mutually exclusive.

5.2 Food Wanting and Regulation task

Behavioral task effects

Task validation. We observed the expected effect of the task manipulation such that reported food wanting was substantially lower in regulation trials than during passive observation. However, we asked participants to think about negative health consequences of eating the displayed food and then immediately asked them how much they wanted food. It is reasonable to assume that participants were largely able to guess the intended effect of the regulation, so we must assume that the decrease reported food wanting is partially driven by demand characteristics. This confound is likely present in all tasks employing similar cognitive regulation approaches.

Preliminary analyses of drug effects on food wanting. As expected, there was a small but consistent main effect of drug, such that participants reported slightly higher food wanting in the morphine session. The ability to downregulate food wanting, as indexed by ratings after the regulate blocks, was not significantly altered by morphine however. Our group has previously found behavioral changes after administration of the same morphine dose in healthy volunteers across social, monetary and food reward domains (Chelnokova et al., 2014; Chelnokova et al., 2016; Eikemo et al., 2017; Eikemo et al., 2016). However, the only previous fMRI study looking at responses to high value images in healthy humans after MOR agonist administration did not report any behavioral changes in their reward task (Wardle et al., 2014). There might be several reasons for inconsistencies across studies, including the use

of a different agonist drug, a different reward task, and the low power of that particular study to pick up a potential drug effect (*N*=14).

There was an unexpected interaction between gender and condition, even after controlling for weight, such that males reported slightly higher food wanting than females during passive viewing, but not when regulating food wanting. A previous study has reported a similar effect of gender for passive viewing of food images. However, in that study, males reported lower food wanting during regulation blocks too (G. J. Wang et al., 2009). Gender may thus be an important covariate in future fMRI analyses, along with hunger ratings, weight and possibly ratings of dry mouth since the latter was significantly higher in the morphine condition. Morphine effects can interact with the menstrual cycle (Ribeiro-Dasilva et al., 2011). In this study though, the majority of women were on contraceptives, and the majority of those that were not were tested within the same period of their cycle.

BOLD fMRI activity

Task validation. Whole brain analyses revealed that both drug conditions yielded significant activity from baseline during passive viewing of the palatable food stimuli in visual areas, thalamus, insula, amygdala, and caudate, consistent with previous studies (Sescousse et al., 2013; van der Laan et al., 2011). Furthermore, we observed a pattern of increased activity during cognitive regulation compared to passive viewing in the inferior (IFG) and medial frontal gyrus (MFG), as expected from previous studies of cognitive regulation in humans (Buhle et al., 2014; Johnstone et al., 2007; Kober et al., 2010). As with the results from the *observe* condition alone, both the placebo and morphine group analyses yielded similar activation patterns for the *regulate* > *observe* contrast. Inferior frontal cortex is thought to be important for general inhibition (Aron, Robbins, & Poldrack, 2014) while MFG has been shown to be involved in successful emotion reappraisal, especially when using strategies that focus on personal relevance (Ochsner et al., 2004). The reliable activation of the MFG in this study may perhaps indicate that people find it more effective to about future negative consequences compared to thinking about the food items as plastic when attempting to regulate, although this hypothesis is speculative, as we did not ask participants to state which strategy they preferred to use.

Preliminary analysis of drug effects on BOLD. Unexpectedly, there was no significant difference between drugs in the a priori defined "value" striatal ROIs. While this would be consistent with some previous studies investigating MOR agonists in reward tasks (Mei et al., 2010; Wardle et al., 2014), it is not consistent with the assumption that morphine's

effects on self-reported food wanting would be reflected by higher BOLD signal in these value-related regions. We also had an a priori hypothesis that medial parts of OFC and PFC could be influenced by the drug manipulation, as these regions are thought to be involved in value encoding of primary rewards (Sescousse et al., 2013; Sescousse et al., 2010). However, there was no significant effect of drug in the frontal "value" ROI either. This ROI consisted of voxels within ventromedial PFC and medial OFC. Previous studies using systemic MOR manipulations to study reward responses tend to find main effects of drug on BOLD without without detecting changes in behavioral responses (Murray et al., 2014; Wardle et al., 2014). Here we surprisingly report a behavioral main effect of drug on food wanting, but fail to observe a main effect in the BOLD signal.

The only regions showing significantly higher activity with morphine than placebo was parts of the left visual cortex, localized to Ventral V3 according to Juelich atlas. This pattern was present for both the *observe* and the *regulate* conditions (compared to rest; see figure 10B). Higher activity in V3 with morphine was also found in the checkerboard control task, although visual inspection of the contrast masks revealed the regions to be non-overlapping across tasks. Thus, the effects of morphine on visual activation appear to be non-specific. Since the visual checkerboard task was designed with a view to minimizing the emotional impact (one-second blocks instead of the possibly more unpleasant versions of this task that use longer blocks), the morphine effects observed across tasks and condition are unlikely to reflect reward-related processes. The proposed explanation of general attention increases after low-dose morphine administration may be the most likely.

One brain region, a lateral section of the left OFC, was identified as more active during placebo than morphine for the *regulate* blocks. Wardle et al. (2014) have previously reported that systemic μ-opioid agonism decreased lateral OFC responses to positive images, but in the *right* side of the brain. It is unclear why the lateral OFC would be modulated during exposure to palatable food images. Indeed, lateral OFC activation have more commonly been associated with either stimuli of negative valence (Kringelbach, 2005), or with more secondary rewards (Sescousse et al., 2010). However, the functional role of the lateral OFC is still unclear and under debate (Stalnaker, Cooch, & Schoenbaum, 2015).

The most extensive drug differences in fMRI signal was identified during the preliminary analysis of the *regulate > observe* contrast. Two regions in the lateral PFC (figure 10B, right) were more strongly activated during active regulation than passive viewing (*regulate > observe*) in the *morphine > placebo* contrast. This pattern of drug effects does not

correspond to the observed pattern of drug effects on food wanting ratings, however (See figure 8B). Morphine increased self-reported desire for the palatable food images to a similar extent across the *observe* and *regulate* conditions, and we found no evidence that morphine interfered with the ability to downregulate food wanting. The increased food wanting in the morphine session may suggest the following, however: The increased wanting experienced in the morphine session may be more difficult for participants to regulate. It may be that increased prefrontal resources are recruited in the morphine condition, and this increase allows participants to successfully downregulate wanting by the same amount as in the placebo session.

A previous study by Kober et al. (2010) on BOLD responses during cognitive regulation of cigarette craving in smokers suggested that the left dlPFC mediated a relationship between decreased ventral striatal activity and decreased cigarette craving due to cognitive regulation. While we observed no difference between drug conditions in ventral striatum in these preliminary analyses, for the *regulate > observe* contrast this might be because participants are successfully mustering extra resources to downregulate activity in this region during regulation. A future analysis should assess the connectivity between ventral striatum and these prefrontal regions in the morphine and placebo conditions.

Since this is a study of systemic administration of a MOR agonist, it is difficult to determine the position of MOR in the causal chain from drug uptake to its effect on food wanting. Our results regarding food wanting can be caused by multiple mechanisms, not mutually exclusive. It is possible that MOR directly mediates feelings and regulation of wanting. For example, one recent review has suggested that MOR in the prefrontal cortex may be directly involved in the regulation of drug craving (Baldo, 2016). It is also possible that MOR signaling influences wanting indirectly, for example by regulating levels of dopamine in the brain (Nestler, 2005), or by influencing digestion in the gastrointestinal system of the gut (e.g. Holzer, 2009). Determining the exact mechanisms by which opioids influence wanting will be important for truly understanding how wanting is processed in the human brain.

5.3 Statistical power

Consideration of the power of a study to pick up effects with appreciable accuracy is an important part of any empirical study. Low power reduces the interpretability of non-

significant findings, and inflates reported effect size (Yarkoni, 2009). In other words, the lower the power of a study, the higher the effect size will have to be to pass the significance threshold traditionally required for publication. This may in turn make it difficult for future research to build on the results of a study. A large scale independent replication project of 100 studies in psychology revealed surprisingly low number of successful replications, and a substantial decrease in average effect across replications compared to original studies (Open Science Collaboration, 2015). The problems of underpowered research may be equally serious or worse in neuroscience (Button et al., 2013; Szucs & Ioannidis, 2017).

In this study, we collected complete datasets from 63 participants. The sample size of the current study is around 3-6 times the typical participant count used in previous fMRI studies on food reward (Sescousse et al., 2013; van der Laan et al., 2011). We also incorporated a within-subject design, which improves statistical power when between-subjects variability is larger than within-subject variability on relevant measures. As one example, in this study it allowed us to deal the large inter-subject variability in the drug effects of interest, which may obscure drug effects in between-subjects designs. As one post-hoc estimate of the power of this study, using the G*Power software (v. 3.1.9.2), a sensitivity analysis for simple within-subject t-tests, assuming α=.05 and a sample size of 63, revealed a power of .80 for effects as low as d_z=.32. Still, the ideal approach would have been to conduct an a priori power analysis based on pilot data, and set a target sample size based on this. Future studies can make use of recent software developments for calculating power in fMRI a priori using pilot data (Mumford, 2012).

5.4 Limitations

Perhaps the most serious disadvantage of the current study is the lack of a control image condition of either non-food items, non-palatable food items, or both, to contrast the palatable food items with. This was a conscious trade-off to enable testing of the most pertinent hypotheses within a sensible time frame given the half-life of morphine. However, it does severely limit the ability to separate activity specific to reward processing in from specific food-associated activity, and activity simply related to the visual nature of the task. In addition to the lack of a good control condition, all food images displayed in this study were high-caloric and highly palatable. It is possible that the judged value of a food item is context

dependent. While all the food items in this study were designed to be objectively palatable, it is possible that the subjectively judged value of the food would increase if less palatable food items were presented for comparison. A future study of MOR reward regulation might benefit greatly from deliberately implementing images of low-value equivalent objects and non-related but equally visually complex objects (Murdaugh, Cox, Cook, & Weller, 2012; van der Laan et al., 2011), and ideally also a high-value non-food condition to better tease apart the reward- and food-specific activation. Further, including an upregulation condition in the design (to increase food wanting) could enable further probing of interactions between cognition and reward/MOR-related processing.

Another less serious limitation regarding the food wanting task is the lack of control over participants' food intake before arriving. Although we asked participants to eat within a few hours of arriving, we did not know how much they had eaten, or whether some participants did not comply. Since we asked participants to report their hunger level before entering the scanner, we were able to in some extent control for this, but due to the large effects that variation in satiety can have on behavioral and neural responses to food reward images (Siep et al., 2009), many researchers exert much tighter control over participants' food intake in the study of food reward processes (van der Laan et al., 2011).

Another limitation of the food reward task is the lack of a behavioral task which could link ratings and brain activity to e.g. food consumption. Although it is very common to apply this type of design in fMRI studies of reward (Sescousse et al., 2013), the validity of self-report as sole measures of mental processes has received longstanding criticism (Nisbett & Wilson, 1977). In the case of a regulation task, it is of particular interest to assess whether the regulation strategies employed could reduce how much participants would eat.

Future studies should also consider using arterial spin labeling (ASL) MRI to obtain a non-invasive quantitative measure of cerebral blood flow at baseline during each drug session. Although we did implement the checkerboard task to gauge potential global effects of morphine on BOLD, it is recommended to more formally control for non-neural components of the BOLD signal such as cerebral blood flow (Murphy & Mackay, 2011). ASL has both spatial, temporal, and signal-to-noise limitations. It is nevertheless a widely-recommended control measure in phMRI (Bourke & Wall, 2015; Iannetti & Wise, 2007; Murphy & Mackay, 2011).

Finally, the chosen drug administration procedure has limitations worth noticing. First is the administration of a single dose vs placebo, which makes hypotheses about linear effects of

dose increase unavailable. Second is the use of per oral administration vs intravenous (IV). In our study, while we did control for plausible covariates like weight in the analysis of behavioral data, we administered the same dose to all participants and did not have control over the actual relationship between dose and drug concentration in each participant. IV administration allows one to customize the drug dose to each participant, often using a dose/kg calculation, and to keep concentration stable by using a continuous infusion throughout the experiment. IV infusion also offers a much faster drug effect onset time than per oral administration, especially with a fast-acting MOR agonist like remifentanil (Egan, 1995; McQuay, 1999).

5.5 Conclusion:

The current study was designed to investigate the role of the MOR system in motivational behavior and neural activity approximated by BOLD fMRI. The main aims were (1) to establish the internal validity of results from the drug manipulation, and investigate potential confounds in behavior and brain physiology, and (2) to assess whether the Food Wanting and Regulation task elicited the expected effects on behavior and BOLD signal. There was no major influence of drug on general subjective state, heart- or respiration rate, eye-hand coordination, or BOLD signal in early visual areas low in MOR. The relatively large sample size of the study strengthens the interpretation of these results as true non-differences. In addition, the main task elicited the expected effects of exposure to palatable food images and actively regulating wanting, irrespective of drug manipulation, both on participants' behavior and neural response to the task. We therefore conclude that there is a solid foundation for analyzing the effect of MOR manipulation on relevant task measures. Preliminary analyses confirm our initial hypothesis that MOR agonism increase reported food 'wanting', but do not confirm the hypothesis that this increase corresponds with increased activity in value-encoding regions like medial PFC and striatum. Further analyses are required to reach firm conclusions regarding the effects of systemic MOR manipulation in healthy humans on food wanting and on the ability to downregulate food wanting by using cognitive strategies.

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Appendix

Table 2: Activation peaks and cluster extents for significant activations during stimulus presentation in the checkerboard task. Z threshold = 2.3, FWE=.05

Drug	Region	X (mm)	Y (mm)	Z (mm)	Cluster Extent	Max Z
Morphine	Occipital Lobe	-20	-92	-2	75439	11.6
Cluster peaks	*L Occipital Pole*	-20	-92	-2		11.6
	R Lingual Gyrus	4	-78	-2		11.5
	L Frontal Pole	-40	48	28	1067	5.04
Placebo	Occipital Lobe	10	-84	-10	75384	11.2
Cluster peaks	*R Lingual Gyrus*	10	-84	-10		11.2
	L Occipital Fusiform	-24	-88	-4		10.7
Morhine > Placebo	L Visual Area V3	-4	-78	-4	1247	4.8
Cluster peaks	*L Ventral Visual V3*	-4	-78	-4		4.8
	R Ventral Visual V3	26	-86	-16		4.35
Placebo > Morphine	—					

Table 3: Activation peaks and cluster extents for significant activations during image viewing in the Food Wanting and Regulation task. Table displays significant activations for each drug session in the *observe* condition. These were used to make sure that passively viewing the images activated expected reward regions. Z threshold = 3.1, FWE=.05

Condition	Drug	Region	X (mm)	Y (mm)	Z (mm)	Cluster Extent	Max Z
Observe	Morphine	R Occipital Pole	16	-94	-4	45212	11.5
	Cluster peaks	*L Occipital Pole*	-18	-94	-2		11.2
		R Superior Parietal Lobule	32	-54	56		7.6
		L Superior Parietal Lobule	-28	-56	60		8.0
		R Fronal Pole	26	36	-14	507	6.8
		L Frontal Pole	-46	38	16		7.1
		Superior Frontal Gyrus	2	14	54		6.8
		R Insula	38	-2	14		6.7
		L Insula	-36	-4	12		7.2
		R Thalamus	22	-24	-2	202	6.1
		L Thalamus	-20	-22	-4	208	6.3
Observe	Placebo	L Occipital Pole	-20	-98	2	50636	11.5
	Cluster peaks	*R Occipital Pole*	20	-94	-6		10.9
		R Superior Parietal Lobule	32	-52	56		7.5
		L Superior Parietal Lobule	-32	-54	56		7.6
		R Fronal Pole	26	38	-2	645	6.5
		L Frontal Pole	-30	38	-12		6.4
		Superior Frontal Gyrus	2	14	54		6.4
		L Insula	-36	-4	12		6.2
		R Thalamus	18	-24	-2		5.4
		L Thalamus	-6	-24	-2		4.5
		L Caudate	-10	18	2		3.9

Table 4: Activation peaks and cluster extents for significant activations during image viewing in the Food Wanting and Regulation task. Table displays significant activations and condition contrasts in the placebo session. These were used to make sure that regulation activated expected prefrontal control regions compared to passive viewing. Z threshold = 3.1, FWE=.05

Drug	Condition	Region	X (mm)	Y (mm)	Z (mm)	Cluster Extent	Max Z
Placebo	Observe	L Occipital Pole	-20	-98	2	50636	11.5
		R Occipital Pole	20	-94	-6		10.9
		R Superior Parietal Lobule	32	-52	56		7.5
		L Superior Parietal Lobule	-32	-54	56		7.6
		L Insula	-36	-4	12		6.2
		R Thalamus	18	-24	-2		5.4
		L Thalamus	-6	-24	-2		4.5
		L Caudate	-10	18	2		3.9
		R Amygdala	26	0	-22		3.7
		L Amygdala	-30	-4	-18		3.6
		R Fronal Pole	26	38	-2	645	6.5
		L Frontal Pole	-30	38	-12		6.4
		Superior Frontal Gyrus	2	14	54		6.4
Placebo	Regulate	L Occipital	-20	-98	2	68626	11.3
		R Occipital	20	-92	-6		11.1
		R Superior Frontal Gyrus	2	10	56		8.0
		R Frontal Pole	52	40	16		6.0
		L Frontal Pole	-46	38	14		7.3
		R Insula	40	0	6		5.0
		L Insula	-36	-4	10		6.7
		R Thalamus	24	-20	-4		6.3
		L Thalamus	-16	-24	-4		6.1
		R Putamen	16	10	0		5.0
		L Caudate	-16	16	6		5.4
		R Amygdala	-20	0	-18		4.2
		L Amygdala	20	0	-22		4.2
Placebo	Observe > Regulate	R Posterior Cingulate	8	-52	28	321	3.89
Placebo	Regulate > Observe	L Inferior Frontal Gyrus	-50	18	-4	2044	4.28
		R Cerebellum	4	-78	-32	2037	4.31
		Superior Frontal Gyrus	0	30	66	1045	5.06
		R Frontal pole	58	38	0	309	4.1

		L Frontal Pole	-48	30	10	3.8
		L Lateral Occipital	-24	-72	46	298	4.5
		L Inferior Temporal Gyrus	-52	-64	-22	282	4.9
		L Middle Frontal Gyrus	-46	6	42	250	3.94

Table 5: Activation peaks and cluster extents for significant activations during image viewing in the Food Wanting and Regulation task. Table displays significant activations in all drug contrasts. Z threshold = 3.1, FWE=.05

Condition	Drug	Region	X (mm)	Y (mm)	Z (mm)	Cluster Extent	Max Z
Observe	Morphine > Placebo	R Lingual Gyrus	12	-84	-14	141	6.0
	Placebo > Morphine	L Orbitofrontal Cortex	-40	36	-14	164	4.6
Regulate	Morphine > Placebo	R Occipital Fusiform Gyrus	14	-82	-14	171	6.0
	Placebo > Morphine	–					
Regulate > Observe	Morphine > Placebo	R Inf. Frontal Gyrus	54	24	12	822	6.0
		L Inf. Frontal Gyrus	-52	36	0	655	5.5
		R Middle Dorsal Cerebellum	26	-74	-22	940	5.7
		R L Superior Frontal Gyrus	0	14	58	709	5.4
		R Caudate	18	22	6	442	5.1
		L Anterior Occipital Cortex	-38	-56	0	315	5.0
		L Lateral Occipital	-52	-64	32	302	4.8
		L Frontal Pole	-18	52	28	245	5.0
Observe > Regulate	Morphine > Placebo	–					